Effects of Tetrastarch Administration on Hemostatic, Laboratory, and Hemodynamic Variables in Healthy Dogs and Dogs with Systemic Inflammation

by

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ABSTRACT

EFFECTS OF TETRASTARCH ADMINISTRATION ON HEMOSTATIC, LABORATORY, AND HEMODYNAMIC VARIABLES IN HEALTHY DOGS AND DOGS WITH SYSTEMIC INFLAMMATION

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Hydroxyethyl starches (HES) are the most routinely used synthetic colloids during fluid resuscitation and have reported effects on coagulation. The overall goal of the investigation in this thesis was to evaluate the effects of tetrastarch administration on hemodynamic, laboratory, and hemostatic variables in healthy dogs and dogs with systemic inflammation. The objectives were to compare hemodynamic and laboratory variables in dogs receiving an isotonic crystalloid (0.9% NaCl) or tetrastarch during health and after induction of systemic inflammation; to compare the hemostatic effects of an isotonic crystalloid (0.9% NaCl) and synthetic colloid (tetrastarch) in healthy dogs and dogs with induced systemic inflammation; to compare two different protocols for TEG® activation and to determine the correlation between TEG® variables and traditional coagulation test results.

Sixteen adult purpose-bred Beagles were randomized into one of two groups receiving fluid resuscitation with either 40 mL/kg IV isotonic crystalloid (0.9% NaCl) or synthetic colloid (tetrastarch) after administration of lipopolysaccharide (LPS; 5 µg/kg, IV) or an equal volume of placebo (0.9% NaCl, IV). Blood samples, for analysis, were collected at 0, 1, 2, 4, and 24 hours from the time of fluid resuscitation. After a 14-day
washout period, the study was repeated such that dogs received the opposite treatment (LPS or placebo) and the same resuscitation fluid. Resuscitation with equal volumes of 0.9% NaCl and tetrastarch caused similar changes in hemodynamic and laboratory variables in dogs with LPS-induced systemic inflammation; however, larger increases in HR and blood pressure were seen within the first 2 hours following tetrastarch administration compared to 0.9% NaCl. Tetrastarch administration increased COP in all dogs, despite a decrease in TS. Tetrastarch bolus administration to dogs with LPS-induced systemic inflammation also resulted in a transient hypocoagulability characterized by a prolonged PTT, decreased clot formation speed and clot strength, and acquired type 1 von Willebrand disease.

Considering the limited additional benefit of tetrastarch administration on hemodynamic variables demonstrated, as well as the transient adverse hemostatic effects of tetrastarch administration, the increased cost associated with the use of tetrastarch likely negates its use as a first line treatment during fluid resuscitation in dogs.
ACKNOWLEDGEMENTS

I would like to sincerely thank my DVSc Advisory Committee, Drs. Marie Holowaychuk, Carolyn Kerr, Alexa Bersenas and Darren Wood, for their guidance and support throughout the research process. I also want to extend my gratitude to Drs. Anthony Abrams-Ogg and Kate Hopper for participating on my DVSc Examination Committee. I am also grateful to the Ontario Veterinary College Pet Trust Fund for providing the necessary funds required to complete this project successfully.

I would like to extend my gratitude to Jessica Leader, Mandy Hathway, and Hiroshi Fujita for their invaluable assistance with data collection, as well as Gabrielle Monteith and William Sears for their help with statistical analyses. I would also like to thank my fellow resident mates and the ICU technicians for their moral support.
DECLARATION OF THE WORK PERFORMED

I declare that with the exception of the items below, all work in this thesis was performed by me.

The pre-study complete blood counts, serum biochemistry profiles, prothrombin times, and partial thromboplastin times as well as the study platelet counts and white blood cell counts were performed at the Animal Health Laboratory of the University of Guelph. The study prothrombin times and partial thromboplastin times were performed at the coagulation laboratory at the Foster Hospital for Small Animals at Tufts University. Finally, factor VIII activity, von Willebrand factor antigen and collagen binding activity, and D-dimer concentration were measured at the Comparative Coagulation Section of the Animal Health Diagnostic Center at Cornell University.

The care of the research dogs was provided by the Central Animal Facility of the University of Guelph. Technicians from the Department of Clinical Studies as well as summer students and volunteers participated in restraint during blood collection and data collection of vital parameters. Drs. Marie Holowaychuk and Hiroshi Fujita performed measurements of colloid osmotic pressure, packed cell volume, total solids, and serum lactate concentration.
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ABBREVIATIONS:

ACT  Activated clotting time
α    Angle α
CI    Coagulation index
CL30% Clot lysis index after 30 minutes
CL60% Clot lysis index after 60 minutes
COP  Colloid osmotic pressure
CT    Closure time
DIC  Disseminated intravascular coagulation
Fg    Fibrinogen
FII   Thrombin
FV    Factor V
FVII  Factor VII
FVIII Factor VIII
FIX   Factor IX
FX    Factor X
FXI   Factor XI
FXIII Factor XIII
FDP   Fibrin degradation products
G     Global clot strength
HES   Hydroxyethyl starch
IL-1  Interleukin-1
IL-6  Interleukin-6
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<td>INR</td>
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<td>K</td>
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<td>PTT</td>
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<td>PFA-100</td>
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<td>PT</td>
<td>Prothrombin time</td>
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<td>R</td>
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<td>TAFI</td>
<td>Thrombin activatable fibrinolysis inhibitor</td>
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<td>TEG®</td>
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<td>TF</td>
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<td>TF pathway inhibitor</td>
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<td>Tumor necrosis factor</td>
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<td>Total solids</td>
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CHAPTER 1: LITERATURE REVIEW

1.0 Introduction

Sepsis is a common disease in dogs and is associated with high mortality rates. Also, dysfunction of the coagulation system is independently associated with increased likelihood of death in dogs with sepsis. Fluid therapy is part of the mainstay of treatment and synthetic colloids are sometimes used. General indications for colloid therapy include intravascular volume expansion for patients with hypovolemic or distributive shock, as well as patients with hypoalbuminemia and decreased oncotic pressure. Hydroxyethyl starches (HES) are the most routinely used type of synthetic colloids, but they are associated with adverse effects such as coagulopathies. Newer generation HES products such as tetrastarch have been developed, with fewer reported side effects. However, the effects of tetrastarch on hemostasis have not been investigated in dogs. Thromboelastography (TEG®) is a method for global assessment of hemostasis and allows for detection of both hypo- and hypercoagulability. No in vivo study has been performed in dogs using TEG to assess the effect of HES administration on coagulation. In this review, the cell-based model of coagulation, the evaluation of hemostasis with traditional coagulation tests and TEG®, the interactions between inflammation and hemostasis, the hemostatic changes during sepsis will be discussed. Lipopolysaccharride (LPS) as a model for canine sepsis, the use of HES solutions during sepsis and the coagulation abnormalities associated with their use, as well as the changes on colloid osmotic pressure (COP) associated with the HES will also be reviewed.

1.1 The Cell-Based Model of Coagulation

Hemostasis is defined as the stoppage of bleeding or the interruption of blood flow through a vessel. Normal hemostasis relies on complex interactions of several
components including platelets, coagulation factors, fibrinolytic factors, and blood vessels (Stockham et al. 2008).

For normal hemostasis to occur there must be thrombin (FII) generation sufficient to convert fibrinogen (Fg) to fibrin and to make the clot impermeable to blood and temporarily resistant to fibrinolysis (Roberts et al. 2006). Traditionally, the cascade model has been used to explain the sequence of events leading to fibrin formation (Figure 1.1.1). This model consists of a series of steps where enzymes cleave zymogen substrates, or proenzymes, to generate the next enzyme in the cascade (Hoffman 2003b, Smith 2009).

**Figure 1.1.1 - Cascade Model of Coagulation.** The intrinsic and extrinsic pathways can both redundantly lead to generation of FXa. The common pathway results in formation of thrombin and cleavage of fibrinogen to fibrin.
Most of these steps require calcium and take place on phospholipid membrane surfaces, with the exception of Fg cleavage by thrombin. The cascade model is divided into the extrinsic (outside the blood) and intrinsic (within the blood) pathways. The extrinsic pathway consists of tissue factor (TF) and factor VII (FVII). The intrinsic pathway involves contact activation of factor XII (FXII) on negatively charged surfaces, with subsequent sequential activation of factors XI (FXI), IX (FIX), and VIII (FVIII). Both pathways then activate factor X (FX) and V (FV) through the common pathway, which leads to generation of thrombin that cleaves Fg to fibrin (Smith 2009). The cascade model is mostly useful for interpretation of traditional plasma-based coagulation tests (prothrombin time (PT), partial thromboplastin time (PTT)). One of the major gaps in the cascade model is that it presents the extrinsic and intrinsic pathways as independent and redundant pathways. This model is unable to explain how a deficient enzyme in either pathway can be associated with increased bleeding, while another missing enzyme has no clinical significance. For example, although FXII deficiency reported in cats causes significant prolongation of PTT, it is not associated with an increased tendency for bleeding (Kier et al. 1980). This questions the importance of FXII in initiating coagulation, which is emphasized in the cascade model.

A new cell-based model of coagulation has been developed in recent years and better represents what occurs in vivo. This model also incorporates the additional functions attributed to certain coagulation proteases during inflammation (Hoffman et al. 2001, Smith 2009). It is based on the fact that appropriate hemostasis requires that it be localized specifically to the site of injury (Hoffman et al. 2001, Smith 2009), which is achieved primarily by the participation of membrane surfaces during hemostasis. Binding
of the different coagulation proteins to the membrane surface allows for their alignment, which enhances the speed of the different enzymatic reactions. TF is the only coagulation protein that is permanently attached to the membrane surface (Hoffman et al. 2001, Smith 2009). There is no detectable TF activity in the blood of healthy animals, although very low levels of circulating TF have been detected in healthy humans (Butenas et al. 2005, DelGiudice et al. 2009). Other coagulation proteins circulate in blood and contain glutamic acid residues that are carboxylated through the vitamin K cycle in the liver, allowing them to bind to a membrane surface by interacting with calcium and negatively charged phospholipids (Hoffman et al. 2001, Smith 2009). Cofactors such as FV and FVIII interact directly with the lipid membrane bilayer that surrounds every cell based on the particular arrangement of proteins in activated state which differs from the one observed during the resting state (Hoffman et al. 2001, Smith 2009). During activation or following cell injury, the neutral phospholipids (e.g., phosphaditylcholine, sphingomyeline, sugar-linked phospholipids) located on the outer layer of the cell membrane, and phosphatidylserine and phosphatidylethanolamine found on the inner layer are shuffled by the action of a variety of ATP-dependent enzymes (e.g., flippase, floppase, and scramblase) (Hoffman 2001 et al., Smith 2009). This ultimately changes the cell membrane into a procoagulant surface to which the expression of thrombin is limited to (Hoffman 2001 et al., Smith 2009).

Also found in the circulation and actively involved with coagulation are microparticles. These are primarily derived from endothelial cells, platelets, and monocytes, but also occasionally from granulocytes and erythrocytes (Morel et al. 2006). They are membrane shed fragments (<1 μm) that form vesicles and circulate in plasma.
(Enjeti et al. 2008). They are found during states of both health and disease and possess phosphatidylserine on their outer layer, which makes them procoagulable. Microparticle formation is stimulated by cytokines (e.g., tumor necrosis factor (TNF), interleukin-6 (IL-6)), thrombin, shear stress, and hypoxia; under these conditions, microparticles contain proteins (e.g., ultra large von Willebrand factor monomers, P-selectin, TF) that participate in coagulation reactions (Morel et al. 2006, Smith 2009). The TF derived from microparticles plays an important role in the initiation and propagation of coagulation. TF containing microparticles also bind to P-selectin expressed by activated platelets and promote platelet recruitment and aggregation (Furie et al. 2008). Some microparticles exhibit anticoagulant activity through the presence of protein C in their content (Enjeti et al. 2008).

In the cell-based coagulation model, fibrin formation occurs in three steps (Hoffman 2003a) (Figure 1.1.2). The initiation phase occurs when injury exposes the TF-bearing cell to the blood. FVIIa, the only coagulation protein that circulates in its active form (1% of total FVII), rapidly binds to the exposed TF. The TF-FVII complex activates additional FVII as well as FIX and FX. The activated FX then activates and combines with FV (prothrombinase complex) to ultimately generate small amounts of thrombin. Any activated FX that dissociates from the membrane surface is inactivated by antithrombin or TF pathway inhibitor (TFPI). However, the activated FIX can dissociate from the TF-bearing cell and move to nearby platelets or other cells. TF is generally found outside of the vasculature, which prevents initiation of coagulation when the endothelium is intact (Hoffman 2003a, Smith 2009). However, the ‘idling’ theory suggests that basal generation of low levels of activated factors leads to continual
production of small amounts of thrombin even when the vasculature remains intact (Mann 1992). Endothelial cells, monocytes, tumor cells, and microparticles can express TF in response to stimuli such as cytokines (e.g., TNF, interleukin-1 (IL-1)), endotoxins, and thrombin (DelGiudice et al. 2009), but TF is inactive and encrypted under normal circumstances (Smith 2009, DelGiudice et al. 2009).

Figure 1.1.2 - Cell-based Model of Coagulation. Thrombin generation occurs in various phases. During the initiation phase, TF-bearing cells are exposed to the flowing blood and result in formation of small amount of FIXa and thrombin that diffuse to recruited platelets. During the amplification phase, the small amount of thrombin produced by TF-bearing cells activates platelets, releases vWF, and generates activated FV, FVIII, and FXI. During the propagation phase, the activated enzymes assemble on the surface of the activated platelets to form the tenase complex, which then leads to formation of the prothrombinase complex and subsequent burst of thrombin production.
Other cell types express TF constitutively and include adventitial fibroblasts, smooth muscle cells, keratinocytes of the skin, astroglia of the brain, and myocytes of the heart (DelGiudice et al. 2009).

During the amplification phase, the small amount of thrombin generated during the initiation phase activates platelets recruited to the site of injury, causes release of von Willebrand factor (vWF), and leads to production of activated FV, FVIII and FXI (Roberts et al. 2006). Cleavage of FVIII from vWF makes both available for the amplification phase (Smith 2009). Under high shear conditions, initial platelet adhesion to the endothelium is mediated by collagen and vWF. von Willebrand factor, which is synthesized by endothelial cells and megakaryocytes, also mediates platelet aggregation, and protects FVIII from rapid clearance by protein C by forming non covalent complexes with it (McMichael et al. 2005, Stockham et al. 2008). Platelet activation causes a change in platelet shape and reorganisation of the surface membrane to a procoagulant state (clustering of phosphatidylserine). It also causes release of granule contents including calcium ions (Furie et al. 2008), which altogether promote binding of coagulation proteins to the activated membrane surface (Smith 2009). Platelet aggregation, through ligand interactions, provides the surface required for thrombin generation.

Finally, during the propagation phase, the released platelet granule contents allow for recruitment of additional platelets to the site of injury. The propagation of thrombin generation then takes place on the surface of these platelets. FVIIIa and FIXa (tenase complex) begin to generate FXa. Also, the FXIa generated during the amplification phase activates additional FIXa. The FXa that is generated on the platelet surface then binds to FVa (prothrombinase complex) and cleaves prothrombin to thrombin, thereby generating
a burst of thrombin generation. This large thrombin production leads to cleavage of fibrinopeptide A from Fg. These soluble fibrin molecules subsequently polymerize into strands, resulting in an insoluble fibrin matrix (Hoffman 2003a, Smith 2009). Thrombin also activates FXIII, which leads to cross-linking of the fibrin strands and improves the strength and the elasticity of the fibrin clot (Smith 2009).

Following clot formation, the process must be counteracted to avoid thrombus formation in normal adjacent vasculature. Endothelial cells produce heparin-sulfated proteoglycans that possess a binding site for antithrombin to allow for inactivation of thrombin. Some of the formed thrombin will bind to thrombomodulin, also expressed by endothelial cells, which then activates thrombin activatable fibrinolysis inhibitor (TAFI); this molecule removes binding sites for fibrinolytic proteins (Hoffman 2003b, Roberts et al. 2006, Smith 2009). Binding of thrombin with thrombomodulin is also associated with a 100-fold increase in protein C activation and with fibrin generation from thrombin (Levi et al. 2004). The thrombin-thrombomodulin complex also activates protein C, which then forms a complex with its cofactor protein S. Activated protein C and protein S irreversibly cleave FV and FVIII, thereby preventing further thrombin formation. The protein C and protein S complex is a better inactivator of FV on the endothelial surface than on platelets (Hoffman 2003b, Roberts et al. 2006, Smith 2009). This complex also inactivates plasminogen activator inhibitor-1 (PAI-1), an important inhibitor of fibrinolysis. Finally, endothelial cells also produce TFPI and a small percentage of TFPI is released from platelets in response to thrombin (DelGiudice et al. 2009). TFPI is the main inhibitor of the TF pathway and inhibits FVII and FX by irreversibly binding with them as well as TF, thereby preventing thrombin formation (Smith 2009).
Once clot formation has served its purpose of controlling hemorrhage, the clot must be removed by fibrinolysis in order to restore blood flow. First, plasminogen, which is produced by the liver, is transformed into plasmin by plasminogen activators. Plasmin then degrades polymerized fibrin to form fibrin degradation products (FDP). D-dimer fragments are also produced by degradation of the cross-linked fibrin molecules. Plasminogen activators include tissue plasminogen activator (within the vasculature), which is produced by endothelial cells in response to bradykinin, histamine, acetylcholine, and platelet adhesion factor, as well as urokinase plasminogen activator (extravascular), which is synthesized by fibroblasts, epithelial cells, monocytes, and endothelial cells. Only a small amount of tissue plasminogen is found in circulation, as most of it is bound to its inhibitor PAI-1. Urokinase plasminogen activator is able to activate plasminogen in the absence of fibrin (Weiss et al. 2010). Conversely, fibrinolysis inhibition is achieved mostly by the action of PAI-1, antiplasmin, and TAFI. PAI-1 is the major inhibitor of plasminogen activators and is synthesized by many cell types including megakaryocytes, endothelial cells, and hepatocytes (Weiss et al. 2010). Platelet granules contain large amount of PAI-1. It is released upon platelet activation during systemic inflammation (Levi et al. 2003). Antiplasmin is synthesized by the liver and interferes with plasminogen activation by impairing the plasminogen-fibrin interaction. Finally, TAFI is also synthesized by the liver and is activated by thrombin and plasmin. Its activity is markedly increased (more than 1000-fold) when it is bound to thrombomodulin. It interferes with fibrinolysis by removing the binding sites of plasminogen and plasminogen activators on fibrin (Weiss et al. 2010).
1.2 Traditional Hemostasis Testing

Different aspects of the coagulation system can be assessed by various laboratory methods. Primary hemostasis is traditionally evaluated using platelet count, buccal mucosal bleeding time, platelet function testing (PFA-100), platelet aggregometry, Sonoclot®, thromboelastographic platelet mapping, and flow cytometry. Conversely, activated clotting time (ACT), PT, and PTT are conventionally used to assess secondary hemostasis. The fibrinolytic phase of coagulation is traditionally assessed using FDP or D-dimer concentration. Additionally, many of the endogenous anticoagulant systems (e.g., antithrombin, protein C) can be evaluated by measuring their respective activities. The traditional tests offer a valuable insight into hemostasis; however, they have numerous limitations.

Platelet count is determined by evaluating a standard blood smear microscopically or by using an automated cell counter (Jandrey 2012). Hemorrhage from thrombocytopenia alone is not expected unless the platelet count is less than \( 30 \times 10^3/\mu L \) (Stockham et al. 2008). Several methods to assess platelet function are available, but are beyond the scope of this review and will not be discussed.

ACT is a simple and rapid bedside test that is performed on whole blood and evaluates the intrinsic and common pathways. It is activated with a contact activator (diatomaceous earth, celite, or kaolin), but does not require the addition of reagents. Both automated (magnet detection) and axillary (visual inspection) methods are routinely used and the test is performed at body temperature (37°C). ACT uses platelets as a phospholipid surface for coagulation to take place; therefore, severe thrombocytopenia can cause mild artifactual prolongations (See et al. 2009). ACT is less sensitive than PTT.
for detection of clotting factor deficiencies (Cheng *et al.* 2011) and a prospective analysis of clotting tests in healthy dogs found no correlation between ACT and PT or PTT (See *et al.* 2009). However, because ACT incorporates platelets and clotting factors, its sensitivity in detecting disturbances in hemostasis is increased with certain diseases (Martini *et al.* 2008). Specifically, ACT is prolonged during systemic inflammation and sepsis (Cheng *et al.* 2009), although the exact mechanism by which inflammation prolongs ACT is unknown (Cheng *et al.* 2009, Cheng *et al.* 2011). C-reactive protein has a strong correlation with ACT and only good correlation with PTT. The stronger correlation for ACT is assumed to reflect the more global assessment provided by ACT due to the influence of inflammation on platelets and TF bearing cells (Cheng *et al.* 2009).

PT is a plasma-based assay and traditionally reflects the extrinsic and common pathways. It is performed by adding a high concentration of thromboplastin and calcium to a citrated plasma sample. Prolongation of PT therefore indicates a possible deficiency in Fg concentration or FII, FV, FVII, or FX. Its main indication includes the detection of acquired clotting disorders such as vitamin K deficiency or antagonism, liver disease, or disseminated intravascular coagulation (DIC). PT is relatively insensitive to mild factor depletion, as any single clotting factor must be decreased by greater than 70% before PT is prolonged. Other negative aspects include its inability to detect hypercoagulability and interference by lupus anticoagulants.

PTT is also a plasma-based assay and reflects the intrinsic and common pathways of coagulation. It is activated by celite or kaolin as well as calcium and is also measured using citrated plasma. Prolongation of PTT is caused by deficiencies in Fg, high
molecular-weight kininogen, prekallikrein, or FII, FV, FVIII, FIX, FX, FXI, or FXII. It is routinely used to detect clotting factor deficiencies other than FVII. Possible causes for prolongation include liver disease, vitamin K deficiency or antagonism, hyperglobulinemia, congenital clotting factor deficiency, and vWF deficiency (Stockham et al. 2008, Weiss et al. 2010). Stress (catecholamine) induced increases in FVIII (e.g., trauma, pregnancy) can hide mild factor deficiencies, whereas PTT is significantly elevated with certain factor deficiencies (FXII) that are not associated with clinical bleeding (Chee et al. 2003, Cheng et al. 2011).

For both PT and PTT, it is important to maintain the 1:9 citrate to blood volume ratio as overcitrated samples (i.e., erythrocytosis) may have reduced coagulation activity because of the excess of citrate. Undercitration, such as with severe anemia, results in more plasma for the same volume of citrate is associated with hypercoagulability (Weiss et al. 2010). After collection, the citrated blood should be centrifuged and the plasma separated within 1 hour (Weiss et al. 2010). Lipemia or hyperbiliruninemia can cause artifactual prolongations of PT and PTT, although values remain within reference range (Moreno et al. 1999). Prolongations of PT and PTT can also occur when coagulation is initiated prior to anticoagulation in the citrated tube due to consumption of clotting factors. Examples include venous congestion, traumatic sampling technique, prolonged blood sampling time, frothing from negative pressure aspiration, delay in transfer to anticoagulant, and inadequate mixing of the anticoagulant within the blood tube (See et al. 2009). Shortened PT or PTT is inconsistent and unreliable in predicting hypercoagulability (Donahue et al. 2005). Although PT and PTT are useful to assess clotting processes in vitro, they do not always relate to in vivo coagulation abnormalities.
(Tripodi et al. 2007). Approximately 95% of thrombin formation takes place after fibrin gel formation is detected in routine plasma-based coagulation tests (Smith 2009). As a result, these assays are unable to detect the hemostatic abnormalities that are due to deficiencies following the initial fibrin polymerization (Mann et al. 2003).

Fibrin degradation products are generated non-specifically from the degradation of fibrinogen, soluble fibrin, or cross-linked fibrin through plasmin activation. Latex agglutination assays using serum or citrated plasma are generally employed to measure the FDP (Cheng et al. 2011). Various dilutions of patient plasma are tested to provide a semiquantitative estimation of the FDP concentration (Weiss et al. 2010). Beads coated with human monoclonal anti-FDP antibodies are mixed with citrated blood and agglutination is noted when the patient’s FDP combine with the antibodies (Stockham et al. 2008). In addition to thromboembolic diseases or DIC, FDP are increased during inflammation (sepsis), neoplasia, trauma, internal hemorrhage, and liver or renal disease (Mueller et al. 2002, Griffin et al. 2003, Nelson et al. 2003). Increased FDP are associated with impaired platelet function and prolongation of clotting times through competition with fibrinogen and its binding sites (Stockham et al. 2008).

D-dimers are produced by the activation of plasmin and the degradation of cross-linked insoluble fibrin polymers. Immunoturbidimetry is the most frequently used assay to measure D-dimers, although other methods include latex agglutination and enzyme-linked immunosorbent assay (ELISA). Interpretation of weak reactions can be difficult with latex agglutination and this method is generally considered less sensitive than the other two (Mavromatis et al. 2001, Kelly et al. 2002, Weiss et al. 2010). During immunoturbidimetry, citrated plasma is mixed with a reagent containing latex particles
coated with a monoclonal antibody highly specific for D-dimers. D-dimers contained in the plasma will bind with the coated latex particles and agglutinate. The degree of agglutination is directly proportional to the concentration of D-dimer in the sample and is determined by measuring the decrease of the transmitted light caused by the aggregates. Increased D-dimer concentration can be seen with conditions other than thromboembolic disorders including surgical trauma, hemorrhage, neoplasia, liver disease, renal disease, cardiac disease, hypoalbuminemia, and immune-mediated hemolytic anemia (Weiss et al. 2010, Cheng et al. 2011). As their negative predictive value is usually quite high, the D-dimer assays are generally recommended for ruling out thromboembolic disease or DIC (Nelson et al. 2003, Weiss et al. 2010, Cheng et al. 2011).

Fibrinogen is a positive acute phase protein synthesized by the liver in response to inflammation. Hyperfibrinogenemia is associated with altered blood rheology due to increased plasma viscosity (Stockham et al. 2008, Cheng et al. 2009, Weiss et al. 2010, Cheng et al. 2011). Fibrinogen concentration is measured using the clot-based Clauss assay when a high concentration of thrombin solution is added to a citrated plasma sample. It is affected by reagent variability and requires specialized equipment (Stockham et al. 2008, Cheng et al. 2009, Weiss et al. 2010, Cheng et al. 2011). Acquired fibrinogen deficiencies are usually due to liver disease or increased consumption (e.g., DIC). Thus, concomitant inflammation and increased consumption found with DIC can result in a fibrinogen concentration within the reference interval (Stockham et al. 2008, Cheng et al. 2009, Weiss et al. 2010, Cheng et al. 2011). Increased FDP concentration can also impair fibrinogen function (Stockham et al. 2008, Cheng et al. 2009, Weiss et al. 2010, Cheng et al. 2011).
Antithrombin is produced by the liver as well as endothelial cells; it is a negative acute phase protein. It mostly acts by binding to thrombin and preventing the conversion of fibrinogen to fibrin. It also binds to other activated factors including FIX, FX, FXI, FXII, and partially to FVII. Chromogenic assays are most commonly used to determine its activity, which is reported as a percentage (Weiss et al. 2010). Acquired deficiency is frequent and results from increased consumption (e.g., DIC, sepsis), increased losses (e.g., protein losing nephropathies, protein losing enteropathies, extensive burns), or decreased production (e.g., liver disease, malnutrition). Antithrombin activity is also decreased by heparin therapy as the thrombin-antithrombin complexes become inactive and are subsequently eliminated through phagocytosis (Stockham et al. 2008, Cheng et al. 2011). A negative correlation was identified between antithrombin activity and C-reactive protein, which was likely because antithrombin is a negative acute phase protein (Cheng et al. 2009).

Protein C is a vitamin K dependent protein synthesized by the liver. Functional (clotting or chromogenic) and immunochemical assays are available for its measurement (Weiss et al. 2010). Decreased plasma activity of protein C is associated with an increased risk of thrombosis due to reduced inactivation of FV and FVIII and because of decreased fibrinolysis (Stockham et al. 2008). Reduced protein C activity occurs secondary to hereditary deficiencies, vitamin K antagonism, decreased protein S, liver disease, and increased consumption (e.g., DIC, sepsis) (Stockham et al. 2008).

1.3 Thromboelastography (TEG®)

Considering the lack of sensitivity and availability of some of the traditional coagulation tests, as well as their inability to readily identify hypercoagulability, newer
technologies such as TEG® and thromboelastometry (ROTEM®) have been developed. TEG® was originally developed decades ago (Hartert 1948) but has recently become automated and new activators have been introduced. TEG® combines traditional plasma components of coagulation with the cellular components (i.e., erythrocytes, leukocytes, and platelets) and evaluates all steps in hemostasis including initiation, amplification, propagation, and fibrinolysis (Mallett et al. 1992); it therefore provides a global assessment of hemostasis. Nevertheless, the important role of the endothelium in hemostasis is still not evaluated through the use of TEG®. TEG® and ROTEM® both evaluate global hemostasis through graphic representation of clot formation and subsequent lysis. They differ slightly in their methodology (detector system and initiator of movement between the pin and the cup) and it is generally accepted that reference intervals for each are not directly interchangeable. Finally, their respective terminology is different although the same parameters are evaluated (Luddington 2005, Wiinberg et al. 2010).

Anticoagulated citrated blood should be used for TEG®, to which various activators (kaolin, celite, or recombinant TF) and calcium are added (Kol et al. 2010). Kaolin, which is a buffered solution of phospholipids, provides a negatively charged surface that induces coagulation through the contact pathway (FXII activation) (Johansson et al. 2008). TF is thought to better represent the initiation of in vivo coagulation, but the concentration used for activation has not been standardized (Wiinberg et al. 2005, Wagg et al. 2009, Wiinberg et al. 2009). Kaolin vials are commercially available, while dilution of TF must be made prior to its use leading to potential pre-analytical error. TEG® measurements using blood samples activated with TF demonstrate coagulation
abnormalities via activation of the extrinsic pathway, whereas measurements using kaolin-activated blood samples reflect abnormalities associated with the intrinsic pathway (Martini et al. 2008). In healthy people, there is no significant difference between any of the TEG® parameters when whole blood is activated by kaolin or TF (Johansson et al. 2008). However, variation of the TEG® measurements is reportedly lower with TF activation than with kaolin activation in humans (Bolliger et al. 2012). However, when comparing fresh non-citrated and citrated blood samples, significant differences are found. Specifically, citrated samples produce hypercoagulable profiles except with kaolin activation (Wasowicz et al. 2008). A high degree of intraassay variation is also found when native or non-activated blood is used (Kol et al. 2010). The native assay (fresh non-activated whole blood) is associated with a prolonged lag time before initiation of clot formation and must be performed within 4-6 minutes of blood collection (Wiinberg et al. 2010). Activators are therefore generally used to reduce lag time, increase sensitivity, and decrease analytical variation (Wiinberg et al. 2010). Reference intervals have been established for citrated non-activated, kaolin-activated, and TF-activated TEG® in dogs (Wiinberg et al. 2005, Vilar et al. 2008, Bauer et al. 2009, Flint et al. 2012). The different results suggest that reference ranges for TEG® established with different activators are not interchangeable.

When fresh whole blood analysis is not possible, citrated whole blood (with recalcification before analysis) is recommended (Chandler 1995). Recalcification is achieved by adding calcium chloride to the blood sample in order to initiate coagulation. Once the TEG® has begun, a torsion wire is inserted into a preheated cup (37°C)
containing a 360 μL aliquot of the blood sample. The cup continuously rotates through an arc of 4°45´ in 10-second cycles (Donahue et al. 2005) (Figure 1.3.1). Tension is exerted on the wire as the blood clots and is transmitted as an electrical signal that generates a graphic display where time is expressed in minutes on the x-axis and amplitude is expressed in millimeters on the y-axis (Kol et al. 2010) (Figure 1.3.2). The tracing is representative of the rate of clot formation, clot strength, and fibrinolysis (Donahue et al. 2005).

The blood sample should be processed within 2 hours of collection, after which a tendency toward hypercoagulation is observed (statistically significant decreases in reaction time (R) and clotting time (K) and increases in angle α (α) and maximum amplitude (MA)) (Wiinberg et al. 2005). Incomplete inhibition of thrombin formation in

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**Figure 1.3.1 - TEG® machine and cup.** A torsion wire is inserted into the preheated cup containing the blood sample. The cup oscillates through an arc and tension exerted by the clot formation is transmitted as an electrical signal that generates a graphic display.
Figure 1.3.2 - TEG® tracing. Standard TEG® values with main factors influencing each variable.

citrated whole blood has been suggested as the most likely cause (Camenzind et al. 2000). However, hypercoagulability of the TEG® profile was not detected in activated (kaolin or TF) samples with delayed analysis and fresh non citrated samples analyzed within 5 minutes of collection in an experimental trial performed in healthy humans (Wasowicz et al. 2008). Duration of blood storage should be standardized and a minimum interval of 30 minutes before analysis is recommended to decrease the variance of the TEG® measurements (Camenzind et al. 2000, Bowbrick et al. 2000, Vig et al. 2001).

The TEG® analysis is performed for a maximum of 90 minutes or minimally until R, K, α, MA, global clot strength (G), clot lysis index after 30 minutes (CL30%), and clot lysis rate after 30 minutes (LY30%) values are obtained (Donahue et al. 2005). R, reaction or pre-coagulation time, represents the time from starting the test until detectable clot formation, identified as an increase in amplitude of 2 mm. It primarily relates to plasma clotting factors and inhibitor activity. It evaluates the intrinsic pathway and is
influenced by FVIII, FIX, FXI, and FXII (Franz et al. 1981, Chandler 1995, Donahue et al. 2005). K, clot formation time, is the time for the blood to achieve a fixed viscoelasticity, measured from R to when the tracing amplitude reaches 20 mm. It is influenced by FII, FVIII, platelet count and function, thrombin formation, fibrin precipitation, fibrinogen concentration, and hematocrit (Franz et al. 1981, Chandler 1995, Donahue et al. 2005). Angle α represents the speed of clot formation and is affected by the same factors as K. MA is the maximal distance in millimeters between the two branches of the tracing. It indicates the clot strength and is affected by fibrin and fibrinogen concentration, platelet count and function, thrombin formation, FXIII, and hematocrit (Franz et al. 1981, Donahue et al. 2005). G represents the global clot strength, is calculated from MA (G = 5,000 x MA/ [100-MA]), and is a measure of the overall coagulant state (i.e., hypo-, normo-, or hypercoagulable) (Donahue et al. 2005, Haemoscope 2007, Kol et al. 2010). G has a positive predictive value of 89% and a negative predictive value of 98% in correctly identifying dogs with clinical signs of bleeding (Wiinberg et al. 2009). Similarly, the coagulation index (CI = 0.1227 (R) + 0.0092 (K) + 0.1655 (MA) – 0.0241 (α) – 5.00220) can be calculated to assess the global coagulability of a patient (Donahue et al. 2005). Based on human data, a CI above +3.0 is consistent with hypercoagulability, while a CI below –3.0 indicates hypocoagulability (Donahue et al. 2005). Values reported in dogs with immune-mediated hemolytic anemia ranged between -4.0 and +4.0 (Sinnot et al. 2009). CL30% and LY30% are both measures of fibrinolysis. CL30% corresponds to the amplitude of the tracing in relation to MA 30 minutes after MA is reached, while LY30% represents the percent lysis at 30 minutes after MA is reached (area under the curve) (Donahue et al. 2005, Haemoscope
Both measurements are inversely related to each other; generally, when LY30% is high due to a high fibrinolytic activity, CL30% is low, and vice versa (Donahue et al. 2005, Haemoscope 2007, Kol et al. 2010). Values at 60 minutes for clot lysis index (CL60%) and clot lysis rate (LY60%) are also sometimes reported.

All TEG® parameters are used to determine the global coagulation status. Hypercoagulability is recognized as a combination of decreases in R and/or K, and increases in α, MA, G and/or CI. Evidence of hypercoagulability using TEG® in humans has been shown to predict thromboembolic events; however this is highly variable (McCrath et al. 2005, Dai et al. 2009, Kol et al. 2010). Studies assessing the ability of TEG® to predict thromboembolic events in dogs are not currently available (Kol et al. 2010). Hypercoagulability measured using TEG® occurs in dogs with parvoviral infection, neoplasia, DIC, and immune-mediated hemolytic anemia, as well as in dogs admitted to an intensive care unit (Otto et al. 2000, Kristensen et al. 2008, Wiinberg et al. 2008, Sinnott et al. 2009, Wagg et al. 2009, Fenty et al. 2011). Hypocoagulability is recognized as a combination of increases in R and/or K, and decreases in α, MA, G and/or CI. The ability of TEG® to detect hypocoagulability and predict hemorrhage is superior to plasma-based assays in dogs (Prasad et al. 2008, Wiinberg et al. 2009). G has high positive and negative predictive value for predicting bleeding and is more accurate than the combination of platelet concentration, PT, PTT, D-dimer concentration, and fibrinogen concentration (Prasad et al. 2008, Wiinberg et al. 2009). Also, hypocoagulability measured by TEG® is associated with a poorer outcome in dogs with clinical suspicion of DIC (Wiinberg et al. 2008). This is consistent with human data.
identifying hypocoagulability as evaluated by TEG® as an independent risk factor for mortality at 30 days in people admitted to an intensive care unit (Johansson et al. 2010).

Although TEG® is useful to assess the interaction between platelet GPIIb/IIIa receptors and fibrinogen, it fails to detect primary or drug-mediated platelet dysfunction (Brainard et al. 2007, Brooks et al. 2009). When compared to flow cytometry and aggregometry, TEG® is insensitive in assessing platelet function, but might play a role in identifying marked thrombocytopenia and/or widespread inhibition of platelet aggregability (Bowbrick et al. 2003). The contribution of platelets to the MA measurement is approximately 55% with the rest being due to the fibrinogen concentration (Tynngård et al. 2006). Because of the TEG® methodology, the maximal torque likely occurs before the maximum clot strength is developed and therefore leads to an underestimation of the contribution of platelets to the clot strength (Tynngård et al. 2006).

TEG® is sensitive, but not specific, in identifying hyperfibrinolysis. In other words, a hyperfibrinolytic TEG® pattern must be differentiated from platelet retraction (Katori et al. 2005). Dissociation of fibrin strands from the cup wall decreases the torque between the pin and the cup, as does degradation of fibrin by fibrinolysis. Hyperfibrinolysis is suspected when the decrease in amplitude over 1 hour is greater than 15% of the MA (Bolliger et al. 2012). Notable causes of a hyperfibrinolytic TEG® profile include liver failure (e.g., decreased production of fibrinolytic inhibitors, impaired elimination of activated clotting factors) and increased release of endogenous tissue plasminogen activator (e.g., hemorrhagic shock, neoplasia (e.g., hemangiosarcoma, prostatic carcinoma), parasitic infestation (e.g., Angiostrongylus vasorum)) (Vilar-Saavedra et al. 2005).
2011). On the contrary, clot retraction is suspected when the decrease is less than 15% of the MA and likely occurs secondary to the dissociation of fibrin strands from the cup wall due to powerful interaction between fibrin and platelets GPIIb/IIIa receptors. Clot retraction is rarely seen after platelet inhibition or hemodilution, but becomes more prominent with a platelet count greater than 200,000/µL (Katori et al. 2005, Bolliger et al. 2012). Its purpose in vivo has been suggested to be for the promotion of recanalization of blood vessels after hemostasis and tissue healing are achieved (Katori et al. 2005).

Some canine and human studies inconsistently show significant positive correlations between R and PT or PTT, as well as platelet count and fibrinogen concentration with K, MA, and G (Wagg et al. 2009, Alexander et al. 2010). In dogs, the correlation between platelet count and MA or G only occurs when the platelet count is within reference range or decreased, whereas, thrombocytosis (> 400,000 platelets/µL) is not correlated with either TEG® variable (Wiinberg et al. 2008). An association between the fibrinolytic TEG® variables and FDP or D-dimer concentration also occurs in humans (Levrat et al. 2008, Park et al. 2009, Kol et al. 2010). Conversely, D-dimer concentration and fibrinolytic TEG® variables are not associated in dogs with DIC (Wiinberg et al. 2008).

Hematologic measurements can affect thromboelastographic measurements and need to be considered when interpreting TEG® results. These include platelet count, red blood cell mass, and fibrinogen concentration (Tynngård et al. 2006, Rogers et al. 2010). Significant changes in K (prolonged) and MA (decreased) are observed in people when platelet count is significantly low (< 66 x 10^9/L) (Oshita et al. 1999). Platelet count is similarly correlated with thromboelastometry measurements in healthy dogs (Smith et al. 2012). Additionally, serial in vitro dilution with plasma of whole blood samples obtained
from healthy dogs to alter the hematocrit value is associated with hypercoagulable TEG® results (Vilar et al. 2008[abstract], Jacquith et al. 2009). Similar results are found with non diluted whole blood samples obtained from healthy dogs using thromboelastometry (Smith et al. 2012). Conversely, higher hematocrit is associated with significantly decreased MA in Greyhounds (Vilar et al. 2008). TEG® is performed under low shear conditions and the red blood cell mass within the fibrin clot normally interferes with the spreading of fibrin strands or with the interaction of fibrin and platelets GPIIb/IIIa receptors (Bolliger et al. 2012). A lower hematocrit can therefore allow for greater platelet and fibrin interactions leading to hypercoagulability. Finally, fibrinogen concentration can also affect thromboelastographic and thromboelastometric parameters (Nielsen 2005a, Smith et al. 2012). In healthy dogs, fibrinogen concentration has strong negative correlation with clot formation time and positive correlation with α angle and maximal clot firmness using thromboelastometry (Smith et al. 2012). Similarly, in humans α and MA are highly correlated with fibrinogen concentration, whereas R is only affected when fibrinogen decreases to very low concentrations (< 2.2 μmol/L) (Nielsen et al. 2005b).

Preanalytical factors such as blood collection site and technique, prolonged venous stasis, the material used (needle size, collection system (syringe or evacuated tube collection)), inappropriate filling and mixing of blood tubes, delay between blood collection and analysis, sample temperature, and intra- and inter-individual variation for processing the sample must be considered when interpreting TEG® results (Kol et al. 2010, Walker et al. 2012). Similar results for traditional coagulation tests have been obtained when using catheter sampling versus direct venipuncture (Millis et al. 1995).
Conversely, significant differences in thromboelastographic results for blood samples collected via different collection sites and methods occur in healthy dogs (Walker et al. 2012). Although the differences are small and the values remain within reference intervals, the authors recommend that blood collection be standardized for research purposes or for repeated analyses in a single patient (Walker et al. 2012). Also, induction of moderate to severe in vitro hemolysis in canine citrated blood samples is associated with hypocoagulability characterized by significant decreases in MA and G (Bauer et al. 2010).

Some human studies show that TEG® parameters are significantly different for neonates compared to children and adults (Edwards et al. 2008, Oswald et al. 2010, Sucker et al. 2011). The influence of sex has also been demonstrated in people, leading to recommendations of reporting different reference intervals for each sex (Theusinger et al. 2010, Sucker et al. 2011). However, sex differences do not affect TEG® measurements in dogs (Bauer et al. 2009). Interestingly, pregnant women have a more hypercoagulable profile compared to non-pregnant women so unique reference ranges have also been established for that population (Armstrong et al. 2011, Polak et al. 2011). Conversely, the influence of age and reproductive hormones on TEG® in intact animals has not been investigated in veterinary studies. Finally, breed differences have been identified for various coagulation parameters including TEG® measurements in Greyhounds (Vilar et al. 2008). In summary, it is recommend that any institution using TEG® for clinical or research purposes should perform appropriate-sized studies to determine reference intervals for its target population (Scarperlini et al. 2009).
TEG® is therefore a sensitive method for assessing the different phases of hemostasis and, contrary to traditional coagulation tests, single handedly allows for detection of both hypo- and hypercoagulable states.

1.4 Interactions Between Inflammation and Hemostasis

Close interactions exist between inflammation and hemostasis; therefore any factor or treatment affecting hemostasis in inflammatory conditions such as sepsis is of great importance.

Acute inflammation can induce a hypercoagulable state by activating coagulation, down-regulating endogenous anticoagulants, and inhibiting fibrinolysis (Cheng et al. 2011). Hypercoagulability results from an increased availability of components of primary (e.g., platelets) or secondary (e.g., clotting factors, fibrinogen) hemostasis (Franz et al. 1981, Donahue et al. 2005). Expression of pro-coagulant material by inflammatory cells can lead to activation of coagulation and the thrombin generated will activate platelets and result in clot formation. During inflammation, cytokine-induced platelet activation and TF expression on cytokine-activated mononuclear cells lead to the systemic activation of coagulation (Burstein et al. 1996, Levi et al. 2004). Several mediators such as IL-6, TNF, lipoproteins, C-reactive protein, and complement proteins also exert a direct pro-coagulant effect by stimulating TF expression on endothelial cells, circulating monocytes, and macrophages (Levi et al. 2004, Cheng et al. 2011). Activated monocytes can also directly activate FX to generate thrombin, independently of the TF pathway (Cheng et al. 2011). Several inflammatory cytokines and mediators (e.g., IL-6, bacterial endotoxin, thromboxane A₂, platelet activating factor) can stimulate platelet production and activation. The activated platelets then provide the negatively charged
surface required for secondary hemostasis (Weiss et al. 2010, Cheng et al. 2011). The activated platelets can also synthesize IL-1β, which enhances the adhesive properties and interacts with endothelial cells to recruit additional inflammatory cells (Cheng et al. 2011). IL-6 administration in an in vitro canine model induces hypercoagulability characterized by increased TF and vWF expression, fibrinogen concentrations, platelet responsiveness to thrombin, and complement activation, with secondary inhibition of protein S (Burstein et al. 1996, Esmon 2008).

The vascular endothelium also plays a key role in inflammation-induced coagulation activation. Specifically, endothelial cells release cytokines and respond to cytokines released by inflammatory cells. Also, endothelial cells can express leukocyte adhesion molecules and growth factors that play a role in inflammation (Levi et al. 2003). Endothelial injury leads to activation and degranulation of neutrophils, which then release reactive oxygen species and proteases that participate in endothelial injury. Overall, endothelial dysfunction manifests as down-regulation of thrombomodulin expression, release of platelet activating factor and vWF, leukocyte adhesion protein expression, and TF expression (Weiss et al. 1998). When regulatory mechanisms of hemostasis are exceeded, acute DIC develops with utilization of blood coagulation proteins and platelets and subsequent hemorrhage (Furie et al. 2006).

The three major anticoagulant systems (i.e., antithrombin, protein C, and TFPI) are also impaired during inflammation (Nawroth et al. 1986, Seitz et al. 1989, Heyderman et al. 1992, Fisher et al. 2000). During inflammation, antithrombin is inactivated by proteolysis. Its activity is further reduced by the decreased expression of endothelial heparin sulfated glycoaminoglycans as a consequence of neutrophil release products and
inflammatory cytokines (Weiss et al. 2010, Cheng et al. 2011). Antithrombin is also decreased due to increased consumption from ongoing thrombin generation and decreased production as a result of the negative acute phase response (Levi et al. 2004). The protein C pathway is down-regulated by the inhibition of thrombomodulin and endothelial protein C receptor transcription (Esmon 2008). Hepatic dysfunction associated with inflammation is another factor linked with protein C impaired production and increased consumption (Esmon 2008). Thrombomodulin activity is also reduced through its cleavage from the endothelial cells by neutrophil elastases (Cheng et al. 2011).

Inflammation initially promotes fibrinolysis by increasing the release of plasminogen activators (i.e., tissue plasminogen activator, urokinase plasminogen activator). Fibrinolysis later becomes impaired following the increased production of PAI-1 as well as TAFI, which inhibits fibrinolysis through an indirect inhibition of plasminogen activation (Cheng et al. 2011).

In summary, the effects of inflammatory cytokines and mediators on coagulation include inhibition of endothelial protein C receptors, thrombomodulin, and tissue plasminogen activator, as well as the up-regulation of TF expression and PAI-1 by TNF. Increased TF expression, platelet count, and platelet aggregability from the action of IL-6 and decreased production of antithrombin, TFPI, and thrombomodulin associated with neutrophil elastases also contribute to this interaction. Additionally, TF expression and complement activation lead to activation of neutrophils, cytokine production, and C-reactive protein expression, which inactivates protein S (Cheng et al. 2011).
While inflammation activates coagulation, coagulation also considerably affects inflammation. Activation of coagulation and fibrin deposition are part of the host defence against infectious agents, in an effort to contain the invading organism and the subsequent inflammatory response to a limited area (Levi et al. 2004). Activation of coagulation results in production of proteases that interact with coagulation proteins, as well as with specific cell receptors to induce signalling pathways that mediate inflammatory responses (Levi et al. 2004). Thrombin, TF, and FVII and FX activate protease-activated receptors located on endothelial cells, mononuclear cells, platelets, fibroblasts, and smooth muscle cells that induce expression of pro-inflammatory cytokines (TNF, IL-1, IL-6) (Levi et al. 2004, DelGiudice et al. 2009, Weiss et al. 2010, Cheng et al. 2011). Thrombin also stimulates expression of leukocyte adhesion molecules and chemotactic factors (macrophages and neutrophils), as well as production of platelet activating factor (neutrophil agonist) (Esmon 2008, Weiss et al. 2010, Cheng et al. 2011). The TF-FVII complex, fibrinogen, and fibrin similarly increase the expression of leukocytes adhesion molecules and chemotactic factors (Weiss et al. 2010, Cheng et al. 2011). Also, the activated platelets that adhere to the endothelial cell wall allow for leukocyte adhesion through ligand interactions and release many pro-inflammatory cytokines and chemotactic factors (Levi et al. 2004).

The different natural anticoagulants also exert some degree of control over inflammation. Antithrombin has anti-inflammatory effects through increased prostacyclin formation, decreased nuclear factor κB (NFκB) activation (transcription gene for cytokine production), and reduced leukocyte activation and adhesion. Protein C becomes activated when bound to the thrombin-thrombomodulin complex (Esmon 2008).
Activated protein C manifests anti-inflammatory properties when bound to its endothelial protein C receptor. It then inhibits apoptosis, decreases NFκB expression, and decreases expression of adhesion molecules and inducible TF. Thrombomodulin inhibits leukocyte adhesion and blocks cytokine production (Esmon 2008, Weiss et al. 2010, Cheng et al. 2011). Similarly, TFPI has reported anti-inflammatory properties including decreased leukocyte activation as well as blunted TNF expression (Weiss et al. 2010, Cheng et al. 2011). Finally, the fibrinolytic agents also affect inflammation in multiple ways. Tissue and urokinase plasminogen activator stimulate cytokine production and increase leukocyte activation and adhesion. Also, TAFI inactivates complement proteins (C5a) while PAI-1 decreases cytokine production and leukocyte activation and adhesion (Levi et al. 2004, Cheng et al. 2011).

1.5 Hemostatic Abnormalities During Sepsis and Endotoxemia

Sepsis is defined as a systemic inflammatory response to infection, most often of bacterial origin. Systemic inflammatory response syndrome manifests as two or more of the following: hyper- or hypothermia, tachycardia, tachypnea, or an abnormal leukogram (leukocytosis, leukopenia, or increased band neutrophils) (Bone et al. 1992, Hauptman et al. 1997). Severe sepsis is complicated by organ dysfunction (i.e., liver, kidney, lung), alterations in hemostasis, hypoperfusion, or hypotension (Tsiotou et al. 2005). Septic shock describes the most severe form of sepsis and is characterized by persistent systemic hypotension despite appropriate fluid resuscitation (Tsiotou et al. 2005). Sepsis can occur secondary to infection with gram-negative or gram-positive bacteria, fungi, or viruses. It can also develop secondary to microbial toxins without clinical evidence of infection (Tsiotou et al. 2005).
Sepsis is the leading cause of death in human non-coronary intensive care units and the 10th leading cause of death in the United States overall (Martin *et al.* 2003, NCHS 2010). Similarly, sepsis is a common cause of death in veterinary intensive care units with approximately 50% of septic dogs surviving to hospital discharge (Kenney *et al.* 2010). About 22% of septic dogs develop cardiovascular dysfunction characteristic of septic shock, of which only 10% survive (Kenney *et al.* 2010). Dysfunction of the coagulation system characterized by PT or PTT > 25% above the upper reference limit, platelet count ≤ 100,000/μL, or both, is independently associated with increased likelihood of death in dogs with sepsis (Kenney *et al.* 2010).

Clinically overt infection-induced activation of coagulation is reported in 30-50% of human patients with gram-negative sepsis (Gando *et al.* 1995, Baglin 1996) and might be equally common in patients with gram-positive sepsis (Levi *et al.* 2003). Excessive activation of inflammation including the release of cytokines and activation of plasma protein cascade systems, such as the complement, contact phase, coagulation, and fibrinolytic systems, occurs during sepsis (Tsiotou *et al.* 2005, Zeerleder *et al.* 2005). Simultaneously, decreased anticoagulant capacity and inhibited fibrinolysis occur, causing overwhelming activation of coagulation (Grant *et al.* 1997, Zeerleder *et al.* 2005). Concentrations of procoagulant factors and anticoagulant proteins are altered during sepsis, which is often associated with hypocoagulability and DIC, although the hemostatic abnormalities begin with a hypercoagulable state (de Laforcade *et al.* 2003). Decreased activity of coagulation factors leads to increased bleeding, whereas increased FVIII and fibrinogen, as well as decreased protein C and antithrombin activity cause a prothrombotic state (Collins *et al.* 2006). In severe sepsis, circulating mononuclear cells,
stimulated by pro-inflammatory cytokines (e.g., TNF, IL-1, IL-6) and inflammatory mediators (e.g., LPS, activated complement, immune complex) express TF, causing systemic activation of coagulation (Franco et al. 2000, Esmon 2008, DelGiudice et al. 2009). Septic patients have increased activation of hemostasis as evidenced by increased D-dimer concentration, prothrombin fragments 1 + 2, and thrombin-antithrombin complexes, causing microvascular thrombosis and multiple organ failure (Collins et al. 2006, Levi et al. 1999). DIC occurs in approximately 30% of human patients with severe sepsis and is associated with the development of multiple organ failure and mortality (Sivula et al. 2009).

The various mechanisms involved in the development of coagulation abnormalities associated with sepsis include TF-mediated generation of thrombin, subsequent conversion of fibrinogen to fibrin, platelet activation, and impairment of all major physiological anticoagulant mechanisms including reduced protein C (Madden et al. 1989) and antithrombin activities (de Laforcade et al. 2003). Thrombin also has the ability to augment leukocyte adhesion and activation, thereby contributing to amplification of the inflammatory response (Esmon 2008). Platelet activation manifests during sepsis as decreased platelet survival, platelet recruitment at sites of inflammation, platelet hyper-reactiveness, as well as platelet activation by-products and platelet and leukocyte aggregates in circulation (Weiss et al. 1998). Protein C deficiency develops during sepsis through the combined effects of increased destruction by neutrophil elastases, increased consumption in response to the procoagulant state, decreased synthesis by the liver, and possibly acquired vitamin K deficiency (Esmon 2001, Faust et al. 2001, Hopper et al. 2005). Additionally, increased oxidative injuries occurring during
sepsis lead to impairment of thrombomodulin activity, but preserved activation of TAFI, a potent inhibitor of fibrinolysis (Hopper et al. 2005). Finally, neutrophil elastase is increased during DIC and inactivates antithrombin and cleaves TFPI, thereby contributing to the hypercoagulable manifestations of sepsis (Zeerleder et al. 2005, DelGiudice et al. 2009). Alteration of the shape and rheologic properties of the red blood cells also occurs and contributes to microvascular dysfunction (Hinshaw 1996, Casey 2000, Taylor et al. 2001). The combination of decreased fibrinolysis, activation of coagulation, and excessive production of proinflammatory mediators all contribute to development of DIC and multiple organ dysfunction (DelGiudice et al. 2009).

The TF pathway is the primary initiator of coagulation during sepsis. Human sepsis studies demonstrate that TF production exceeds that of TFPI, which remains at a normal or slightly increased level (DelGiudice et al. 2009). Conversely, antithrombin activity is decreased during sepsis due to the down regulation or inactivation of heparin sulfated glycoaminoglycans during acute inflammation (Levi et al. 2003). The magnitude of reduction in plasma antithrombin activity correlates with the severity of illness and mortality in people (Lorente et al. 1993, Hopper et al. 2005) and is associated with decreased antithrombin anti-inflammatory properties (Esmon 2008). Septic patients also have decreased tissue plasminogen activator and increased PAI-1 that lead to suppression of fibrinolysis (Levi et al. 2004, DelGiudice et al. 2009).

Hemostatic abnormalities in dogs with sepsis are characterized using traditional coagulation tests and coagulation factor activity. Septic dogs have significantly lower platelet counts compared to healthy dogs (Hauptman et al. 1997), although this finding is not always consistent (de Laforcade et al. 2003). Conversely, dogs with sepsis have
increased vWF antigen concentration compared to healthy dogs, likely due to endothelial activation (Rogers et al. 2010). Additionally, protein C and antithrombin activity are decreased in septic dogs during the first 2 days of hospitalization (de Laforcade et al. 2003, de Laforcade et al. 2008) and progressively increase in survivors prior to discharge from the hospital (de Laforcade et al. 2008). Similarly, deficiencies in protein C and antithrombin are present in dogs with septic peritonitis. Also, nonsurvivors have prolonged PTT and a more hypocoagulable TEG® profile (i.e., decreased MA, α, and CI) compared to survivors, with MA being the most specific predictor of survival (Bentley et al. 2013). Evidence of DIC and fibrinolysis characterized by increased PT, PTT, D-dimer, and FDP are also more common in dogs with sepsis, compared to healthy dogs (de Laforcade et al. 2003).

Traditional coagulation tests in human patients with severe sepsis show a variety of changes including thrombocytopenia, prolonged clotting times, reduced natural anticoagulants, and impaired fibrinolysis (Sivula et al. 2009). Specific changes include prolonged PT and/or PTT, as well as decreased FII, FV, FVII, FX, and FXII activities (Collins et al. 2006). FXII is the most affected clotting factor, which explains the prolonged PTT measured in many patients. FVIII activity and fibrinogen concentration are significantly increased, while antithrombin and protein C activity are decreased. These changes are attributed to the acute phase response, with the exception of FVIII due to its lack of correlation with C-reactive protein (Collins et al. 2006). Although routine coagulation tests (PT and PTT) are often prolonged in human septic patients, they fail to correctly reflect an increased risk of bleeding (Chowdhury et al. 2004). This might be because the assays measure only the initiation phase of hemostasis and supra-physiologic
concentrations of activators are used (Schöchl et al. 2011). Additionally, contact activated assays might be deceptive in septic patients; although contact activation can occur during sepsis, depletion of the contact factors does not tend to affect \textit{in vivo} hemostasis (Collins et al. 2006). Conversely, sepsis-induced coagulation results in part from increased TF expression and because most routine coagulation tests are plasma-based, centrifugation leads to removal of those TF expressing cells (Schöchl et al. 2011).

Viscoelastic devices show variable coagulation profiles in septic patients, with limited studies published in the veterinary literature. Conversely, there are many studies investigating TEG® or thromboelastometry in septic people with conflicting results. In some human patients with sepsis, thromboelastometric parameters remain within the reference range and those patients with a hypercoagulable profile as evidenced by a shortened coagulation time, accelerated clot formation time, and increased clot firmness have less organ dysfunction at hospital discharge (Daudel et al. 2009). However, marked hypocoagulability occurs in human patients with severe sepsis and overt DIC, as opposed to trends toward hypercoagulability (increased maximum clot firmness) in patients with non overt DIC. The latter is likely secondary to hyperfibrinogenemia as a consequence of the acute phase response. Hyperfibrinolysis is not detected using thromboelastometric variables in human septic patients (Sivula et al. 2009). Interestingly, TEG® (hypo- or hypercoagulable profile) is more sensitive than white blood cell count or platelet concentration in detecting early sepsis in pediatric patients (sensitivity and specificity of 96%) (Grant et al. 1997).

Similar to naturally occurring sepsis, endotoxemia induced by lipopolysaccharide (LPS) administration also leads to systemic activation of the coagulation system and
fibrinolysis in people (van Deventer et al. 1990). LPS induces a number of signalling pathways leading to activation of the NFκB system, which induces the transcription of genes leading to production of pro-inflammatory cytokines (i.e., TNF, IL-1, IL-6) and TF (Bohrer et al. 1997, Weiss et al. 1998, Levi et al. 2003). IL-6 appears to be the major mediator of activation of coagulation, and TNF is the major initiator of the fibrinolytic response (Weiss et al. 1998). Administration of LPS also stimulates IL-8 production, which has procoagulant effects (Levi et al. 2003). TF expression is increased 125-fold in healthy people with experimentally induced low dose endotoxemia (Levi et al. 2004). Similarly, LPS administration is associated with TF expression in an in vitro canine model (Burstein et al. 1996). Fibrinolysis is initially induced after LPS administration due to increased plasminogen activator production, but within 1-2 hours becomes suppressed by PAI-1. LPS also directly activates platelets and other inflammatory cytokines leading to further platelet activation by platelet activating factor (Weiss et al. 1998, Levi et al. 2003).

Thromboelastometry of human blood incubated with LPS reveals procoagulation characterized by decreased clotting time, but unchanged clot formation time and maximum clot strength. The procoagulant effect of LPS is likely a result of TF synthesis since blockade of TF gene expression inhibits the hemostatic changes that occur with LPS administration (Zacharowski et al. 2006). Similarly, blocking TF activity completely inhibits inflammation-induced activation of coagulation in models of experimental endotoxemia or bacteremia (Taylor et al. 1991, Levi et al. 1994, DelGiudice et al. 2009). Likewise, low dose endotoxemia in humans produces shortened clotting time but unaltered clot formation time, suggesting that initiation but not propagation of
coagulation is affected (Spiel et al. 2006). The unchanged clot strength is likely explained by the mild decrease in platelet concentration and significantly increased vWF concentration (Spiel et al. 2006). Short acting activation of fibrinolysis is also noted following low dose administration of endotoxin (Spiel et al. 2006). Conversely, another low dose endotoxemia study in pigs reveals that maximum clot firmness is reduced during the infusion and is attributed to DIC-associated platelet and fibrinogen consumption (Schöchl et al. 2011). Increased tissue plasminogen activator concentration is also seen, but not activation of fibrinolysis, likely due to increased PAI-1 (Schöchl et al. 2011).

Few studies investigate coagulation in dogs with LPS-induced endotoxemia. Dogs given low-dose LPS to induce endotoxemia exhibit significant prolongation of PT and PTT, increased D-dimer concentration, and decreased antithrombin and protein C activities, but no change in FVIII activity (Eralp et al. 2011). Similar findings for traditional coagulation tests are documented in dogs with high-dose LPS induced endotoxemia (Madden et al. 1989). Additionally, TEG® reveals mild hypocoagulation characterized by decreased α and MA, but no evidence of hyperfibrinolysis in dogs given low doses of LPS (Eralp et al. 2011). The variable coagulation test results likely occur as a result of different thromboelastographic techniques, as well as different doses and types of LPS used.

Various hemostastic changes are therefore encountered during sepsis and endotoxemia, and are readily identified by viscoelastic devices such as TEG®.
1.6 Endotoxemia as a Model for Canine Sepsis

Many animal models replicate the signs and laboratory findings seen during sepsis. Three categories of sepsis models exist and include exogenous administration of endotoxin (LPS), exogenous administration of a viable pathogen (administration of live bacteria), and alteration of the animal’s endogenous protective barrier (bacterial translocation). Examples of these models include intravascular infusions of endotoxin or live bacteria, bacterial instillation causing peritonitis or pneumonia, and cecal ligation and perforation (Garrido et al. 2004). The fundamental goal of these models is to closely replicate the clinically relevant pathogenesis of naturally occurring sepsis. Sepsis tends to occur in 2 phases: the early hyperdynamic phase characterized by decreased systemic vascular resistance and increased cardiac output and the later hypodynamic phase when cardiac output declines. (Buras et al. 2005) The different models are not directly comparable as different cytokine responses, hemodynamic profiles, and mortality rates have been noted for each (Zanotti-Cavazzoni et al. 2009). Also, due to interspecies differences, notably with regards to the sensitivity to endotoxin and strains, doses used are not necessarily equivalent (Zanotti-Cavazzoni et al. 2009).

The LPS model does not necessarily represent naturally occurring sepsis as bacteria are generally released continuously, rather than over a short period. LPS bolus administration, therefore, does not reproduce the complex physiological changes observed with live pathogens. LPS is the principal component of gram-negative bacterial cell walls and is composed of an O-polysaccharide chain, a core sugar, and lipophilic fatty acid. The latter component, also termed the lipid A portion, induces gene expression of inflammatory cytokines through the activation of the nuclear transcription factor NFkB
Bolus injection of LPS leads to an overwhelming innate immune response and release of inflammatory mediators such as TNF (Buras et al. 2005), which usually occurs over a longer period during naturally occurring sepsis (Zanotti-Cavazzoni et al. 2009). Higher doses of LPS are associated with rapid cardiovascular collapse and death, while lower doses generate hyperdynamic changes such as increased cardiac output (Garrido et al. 2004).

LPS administration has been validated in dogs, as well as other species, as a model for sepsis and systemic inflammation (Schultz et al. 2002). LPS is a stable and relatively pure compound available in a lyophilized form that can be administered by many routes including intravenous, intraperitoneal, and intratracheal (Freise et al. 2001, Garrido et al. 2004, Otto et al. 2007, Zanotti-Cavazzoni et al. 2009). Advantages of the LPS model include its technical ease and the homogenous response making it easily replicable, as well as the fact that it induces many of the hemodynamic and cytologic changes encountered during naturally occurring sepsis. Unfortunately, it does not necessarily represent the variable clinical syndrome (Freise et al. 2001, Garrido et al. 2004, Otto et al. 2007, Zanotti-Cavazzoni et al. 2009). Other benefits of the LPS model include not inducing an actual infection and having only transient effects since the signs last only 2-6 hours and death does not typically occur with lower dosages (DeClue et al. 2008, Holowaychuk et al. 2012). Administration of LPS to healthy dogs results in systemic inflammation characterized by fever, tachycardia, tachypnea, hypotension, abdominal discomfort, lethargy, and typically, vomiting or diarrhea (DeClue et al. 2008, Holowaychuk et al. 2012, Eralp et al. 2011). Recent veterinary studies have used the LPS model in dogs to investigate the effects of drugs on systemic inflammation and to

The hemodynamic response to intravenous administration of LPS depends on the host species, the dose administered, the method of administration (bolus versus slow infusion), and the extent of resuscitation (Fink et al. 1989). Bolus administration of LPS (0.02 mg/kg) to dogs consistently causes hyperthermia, leukopenia, and thrombocytopenia, which are consistent with the induction of systemic inflammation (Eralp et al. 2011). Administration of higher doses of LPS to dogs (2 mg/kg, IV) results in a severely hypodynamic state characterized by a decrease in arterial pressure, cardiac output, hepatic blood flow and an increase in systemic vascular resistance and blood lactate (Epstein et al. 1993, Spapen et al. 1999). LPS administered to dogs at doses above 2 mg/kg are consistently lethal, with higher doses (6 mg/kg) causing overwhelming inflammation, circulatory collapse, massive cytokine release, and rapid mortality (Deitch 1998). Deaths are also reported in dogs with doses as low as 0.1 μg/kg (Flatland et al. 2011), which emphasizes that not all LPS doses are equivalent and the effect varies dramatically depending on the type and strain of bacteria used (Deitch 1998, Flatland et al. 2011).

Several cytologic and immunologic changes are anticipated after LPS administration in dogs. Increases in IL-1, IL-6, and TNF concentrations are noted (Miyamoto et al. 1996, DeClue et al. 2012), while interleukin-10 decreases (DeClue et al. 2012). LPS administration to dogs generally causes thrombocytopenia due to sequestration in pulmonary and hepatic capillary beds or eventually also due to DIC (Wagner et al. 1999). Sequestration of platelets, by up to 50% compared to controls, occurs within minutes of
LPS administration (Davis et al. 1960, Gutmann et al. 1987). Increases in hematocrit also occur, likely due to hemoconcentration. LPS administration also induces a leukopenia up to 6 hours after its administration followed by leukocytosis at 24 hours. This is characterized by a neutropenia that initially develops due to margination and sequestration of neutrophils in pulmonary and hepatic capillary beds. Inflammatory mediators and cytokines will also cause neutrophil release leading to neutrophilia that then persists in response to granulocyte-colony stimulating factor secretion (Wagner et al. 1999).

The LPS model, although imperfect, is therefore useful in replicating the signs and laboratory findings of naturally occurring sepsis.

1.7 Fluid Resuscitation and Colloid Osmotic Pressure (COP) During Systemic Inflammation and Sepsis

Fluid resuscitation is an essential part of the management of critically ill patients. During sepsis and septic shock, a relative and absolute intravascular volume deficit occurs. The relative deficit is due to vasodilation, venous pooling, and alterations in the endothelial barrier, while the absolute deficit results from increased sensible (i.e., vomiting, diarrhea, body cavity effusion) and insensible losses (e.g., fever, respiration) (Vincent et al. 2004, Marx et al. 2007). Early goal-directed therapy including appropriate fluid resuscitation within the first 6 hours reduces mortality in human patients with severe sepsis and septic shock (Rivers et al. 2001). Goals for fluid therapy in septic patients include improving tissue perfusion, lactate clearance, and central venous oxygen saturation, as well as reducing the requirement for vasopressors (Rivers et al. 2001, Bayer
et al. 2012). Delayed or inadequate fluid resuscitation is associated with worsened systemic hemodynamics, microvascular flow, and organ dysfunction (Hoffmann et al. 2002).

Considerable debate exists regarding the ideal fluid treatment for patients with systemic inflammation or sepsis, in terms of choosing a crystalloid or colloid. Colloids persist in the intravascular compartment longer than crystalloids and are suggested to be superior in achieving hemodynamic stability (Hillman et al. 1997, Norberg et al. 2005). Additionally, colloids theoretically allow for a less positive fluid balance; the latter is associated with increased 28-day mortality independent of illness severity (Boyd et al. 2011, Alsous et al. 2000, Vincent et al. 2006, Murphy et al. 2009). However, evidence-based reviews of fluid resuscitation in severe sepsis and septic shock show that crystalloids and colloids both allow for successful resuscitation and are equivalent in their effects on preload recruitable stroke volume and oxygen delivery (Vincent et al. 2004). When titrated to the same filling pressure, crystalloids and colloids also restore the same degree of tissue perfusion. Meta-analyses and systematic reviews show no difference in length of stay, pulmonary edema, or mortality in patients treated with crystalloids or colloids (Choi et al. 1999, Perel et al. 2012). Given their lesser cost, crystalloids are therefore considered a first-line option and colloids are used as an adjunctive treatment.

Synthetic colloids have been developed with the benefit of increasing oncotic pressure in patients with systemic inflammation and sepsis. Different synthetic colloids are available, but HES are the most routinely used products in North America (Bayer et al. 2012). Multiple studies in humans have recently compared crystalloids and colloids for fluid resuscitation, but have failed to identify a survival benefit for either fluid type.
(Bayer et al. 2012, Perner et al. 2012, Myburgh et al. 2012). Additionally, dose-related adverse effects have been associated with the use of certain synthetic colloids including induction of coagulopathies, impaired kidney function, and prolonged tissue storage with secondary pruritus (Kozek-Langenecker et al. 2005, Westphal et al. 2009, Dart et al. 2010). Due to concerns regarding safety, HES and other synthetic colloids have become less desirable in human medicine. The Surviving Sepsis Campaign guidelines currently recommend crystalloids be used as the initial fluid of choice, with a recommendation to avoid using HES due to their adverse effects on kidney function (Dellinger et al. 2013).

The main disadvantages of crystalloids include the limited duration of intravascular volume expansion and the effect of lowering COP, which can lead to edema formation (Chan 2008). Colloid osmotic pressure or oncotic pressure is the force that prevents fluid movement outside of the vascular compartment and is generated by particles greater than 30 kD. Albumin is the principal contributor to COP, but immunoglobulins, fibrinogen, and other plasma proteins also contribute (Chan et al. 2001). Plasma oncotic pressure can be estimated or directly measured. Using plasma proteins measured with refractometry in dogs, the following equation predicts COP: \[ \text{COP} = 1.4 \times [\text{total protein}] + 0.22 \times [\text{total protein}]^2 + 0.005 \times [\text{total protein}]^3 \] (Navar et al. 1977). Unfortunately, changes in blood pH, ion binding capacity of proteins, and alterations of the albumin:globulin ratio, cause this formula to become inaccurate (Rudloff et al. 2000).

COP is best measured using a membrane transducer system detecting volume flow across a semipermeable membrane (Treib et al. 1993). The membrane, which simulates the capillary endothelium by only allowing molecules with a molecular weight \( \leq 30 \text{ kD} \) to pass through, separates a reference chamber filled with 0.9% saline and a test chamber.
where the patient sample (serum, plasma, or whole blood) is injected (Figure 1.7.1). The membrane is relatively impermeable to proteins because of their large size, but water will move from the reference chamber into the test chamber because of differences in the oncotic pressure. Because proteins are negatively charged and electroneutrality must be maintained on each side of the membrane, retention of cations in the test chamber occurs and the measured COP is proportional to the number of molecules present (anions and cations). After equilibrium is reached between the two chambers, a negative pressure gradient is formed in the reference chamber. A sensing diaphragm next to the reference chamber is attached to a pressure transducer that converts the small pressure changes in the reference chamber into an electrical signal that is converted to a display on the osmometer and is reported in mm Hg (Silverstein et al. 2009).

Figure 1.7.1 - Osmometer membrane and chambers. A membrane transducer system detects volume flow across a semipermeable membrane that separates a reference chamber filled with 0.9% saline and a sample chamber.

The mean ± standard deviation for COP using whole blood from healthy dogs is 19.9 ± 2.1 mm Hg (Culp et al. 1994). Conversely, mean COP is 13.9 ± 3.1 mm Hg in critically
ill dogs (King et al. 1994). Because whole blood and plasma COP in dogs are significantly different, COP values measured using these different samples cannot be directly compared (Odunayo et al. 2011). Hemolysis and synthetic colloid administration can both increase COP, while anticoagulants, acidemia, and hyponatremia have the opposite effect (Rudloff et al. 2000). Hypoalbuminemia also leads to decreased COP, but while measurement of total solids (TS) is a good surrogate of total protein concentration, (Rackow et al. 1983) TS and COP are poorly correlated (Thomas et al. 1992). In vitro studies show that dilutions of human plasma with synthetic colloids (6% hetastarch and 6% Dextran 70) causes a significant increase in COP with no concurrent change in TS. Therefore, the oncotic response to synthetic colloid therapy should be assessed by measuring COP (Bumpus et al. 1998).

Fluid resuscitation is therefore paramount in managing patients with sepsis and considerations must be given to the potential adverse effects attributed to the chosen fluid type.

1.8 Hydroxyethyl Starch Solutions

HES are composed of large, branched, complex carbohydrates derived from waxy maize or potatoes and modified by the addition of hydroxyethyl groups to amylopectin at the C2, C3, and/or C6 position of glucose to reduce intravascular hydrolysis. HES differ in many aspects including their vehicle, concentration, molar substitution, substitution pattern, and average molecular weight. The concentration of the solution impacts the initial volume effect with higher concentration solutions having a greater volume expansive effect. Commercially available products are 4-6% solutions, which are
considered iso-oncotic or > 6% solutions, which are considered hyperoncotic. HES possess a wide range of molecular weight molecules and can be monodisperse when they include molecules of a single molecular weight, or polydisperse when they contain molecules with a range of molecular weights. The mean molecular weight determines their classification as high-, medium-, or low-molecular-weight solutions. Molar substitution represents the average number of hydroxyethyl groups substituted per glucose subunit. The substitution pattern symbolizes the location of hydroxyethylation at either the C2 or C6 position and is referred to as the C2/C6 ratio. The rate of degradation is influenced by the molar substitution and the C2/C6 ratio with higher values associated with slower degradation. Hydroxyethyl starch molecules typically undergo hydrolyzation by serum amylase and are then excreted by the kidneys (Treib et al. 1999, Ghandi et al. 2007, Hughes et al. 2008, Westphal et al. 2009). Hydroxyethyl groups at the C2 position will inhibit degradation by amylase (Treib et al. 1999, Ghandi et al. 2007, Hughes et al. 2008, Westphal et al. 2009). In addition, a small number of molecules diffuse into the interstitial space where they are slowly broken down by the reticuloendothelial system. For HES, the number of oncotically active molecules determines the oncotic effect, not the molecular size, which determines the plasma and tissue half-lives, as well as the rapidity of excretion by the kidneys. Larger molecules are further away from the renal threshold for excretion (45-60 kD) and therefore persist longer in the body (Treib et al. 1999, Ghandi et al. 2007, Hughes et al. 2008, Westphal et al. 2009).

Tetrastarch, denoted as HES 130/0.4, is a newer generation HES with an average molecular weight of 130 kD, a molar substitution of 0.4, and C2/C6 ratio greater than 8 (Ghandi et al. 2007) (Table 1.8.1). Its lower molar substitution means that a larger
number of anhydroglucose moieties are unsubstituted making those regions more accessible to enzymatic degradation and shortening the intravascular retention time. Due to increased renal excretion compared to high- or medium-molecular weight solutions, plasma accumulation of tetrastarch after multiple doses is minimal and results in markedly reduced tissue storage (Ghandi et al. 2007). Dosing studies reveal that clearance of tetrastarch is at least 23 times greater than hetastarch and almost 5 times that of pentastarch (Westphal et al. 2009). Additionally, the COP for tetrastarch is 37.1 mm Hg, followed by 32.0 mm Hg for pentastarch and 28.7 mm Hg for hetastarch (Humm et al. 2007). Studies show that fluid resuscitation with tetrastarch enables volume repletion comparable to 1.8 times the same volume of crystalloids. This is not consistent with traditional beliefs that at least 3-4 times more volume is required for adequate fluid resuscitation when crystalloids are used as compared to colloids (Hartog et al. 2011).

Adverse effects have been associated with the use of certain synthetic colloids including impaired kidney function, prolonged tissue storage resulting in pruritus, and induction of coagulopathies (Kozek-Langenecker et al. 2005, Westphal et al. 2009, Dart et al. 2010). Several factors must be considered when assessing potential drawbacks of

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<tr>
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<th>Tetrastarch</th>
<th>Pentastarch</th>
<th>Hetastarch</th>
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<tr>
<td>Concentration</td>
<td>6%</td>
<td>6%</td>
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<tr>
<td>Average Molecular Weight (kD)</td>
<td>130</td>
<td>200</td>
<td>450</td>
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<tr>
<td>Molar Substitution</td>
<td>0.4</td>
<td>0.5</td>
<td>0.7</td>
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<tr>
<td>C2/C6 ratio</td>
<td>9:1</td>
<td>6:1</td>
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Table 1.8.1 - Comparison of the physicochemical properties of the commonly utilized commercially available HES products
HES including the type (i.e., concentration, molar substitution, molecular weight, and C2/C6 ratio), daily and cumulative doses used, underlying kidney dysfunction, underlying disease (i.e., surgery, trauma, or sepsis), and level of hydration (Guidet et al. 2012). Hyperoncotic HES products (> 6% solutions) and those with a high degree of substitution (> 0.4) induce kidney injury in patients with septic shock (Schortgen et al. 2001, Schortgen et al. 2008, Brunkorst et al. 2008). Likewise, experimental studies reveal increased histological kidney injury scores and increased neutrophil gelatinase-associated lipocalin (NGAL) concentrations, a biomarker of kidney injury, in rats administered tetrastarch (Schick et al. 2010). Moreover, recent clinical trials in human septic patients reveal dose-related increases in the incidence of kidney insufficiency and higher requirements for renal replacement therapy in patients administered tetrastarch or other HES (Brunkhorst et al. 2008, Bayer et al. 2011, Bayer et al. 2012, Myburgh et al. 2012, Perner et al. 2012). Therefore, the use of HES with molecular weight ≥ 200 kD and/or degree of substitution above 0.4 in patients with severe sepsis or risk of acute kidney injury is not recommended (Reinhart et al. 2012). Recently, the Surviving Sepsis Campaign guidelines recommended against the use of HES including tetrastarch for initial fluid resuscitation of human patients with sepsis or septic shock (Dellinger et al. 2013). The mechanism for colloid-induced kidney injury remains poorly understood. It has been theorized that renal ischemia as a consequence of increased blood viscosity could play a role (Schortgen et al. 2001), although reversible swelling of renal tubular cells has also been proposed (Boldt 2009). Evidence that HES interferes with the reticuloendothelial system and therefore impairs host defenses and participates in organ dysfunction leading to acute kidney injury has also been found (Schildt et al. 1975,
Wiedermann 2008). Thus, tetrastarch is a newer generation HES with reported fewer adverse effects in humans, but its various effects in dogs are largely unknown.

1.9 Coagulation Abnormalities Associated With HES

Adverse effects of HES on hemostasis are characterized by decreased FVIII and vWF activity, dilution of clotting factors, decreased expression of integrin α\text{fib}β3 on activated platelets leading to decreased aggregation, decreased fibrinogen polymerization, acquired fibrinogen deficiency, and impaired fibrinolysis (Kozek-Langenecker et al. 2005, Kozek-Langenecker et al. 2007, Westphal et al. 2009). In vivo hemodilution with crystalloids is associated with hypercoagulability as measured by TEG® (Tuman et al. 1987, Ng et al. 1996, Ruttmann et al. 1996). Although not completely understood, decreased natural anticoagulant activity is a proposed mechanism (Egli et al. 1997). Conversely, while the infusion of large volumes of synthetic colloids also causes hemodilution, there appears to be a reduction in clotting factor activity and a consequent increase in bleeding time (Salmon et al. 1993). These changes are only rarely considered clinically significant unless the patient already has deranged coagulation function (Salmon et al. 1993). Additionally, mild to moderate hemodilution with HES causes increased activation of clotting, possibly secondary to hydroxyethyl starch molecules serving as additional surfaces for activation of coagulation (Kozek-Langenecker et al. 2007). The volume of HES infused is important to consider when interpreting the impact of HES on coagulation. In vitro studies using blood from healthy human volunteers show that PT, PTT, and clotting factor activities are only affected at high dilutions of HES 200/0.5 (Petroianu et al. 2003). But despite HES-associated coagulopathies being tooted as dose
dependent, a systematic review concludes that significant *in vivo* alterations are found even at lower doses (Hartog *et al.* 2011).

The suggested mechanisms for the acquired von Willebrand syndrome that occur after HES administration include decreased release of vWF, increased proteolytic degradation of vWF, and antibody mediated mechanisms (Kozek-Langenecker *et al.* 2005). vWF activity reaches its minimum 1-2 hours after HES infusion and exceeds the expected degree of decline attributed to hemodilution (Jamnicki *et al.* 2000). Decreased release of vWF is unlikely considering the half-life of vWF is 8 hours and both small and large vWF multimers are decreased following HES infusion (Kozek-Langenecker *et al.* 2005). Combination of vWF with larger HES molecules is suggested as an additional mechanism and might lead to accelerated elimination of HES (Dalrymple-Hay *et al.* 1992, Mohri 2006). The physicochemical properties of the HES solutions are important in determining the FVIII and vWF lowering effects. Slowly degradable products (high degree of substitution and/or high C2/C6 ratio) consistently decrease plasma concentrations of FVIII and vWF by up to 80%, a value greater than that expected with dilution alone (Jamnicki *et al.* 2000, Konrad *et al.* 2000, de Jonge *et al.* 2001, Jones *et al.* 2003, Ghandi *et al.* 2005, von Roten *et al.* 2006). Conversely, molecular weight is not an important factor in determining the effect of HES on coagulation (FVIII and vWF) (Madjdpour *et al.* 2005).

Slowly degradable HES products are also associated with platelet dysfunction characterized by decreased expression and activation of platelet surface GPIIb/IIIa (Stögermüller *et al.* 2000, Franz *et al.* 2001). This effect does not appear to be mediated through interference with intracellular signal transduction as intracellular calcium release
is unchanged by HES administration (Gamsjäger et al. 2002). However, blockade of ligand binding sites on platelet surfaces by HES molecules might play a role (Kozek-Langenecker et al. 2005).

Hyperfibrinolysis following HES infusion is shown inconsistently in multiple studies (Kapiotis et al. 1994, Conroy et al. 1996, Jamnicki et al. 1998, Boldt et al. 2002). Decreased FXIII-mediated fibrin polymerization occurs following dilution of plasma samples with various HES solutions (Nielsen 2005a). In vivo hemodilution with HES 130/0.4 in bleeding patients results in hypocoagulability (decreased maximum clot firmness) and possibly occurs secondary to acquired fibrinogen deficiency as ex vivo supplementation corrects the coagulation changes (Fenger-Eriksen et al. 2009).

The carrier crystalloid of the HES might also impact coagulation as products containing high calcium concentrations (Hextend®) stimulate platelet activation (Deusch et al. 2004). However, cardiopulmonary bypass priming solutions containing a balanced (calcium-containing) HES do not preserve coagulation parameters in an ex vivo study using blood from healthy human volunteers (Appelman et al. 2011). Similarly, the carrier solution of HES infusions (0.9% NaCl or Ringer’s lactate) has minimal effect on platelet aggregation and clot formation in human healthy volunteers (Schaden et al. 2012).

Restrictive use of slowly degradable HES solutions is recommended in patients when hemostasis is important such as patients undergoing neurosurgery, patients with preexisting coagulopathies, or patients with overt bleeding (Kozek-Langenecker et al. 2005). Increases in PT, PTT, and bleeding times, as well as decreased FVIII activity occur with hetastarch infusions (Roberts et al. 1998). Pentastarch administration is also
associated with reduced FVIII activity (Roberts et al. 1998). Compared to other traditional coagulation tests, PTT is more sensitive to the adverse coagulation effects of synthetic colloids (Concannon et al. 1992, Chan et al. 2002). All HES products also show variable degrees of impairment of clot strength and clot formation *in vitro* using thromboelastometry (Niemi et al. 1998). Likewise, dilution of whole blood from healthy human volunteers with HES 450/0.7, HES 220/0.45, HES 200/0.5, and HES 130/0.4 causes TEG® changes consistent with hypocoagulability as evidenced by a combination of prolonged R, prolonged K, decreased α, and decreased MA, with no effects on clot lysis at 30 or 60 minutes (Egli et al. 1997, Entholzner et al. 2000, Nielsen 2005a). A systematic review of studies evaluating the effect of tetrastarch on hemostasis using TEG® confirms that tetrastarch administration is associated with hypocoagulability characterized by a weak clot with a less stable fibrin network and decreased platelet aggregation (Hartog et al. 2011).

Maize- or potato-derived HES solutions, and balanced or non-balanced tetrastarch solutions cause similar hemostatic changes (Casutt et al. 2010, Godier et al. 2010). However, conflicting results have supported a possible lesser impact of tetrastarch on coagulation compared to other higher molecular weight HES (Felfernig et al. 2003, Kozek-Langenecker et al. 2005, Sossdorf et al. 2009, Westphal et al. 2009). HES with a high molar substitution and C2/C6 ratio are associated with a greater reduction in factor VIII activity and vWF concentrations compared to products with a lower number of molar substitutions (Boldt 2009). In human orthopedic patients, FVIII and vWF return to normal within 5 hours following infusion of tetrastarch, but not after administration of hetastarch (Jungheinrich et al. 2004). Studies report no deterioration of FVIII with
tetrastarch administered at doses up to 50-70 mL/kg (Kasper et al. 2003, Neff et al. 2003). Similarly, administration of HES 130/0.4 to human patients undergoing major orthopedic surgery results in fewer abnormalities of FVIII and PTT compared to HES 200/0.5 (Langeron et al. 2001). Repeated administration of large doses of the rapidly degradable HES 130/0.4 for up to 28 days results in decreased blood loss and transfusion requirements compared to the slowly degradable product HES 200/0.5 (Neff et al. 2003). Additionally, while tetrastarch is equivalent to hetastarch for volume expansion, it results in fewer effects on coagulation including less decrease in FVIII and vWF activity, less prolonged PT and PTT, and decreased requirements for blood products (Ghandi et al. 2007).

Studies investigating the effects of HES on coagulation in dogs also confirm the development of hypocoagulability. In vitro and in vivo studies demonstrate that high molecular weight HES are associated with platelet dysfunction characterized by prolonged closure time using PFA-100 (Wieranga et al. 2007, Smart et al. 2009). In vitro dilution with HES 130/0.42 also causes hypocoagulability manifested by prolonged closure time using PFA-100 (Classen et al. 2012). However, a recent canine hemorrhagic shock model reveals no difference in platelet function as assessed with PFA-100 after tetrastarch administration (McBride et al. 2012). When using TEG® to investigate the in vitro effects of HES on coagulation, tetrastarch has less impact on coagulation than hetastarch at higher dilutions, but still results in hypocoagulability characterized by changes in K, α, and MA (Bacek et al. 2011). Similarly, in vitro dilution with tetrastarch in dogs causes hypocoagulability secondary to a dose-dependent alteration in fibrinogen
concentration and decreased platelet function assessed by thromboelastometry (Falco et al. 2012).

Interestingly, there are inconsistent findings of whether alterations in coagulation tests coincide with clinical bleeding. Infusion of hetastarch to human patients with septic shock results in decreased platelet count and prolongation of PT and PTT; however, it does not cause a higher incidence of bleeding compared to an albumin infusion (Falk et al. 1988). Conversely, significantly higher postoperative bleeding is seen in cardiopulmonary bypass patients given a high molecular weight HES infusion (Wilkes et al. 2001). And contrary to in vitro studies that show minimal effects of tetrastarch on coagulation and platelet function compared to other HES solutions, randomized controlled trials overwhelmingly suggest that in vivo use of tetrastarch still results in hypocoagulability and blood loss (Raja et al. 2011). Thus, hemostatic changes have been attributed to the use of HES solutions, with newer generation products allegedly being safer. However, studies reveal inconsistent findings and information is minimal regarding the in vivo effects of tetrastarch on hemostasis in dogs.

1.10 Conclusion

A close relationship between inflammation and hemostasis has been well established. A new understanding of hemostasis has been developed in recent years and newer technologies such as TEG® allow for a more global assessment of hemostasis compared to traditional coagulation tests. Sepsis is a common cause of death in veterinary intensive care units with only approximately 50% of septic dogs surviving to hospital discharge. Also, dysfunction of the coagulation system is independently associated with increased likelihood of death in dogs with sepsis. HES are frequently utilized for fluid resuscitation
while treating patients with sepsis and have reported adverse effects on hemostasis. The newer generation product tetrastarch has reported fewer adverse effects on hemostasis in humans, but its effects in dogs are largely unknown. Its effects on laboratory and hemodynamic variables have also not been reported in dogs. Investigation of these knowledge gaps is therefore warranted.
1.1 Objectives and Hypotheses

Systemic inflammation and sepsis are common in critically ill dogs and often necessitate aggressive fluid therapy. Colloids have frequently been utilized during fluid resuscitation. Considering that HES, the most commonly utilized type of synthetic colloids in North America, have amongst other things reported adverse effects on hemostasis, there is considerable interest in determining the effect of the newer generation tetrastarch on coagulation in healthy dogs and dogs with systemic inflammation. Newer technologies such as TEG® provide a more global assessment of hemostasis and have not been utilized to investigate the effect of in vivo administration of tetrastarch on hemostasis in dogs. Finally, the effect of tetrastarch on hemodynamic and laboratory variables such as COP is also largely unknown in dogs.

The objectives of this study were:

1. To compare hemodynamic and laboratory variables in dogs receiving an isotonic crystalloid (0.9% NaCl) or tetrastarch during health and after induction of systemic inflammation.

   Hypothesis: An intravenous bolus of tetrastarch would cause more measurable effects on vital parameters and COP compared to 0.9% NaCl in both healthy dogs and dogs with systemic inflammation.

2. To compare the effects of an isotonic crystalloid (0.9% NaCl) and synthetic colloid (tetrastarch) on coagulation in healthy dogs and dogs with induced systemic inflammation.
Hypothesis: An intravenous bolus of tetrastarch would produce a hypocoagulable TEG® profile compared to 0.9% NaCl administration in both healthy dogs and dogs with systemic inflammation.

3. To compare two different protocols for TEG® activation.

Hypothesis: Blood samples activated with TF would produce a more hypercoagulable TEG® profile when compared to blood samples activated with kaolin.

4. To determine the correlation between TEG® variables and traditional coagulation test results.

Hypothesis: TEG® variables would be correlated with traditional coagulation test results.
1.12 References


cells 1996; 14(s1):154-162.


developed hydroxyethyl starch (HES 130/0.4) compared to hydroxyethyl starches
thrombelastography parameters, secondary and tertiary hemostasis in dogs. J Vet
61. Esmon CT. Protein C anticoagulant pathway and its role in controlling
microvascular thrombosis and inflammation. Crit Care Med 2001; 29(s7):S48-
S51.
62. Esmon CT. Crosstalk between inflammation and thrombosis. Maturitas 2008;
63. Faust SN, Heyderman RS, Levin M. Coagulation in severe sepsis: a central role
for thrombomodulin and activated protein C. Crit Care Med 2001; 29(s7):S62-
S67.
following dilution with hydroxyethyl starch (130/0.4) via thromboelastometry. J
solutions on thromboelastography in preoperative male patients. Acta
66. Fenger-Eriksen C, Tønnesen E, Ingerslev J, Sørensen B. Mechanisms of
hydroxyethyl starch-induced dilutional coagulopathy. Thromb Haemost 2009;
7(7):1099-1105.


84. Griffin A, Callan MB, Shofer FS, Giger U. Evaluation of a canine D-dimer point-of-care test kit for use in samples obtained from dogs with disseminated


111. Kasper SM, Meinert P, Kampe S, et al. Large-dose hydroxyethyl starch 130/0.4 does not increase blood loss and transfusion requirements in coronary artery bypass surgery compared with hydroxyethyl starch 200/0.5 at recommended doses. Anesthesiology 2003; 99(1):42-47


136. Martini WZ, Cortez DS, Dubick MA, Park MS, Holcomb JB. Thromboelastography is better than PT, aPTT, and activated clotting time in detecting relevant clotting abnormalities after hypothermia, hemorrhagic shock...


153. Nelson OL, Andreasen C. The utility of plasma D-dimer to identify


229. Wiinberg B, Jensen AL, Johansson PI, et al. Thromboelastographic evaluation of


CHAPTER 2

Effect of synthetic colloid administration on hemodynamic and laboratory variables in healthy dogs and dogs with systemic inflammation

Abstract

Objective - To compare the effects of administering equal volumes of isotonic crystalloids and synthetic colloids on hemodynamic and laboratory variables in healthy dogs and dogs with systemic inflammation.

Design - Randomized, placebo-controlled, double-blinded study.

Setting - Veterinary teaching hospital.

Animals - Sixteen adult purpose-bred Beagles.

Interventions - Dogs were randomized into one of two groups receiving fluid resuscitation with either 40 mL/kg intravenous (IV) isotonic crystalloid (0.9% NaCl) or synthetic colloid (tetrastarch). Dogs were first randomized to receive either lipopolysaccharide (LPS; 5 µg/kg, IV) or an equal volume of placebo (0.9% NaCl, IV). After a 14-day washout, the study was repeated such that dogs received the opposite treatment (LPS or placebo) and the same resuscitation fluid. Vital signs (heart rate (HR), oscillometric blood pressure) were measured and blood samples were collected for packed cell volume (PCV), total solids (TS), serum lactate concentration, and colloid osmotic pressure (COP) measurements.

Measurements and Main Results - Healthy (placebo) dogs had similar decreases in PCV and TS after administration of either fluid. Tetrastarch administration was
associated with a larger increase in HR, systolic blood pressure (SBP) and mean blood pressure (MBP) compared to 0.9% NaCl. Dogs with systemic inflammation had similar increases in SBP and decreases in PCV, TS, and lactate after administration of either fluid. Tetrastarch administration caused a greater immediate increase in HR and MBP compared to 0.9% NaCl. In all dogs, 0.9% NaCl administration decreased COP and tetrastarch administration increased COP.

**Conclusions** - Resuscitation with equal volumes of 0.9% NaCl or tetrastarch caused similar changes in hemodynamic and laboratory variables in dogs with LPS-induced systemic inflammation; however, larger increases in HR and blood pressure were seen within the first 2 hours following tetrastarch administration compared to 0.9% NaCl. Tetrastarch administration increased COP in all dogs, despite a decrease in TS.

**Abbreviations:**

COP  Colloid osmotic pressure

DBP  Diastolic blood pressure

HES  Hydroxyethyl starches

HR  Heart rate

IV  Intravenous

LPS  Lipopolysaccharide

MBP  Mean blood pressure

RR  Respiratory rate
Introduction

Systemic inflammation and sepsis are common in critically ill dogs (Kenney et al. 2010) and often necessitate aggressive fluid therapy. Considerable controversy exists regarding the safety and efficacy of synthetic colloids (Bayer et al. 2011, Dellinger et al. 2013, Marx et al. 2007), which are commonly used during resuscitative efforts in people and animals. Although multiple studies compare crystalloids and colloids for fluid resuscitation (Perel et al. 2012), little is known as to which improves outcome. A large-scale multicenter randomized controlled trial (CHEST) was recently completed comparing volume resuscitation with 6% hydroxyethyl starch 130/0.4 to 0.9% NaCl on 90-day mortality and other clinically relevant outcomes in a general human ICU population (Myburgh et al. 2012). The authors found no significant difference in 90-day mortality, but more patients receiving tetrastarch required renal replacement therapy (Myburgh et al. 2012). Currently, limited consensus exists regarding the ideal fluid for resuscitating patients with systemic inflammation or sepsis (Bayer et al. 2011, Dellinger et al. 2013, Marx et al. 2007, Perel et al. 2012). Recently, the Surviving Sepsis Campaign guidelines recommended against the use of hydroxyethyl starches (HES) for initial fluid resuscitation of human patients with sepsis or septic shock (Dellinger et al. 2013).

Various synthetic colloids are available, but HES are the most routinely used products in North America (Bayer et al. 2011). Different generations of HES with diverse
physicochemical properties exist, with dissimilar *in vivo* effects. Hydroxyethyl starches are characterized by their average molecular weight and dispersion, degree of substitution, and C2/C6 ratio (Westphal *et al.* 2009, Chan 2008, Goggs *et al.* 2008). By improving COP and remaining in the vasculature longer, HES are expected to have a more positive impact on hemodynamic parameters compared to isotonic crystalloids (Chan 2008, Hanfeln *et al.* 1989, Prough *et al.* 1991). Tetrastarch, the third generation product, has a smaller molecular weight and creates a greater increase in colloid osmotic pressure (COP) compared to other HES (Chan 2008, Humm *et al.* 2007). Due to its more rapid elimination and lack of accumulation, tetrastarch can be administered at higher dosages (50 mL/kg/day) compared to traditionally used HES (Westphal *et al.* 2009, Goggs *et al.* 2008, Van der Linden *et al.* 2005). The effect of tetrastarch on hemodynamic and laboratory variables is largely unknown in dogs.

The objective of this study was to compare hemodynamic and laboratory variables in dogs receiving an isotonic crystalloid (0.9% NaCl) or tetrastarch during health and systemic inflammation. We hypothesized that an intravenous (IV) bolus of tetrastarch would cause more measurable effects on vital parameters and COP compared to 0.9% NaCl in both healthy dogs and dogs with systemic inflammation.

**Material and Methods**

**Animals:** Sixteen healthy adult purpose-bred Beagles were included in this study. All dogs were deemed healthy on the basis of a normal physical examination, CBC, biochemistry profile, prothrombin time, and partial thromboplastin time. The institution
Animal Care Committee approved the study. Dogs were housed and handled according to the guidelines of the Canadian Council on Animal Care, the requirements of the Animals for Research Act Revised Statutes of Ontario, and the institution Animal Care Policy.

**Experimental design:** A randomized, placebo-controlled, double-blinded study was performed. Dogs were randomized into one of two fluid resuscitation groups for the entire study: 8 dogs received an isotonic crystalloid (0.9% NaCl)\(^a\) and 8 dogs received a synthetic colloid (tetrastarch).\(^b\) Dogs were randomized to first receive either 5 µg/kg, IV lipopolysaccharide (LPS, *Escherichia coli* 0127:B8) or an equal volume of placebo (0.9% NaCl)\(^a\) via a cephalic catheter.\(^c\) Thirty minutes later, each dog received 40 mL/kg IV of the designated resuscitation fluid delivered over 30 minutes. The study was repeated with dogs receiving the opposite pre-fluid treatment (LPS or placebo) after a minimum 14-day washout period during which no treatments were administered outside of normal husbandry and care. The first author was blinded to the type of fluid given and randomization was performed using an online number generating program.

Dogs were fasted 12 hours prior to the study and allowed free access to water. Butorphanol\(^d\) (0.2 mg/kg, IM) was given prior to the study for analgesia and was repeated IV at 2 and 4 hours after baseline. A 20-gauge catheter\(^c\) was placed in the cephalic vein to enable IV administration of butorphanol and either placebo or LPS. Vital signs (rectal temperature, heart rate (HR), respiratory rate (RR), blood pressure) were recorded at baseline and every 30 minutes for 6 hours. Heart rate was measured by femoral pulse palpation or cardiac auscultation, RR by visualization of chest movements or thoracic auscultation, and indirect blood pressure (systolic (SBP), diastolic (DBP), mean (MBP) blood pressure) using an oscillometric device.\(^e\) Dogs were also monitored for vomiting or
diarrhea. At the completion of the study period (6 hours after baseline), the cephalic catheter was removed and the dogs were re-housed and offered food.

**Sample Collection and Tests:** Blood samples (2 mL) were collected via jugular venipuncture at baseline (0 h) and 1, 2, 4, and 24 hours after LPS or placebo administration. Blood samples were collected into EDTA and plastic Vacutainer tubes with no additive. Microhematocrit tubes were filled using blood from the EDTA tube and centrifuged at 5400G for 5 minutes for measurement of packed cell volume (PCV) and plasma total solids (TS) using refractometry. The no additive tube was kept at room temperature for 15 minutes (until clot formation) and centrifuged at 700G for 15 minutes. The separated serum was used for measurement of lactate concentration using a handheld device, as well as COP using an osmometer that was calibrated daily per the manufacturer’s instructions.

**Statistical Methods:** A Latin square design with repeated measures over time and a generalized linear mixed-model was employed to analyze the data using proc mixed. The AKAIKE information criterion (AIC) was used to determine an error structure for the auto-regression. The assumptions of the ANOVA were assessed by comprehensive residual analyses. Shapiro-Wilk, Kolmogorov-Smirnov, Cramer-von Mises, and Anderson-Darling tests were conducted to analyze for standard normal distribution. Residuals were plotted against predicted values and explanatory variables (treatment, fluid, time, dogs) to look for outliers or unequal variance. If residual analyses suggested a need for data transformation, logarithmic transformation was done before data analysis. If comparisons of the factors “treatment” and “time” were significant, differences between dogs receiving 0.9% NaCl or tetrastarch were compared using paired t-tests. The level of
significance for the t-tests was corrected using a Tukey’s or Dunnett’s test. Significance was set at $p < 0.006$ for rectal temperature, HR, RR, and blood pressure, and at $p < 0.019$ for PCV, TS, serum lactate, and COP. Commercially available software was used for the statistical analyses$^k$ and graph generation.$^1$

Results:

All dogs were female (7 intact, 9 spayed) ranging in age from 16-44 months (median 18 months). The median body weight was 8.85 kg (range 7.8-11 kg). Baseline values for all vital signs were not statistically different between dogs before administration of placebo or LPS.

Placebo Phase:

There was no significant difference in rectal temperature between dogs receiving either fluid. Compared to dogs given 0.9% NaCl, dogs given tetrastarch had a significantly higher HR between 1 ($p < 0.001$) and 2 hours ($p < 0.001$) (Figure 2.1a), as well as a significantly higher RR ($p = 0.006$), SBP ($p = 0.004$) (Figure 2.2a), and MBP ($p < 0.001$) (Figure 2.3a) at 1.5 hours. There was no significant difference in DBP in dogs given either fluid.

Dogs given tetrastarch had a significantly higher PCV at baseline ($p < 0.001$) and between 2 ($p < 0.001$) and 24 hours ($p = 0.012$) compared to dogs given 0.9% NaCl (Figure 2.4a). Dogs administered tetrastarch had a significantly higher serum lactate concentration at baseline ($p < 0.001$) and 1 hour ($p = 0.018$) compared to dogs given 0.9% NaCl (Figure 2.5a). There were no significant differences in TS between dogs receiving 0.9% NaCl or tetrastarch at any time point (Figure 2.6a).
had a significantly higher COP between 1 (p < 0.001) and 4 hours (p < 0.001) compared to dogs given 0.9% NaCl (Figure 2.6a).

**LPS Phase:**

All dogs demonstrated lethargy and gastrointestinal signs during the first 1-2 hours including hypersalivation and diarrhea within 30 minutes of LPS administration. Dogs had a significant decrease compared to baseline in rectal temperature at 0.5 (p < 0.001) and 1 hour (p < 0.001), followed by a significant increase in rectal temperature at 3 (p < 0.001) and 4 hours (p < 0.001) after LPS administration. There was no difference in rectal temperature between dogs receiving 0.9% NaCl or tetrastarch.

Dogs receiving tetrastarch had a significantly higher HR at 1 (p < 0.001) and 1.5 hours (p < 0.001) compared to dogs administered 0.9% NaCl (Figure 2.1b). There was no difference in RR, SBP (Figure 2.2b), or DBP between dogs given 0.9% NaCl or tetrastarch. Dogs receiving tetrastarch had a significantly higher MBP at 2 hours (p < 0.001) compared to dogs administered 0.9% NaCl (Figure 2.3b).

Dogs receiving tetrastarch had a significantly higher PCV at baseline (p < 0.001) and significantly lower PCV at 1 hour (p = 0.002) compared to dogs administered 0.9% NaCl (Figure 2.4b). There was no significant difference in serum lactate concentration (Figure 2.5b) or TS (Figure 2.6b) between dogs receiving 0.9% NaCl or tetrastarch at any time point. Dogs receiving tetrastarch had a significantly higher COP at 1 (p < 0.001) and 2 hours (p < 0.001) compared to dogs administered 0.9% NaCl (Figure 2.6b).
Discussion:

The results of this study revealed that in healthy dogs, administration of 0.9% NaCl and tetrastarch resulted in parallel decreases in PCV and TS, suggesting similar volume expansion and hemodilution effects. However, tetrastarch administration caused a larger immediate increase in HR, SBP and MBP compared to 0.9% NaCl. Likewise, in dogs with LPS-induced systemic inflammation, administration of 0.9% NaCl and tetrastarch resulted in similar increases in SBP, decreases in PCV and TS, and relatively unchanged lactate concentrations, also suggesting that both fluids resulted in comparable volume expansion and hemodilution effects. However, tetrastarch administration caused a larger immediate increase in HR and MBP compared to 0.9% NaCl. As expected, both healthy dogs and dogs with LPS-induced systemic inflammation had a decrease in COP after 0.9% NaCl administration compared to an increase in COP after tetrastarch administration, confirming the benefit of increasing oncotic pressure with tetrastarch administration compared to the hemodilutional effect and subsequent decrease in COP with 0.9% NaCl administration. Interestingly, despite the positive effect of tetrastarch administration on COP, dogs with LPS-induced systemic inflammation had no significant hemodynamic benefit with tetrastarch administration compared to 0.9% NaCl. Additionally, measurement of TS did not coincide with the change in COP after tetrastarch administration.

The healthy dogs in the present study that received tetrastarch had significantly higher HR, SBP, and MBP within the first 2 hours compared to those dogs receiving 0.9% NaCl. The rapid bolus of either fluid most certainly increased preload and was likely associated with atrial stretching and secondary tachycardia through the Bainbridge
reflex, as well as increased stroke volume, cardiac output, and blood pressure (Hall 2011). However, because an equal volume of 0.9% NaCl and tetrastarch was used in the present study, an overall larger increase in blood volume was likely created by the infusion of tetrastarch, as compared to 0.9% NaCl. A more significant effect on hemodynamic parameters was thus expected and likely caused the larger immediate increase in HR and blood pressure seen following tetrastarch administration.

The volume expanding efficiency of a fluid is known as the efficiency ratio and is calculated as the ratio of increase in blood volume to the volume of infused fluid. To the authors’ knowledge, this value is currently unreported for tetrastarch. In a prospective canine study comparing the effect of infusing 0.9% NaCl (80 mL/kg), 6% hetastarch (20 mL/kg), and Dextran-70 (20 mL/kg) on blood volume expansion, both synthetic colloids caused a larger overall increase in blood volume and thus had a larger efficiency ratio (Silverstein et al. 2005). Although 0.9% NaCl infusion caused the largest immediate increase in blood volume during its administration, this effect declined rapidly at the end of the infusion and resulted in the smallest efficiency ratio, likely due to fluid redistribution to the interstitium. Blood volume continued to increase from the end of the infusion to 30 minutes after the infusion of both synthetic colloids and persisted up to the maximum 240-minute period. A larger efficiency ratio and retention were therefore noted with the infusion of both synthetic colloids. Nevertheless, no significant changes in MBP were seen after the infusion of either 0.9% NaCl or synthetic colloids to those dogs (Silverstein et al. 2005); however, the volume of synthetic colloid administered was smaller than that given during the present study.
Interestingly, there was only a transient increase in HR and MBP in dogs with LPS-induced systemic inflammation after each fluid bolus. This could be explained by the fact that LPS-induced systemic inflammation led to vasodilation, which was counterbalanced by the administered volume that filled the dilated blood compartment. As expected, the increase in HR and blood pressure after tetrastarch administration did not persist beyond 2 hours post-infusion. Due to its small molecular weight and low degree of molar substitution, tetrastarch does not persist long in circulation (Chan 2008, Jungheinrich et al. 2005, Westphal et al. 2009). As such, its effects on hemodynamic parameters are typically short-lived compared to other synthetic colloids.

The fluid bolus dosages for 0.9% NaCl and tetrastarch were selected to create equivalent hemodilution in euvoletic healthy dogs. However, the overall blood volume expansive effect of isotonic crystalloids and synthetic colloids are not equivalent as the latter results in fluid recruitment from the interstitial compartment, in addition to the administered volume (Silverstein et al. 2005). Similar studies comparing the effect of isotonic crystalloids to synthetic colloids have been conducted in other species and designed using either a strategy of equal volume or unequal volume based on the expected relative volume expansion created by each fluid type (Mittermayr et al. 1997, Ruttmann et al. 1998, So et al. 1997). In a recent systematic review of randomized controlled trials in which tetrastarch was used for resuscitation, volume repletion with crystalloids only required 1.8 times the volume used in the colloid groups (Hartog et al. 2011). This finding was not consistent with traditional beliefs that at least 3-4 times more volume is required for adequate fluid resuscitation when crystalloids are used as compared to colloids. Given the lack of literature on the volume expansive effects of
tetrastarch in dogs, a dosage that would give equivalent volume expansion with the crystalloid could not be determined. An equal volume for both 0.9% NaCl and tetrastarch was therefore chosen because the immediate volume increment effect would be equivalent, although ultimately increased and more persistent for tetrastarch. The identical volume of tetrastarch administered and larger increase in blood volume overall, likely created the larger effect on hemodynamic parameters seen in the present study.

Colloid osmotic pressure increased significantly with a peak effect 1 hour after tetrastarch administration, whereas 0.9% NaCl administration caused a significant decrease in COP. The increased COP seen with tetrastarch is likely explained by the oncotic support provided by the hydroxyethyl starch molecules, whereas the decrease in COP seen with 0.9% NaCl is likely due to dilution of the plasma proteins. Starling’s law ($J_V = K_f [(P_c - P_i) - \delta (\pi_c - \pi_i)]$; $J_V$: net fluid movement, $K_f$: filtration coefficient, $P_c$ and $P_i$: capillary and interstitial hydrostatic pressure, $\delta$: reflection coefficient, $\pi_c$ and $\pi_i$: capillary and interstitial COP) describes the forces that regulate fluid movement between the intravascular and interstitial compartments. A negative oncotic gradient between the intravascular and interstitial compartments can ultimately lead to fluid extravasation. Systemic inflammation during sepsis is associated with increased vascular permeability and subsequent capillary leakage (Marx 2003). Hypoalbuminemia occurs commonly as a consequence of transcapillary losses and impaired hepatic albumin synthesis. This leads to decreased COP, which often warrants appropriate fluid therapy to prevent further fluid extravasation (Marx 2003). The increased COP noted with tetrastarch administration in the present study demonstrates its effectiveness at providing oncotic support. The expected in vivo COP following HES administration is not well established in dogs. The
COP of different available synthetic colloids was evaluated in vitro and tetrastarch had the highest COP (37.1 ± 0.8 mmHg) of all HES evaluated (Humm et al. 2007). To the authors’ knowledge, the present study is the first to evaluate the in vivo effect of tetrastarch on COP.

In the present study, TS decreased significantly in response to administration of both tetrastarch and 0.9% NaCl, despite opposite changes in COP. This coincides with findings of a previous study that showed poor correlation between TS and COP (Thomas et al. 1992). Another study investigated the changes in COP and TS associated with progressive dilution of human serum albumin with 6% hetastarch or Dextran-70 (Bumpus et al. 1998). This in vitro study found that both synthetic colloids caused a significant increase in COP with no concurrent change in TS (Bumpus et al. 1998). All synthetic colloids evaluated had a TS measurement by refractometry of 4.5 g/dL (45 g/L) (Bumpus et al. 1998); when the authors measured the TS for tetrastarch using refractometry it was 4.2 g/dL (42 g/L). This suggests that hemodilution with synthetic colloids could potentially cause a change in TS measurements towards that value, irrespective of the observed effect on COP. As such, the response to synthetic colloid administration should be assessed by evaluating hemodynamic changes or by monitoring COP, rather than relying on measurement of TS.

In the present study, healthy dogs receiving tetrastarch had significantly higher serum lactate concentrations at 1 hour compared to dogs receiving 0.9% NaCl. This was an unexpected finding that is difficult to explain in light of the increase in blood pressure seen after tetrastarch administration. Lactate concentration is recognized as a good marker of perfusion (Allen et al. 2008). With its favorable effect on hemodynamic
parameters, tetrastarch should improve global perfusion and decrease lactate concentration. Because lactate concentration was measured using serum samples, a methodologic error could have caused a measurement bias. Delayed measurement of serum lactate when RBCs are present is associated with ongoing lactate production up to 20% during each hour of storage at 25°C in serum clot tubes (Pang et al. 2007). Due to timing of blood collection and performance of several other tests as part of the study protocol, the authors elected to measure serum, rather than whole blood, lactate concentration. Although serum samples were used as soon as possible to measure lactate concentration, timing between blood collection, serum separation, and lactate measurement was not always consistent. Therefore, it is possible that some serum samples were kept at room temperature longer before separation, which might have affected the results. A larger sample size might have helped to remediate this finding and measurements within 5 minutes of collection using whole blood would also be expected to produce more reliable values (Pang et al. 2007).

The present study has some limitations that warrant discussion. First, the small sample size rendered some of the analyses underpowered to detect significant differences. Nonetheless, each dog was used as its own control in order to increase our ability to detect significant differences. Second, differences in baseline PCV and lactate measurements were documented in the tetrastarch group of dogs. This finding is difficult to explain, but might also be a result of the small sample size and lactate measurement methodology. Third, an LPS model was used in this study to mimic the systemic changes encountered during naturally occurring sepsis. While this model has been used successfully to compare the effect of synthetic colloids with isotonic crystalloids (Aksu et
al. 2012, Baum et al. 1990, Hoffmann et al. 2002, Schäper et al. 2008), it does not replicate all changes associated with naturally occurring sepsis. As such, results from the present study cannot necessarily be extrapolated to all dogs with systemic inflammation or naturally occurring sepsis. Finally, the washout period duration was selected based on the plasma terminal half life ($t_{1/2}$) of tetrastarch in humans, which is 12.1 hours following administration of a single dose of 500 mL (Waitsinger et al. 1998). Such information is not available for dogs and the presence of persistent circulating tetrastarch molecules for longer than the 14-day washout period cannot be excluded.

Conclusions:

A 40 mL/kg IV bolus of tetrastarch was well tolerated and caused higher increases in HR and blood pressure compared to 0.9% NaCl administration in healthy dogs; however, the effects on hemodynamic parameters were transient and lasted less than 2 hours. In dogs with LPS-induced systemic inflammation, resuscitation with tetrastarch caused a greater immediate increase in HR and blood pressure with similar changes in other hemodynamic and laboratory variables. Overall, tetrastarch effectively increased COP, but measurement of TS did not coincide with the change in COP. Future studies investigating the effect of other doses or constant rate infusions of tetrastarch on hemodynamic and laboratory variables, as well as in dogs with naturally occurring sepsis, are warranted.

Considering the reported adverse hemostatic and renal effects of tetrastarch found in human studies (Bayer et al. 2011, Dellinger et al. 2013, Marx et al. 2007, Myburgh et al. 2012, Perel et al. 2012, Westphal et al. 2009) and the limited additional benefit of
tetrastarch administration on hemodynamic variables demonstrated in the present study, the increased cost associated with the use of tetrastarch likely negates its use as a first line treatment during fluid resuscitation in dogs. However, the observed positive effect on COP and the transient increase in blood pressure following tetrastarch administration suggest that it might be useful as an adjunctive treatment to crystalloid therapy in patients with low oncotic pressure or refractory shock. A veterinary product recently became commercially available and appears to have the same physicochemical properties as the tetrastarch product investigated in the present study. Therefore, tetrastarch products are becoming more readily available, which will hopefully lead to additional studies investigating its utility in client-owned dogs.
Footnotes:

a. 0.9% NaCl, Baxter, Mississauga, ON, Canada.
b. Voluven, Fresenius Kabi, Mississauga, ON, Canada.
c. BD Insyte Autoguard catheter, BD Medical, Sandy, UT.
d. Torbugesic, Fort Dodge Animal Health, New York, NY.
e. Cardell 9401, Midmark, Versailles, OH.
f. Capiject EDTA micro collection tubes, Terumo, Somerset, NJ.
g. BD Vacutainer plus plastic plasma tubes, Becton, Dickinson, and Company, Franklin Lakes, NJ.
h. Fisherbrand plastic microhematocrit tubes, Fisher Scientific, Hampton, NH.
i. Lactate Pro, FaCT Canada, Quesnel, BC, Canada.
j. Colloid Osmometer Model 4420, Wescor, Logan, UT.
k. SAS Online v.9.1.3, SAS Institute, Cary, NC
l. Prism 5, GraphPad Software, La Jolla, CA.
m. Vetstarch, Abbott Laboratories, North Chicago, IL.
References:


Figure 2.1 – Comparison of HR in 16 dogs receiving 0.9% NaCl or tetrastarch after placebo and LPS administration. Time of administration of 0.9% NaCl or tetrastarch administration represented as ↑. Significant differences (p < 0.006) indicated with *. All values are expressed as mean ± SD. a) Placebo b) LPS.
Figure 2.2 – Comparison of SBP in 16 dogs receiving 0.9% NaCl or tetrastarch after placebo and LPS administration. Time of administration of 0.9% NaCl or tetrastarch administration represented as ↑. Significant differences (p < 0.006) indicated with *. All values are expressed as mean ± SD. a) Placebo b) LPS.
Figure 2.3 – Comparison of MBP in 16 dogs receiving 0.9% NaCl or tetrastarch after placebo and LPS administration. Time of administration of 0.9% NaCl or tetrastarch administration represented as ↑. Significant differences (p < 0.006) indicated with *. All values are expressed as mean ± SD. a) Placebo b) LPS.
Figure 2.4 – Comparison of PCV in 16 dogs receiving 0.9% NaCl or tetrastarch after placebo and LPS administration. Time of administration of 0.9% NaCl or tetrastarch administration represented as ↑. Significant differences (p < 0.019) indicated with *. All values are expressed as mean ± SD. a) Placebo b) LPS.
Figure 2.5 – Comparison of serum lactate concentration in 16 dogs receiving 0.9% NaCl or tetrastarch after placebo and LPS administration. Significant differences (p < 0.019) indicated with *. All values are expressed as mean ± SD. a) Placebo b) LPS.
Figure 2.6 – Comparison of COP and TS in 16 dogs receiving 0.9% NaCl or tetrastarch after placebo and LPS administration. Time of administration of 0.9% NaCl or tetrastarch administration represented as ↑. Significant differences (p < 0.019) indicated with *. All values are expressed as mean ± SD. a) Placebo b) LPS.
CHAPTER 3

Effect of synthetic colloid administration on coagulation in healthy dogs and dogs with systemic inflammation

Abstract

Background – Synthetic colloids are often used during fluid resuscitation and have reported effects on coagulation.

Objective – To compare the effects of an isotonic crystalloid and synthetic colloid on coagulation in healthy dogs and dogs with systemic inflammation.

Animals – Sixteen adult purpose-bred Beagles.

Methods – Dogs were randomized into one of two groups receiving fluid resuscitation with either 40 mL/kg IV 0.9% NaCl or tetrastarch after administration of lipopolysaccharide (LPS) or an equal volume of placebo. After a 14-day washout period, the study was repeated such that dogs received the opposite treatment (LPS or placebo) and the same resuscitation fluid. Blood samples were collected at 0, 1, 2, 4, and 24 hours for measurement of activated clotting time (ACT), platelet count, prothrombin time (PT), partial thromboplastin time (PTT), factor VIII activity, von Willebrand factor antigen (vWFAg) and collagen binding (vWFCBA) activity, D-dimer concentration, and thromboelastography.

Results – Administration of either fluid to healthy dogs and dogs with systemic inflammation resulted in similar increases in PT and ACT. In comparison to saline administration, tetrastarch administration resulted in significantly decreased R in healthy dogs, as well as significantly increased PTT and K and significantly decreased platelet
count, α, MA, CL30%, vWFAg, and vWFCBA in both healthy dogs and dogs with systemic inflammation.

**Conclusions and clinical importance** – Tetrastarch bolus administration to dogs with systemic inflammation resulted in a transient hypocoagulability characterized by a prolonged PTT, decreased clot formation speed and clot strength, and acquired type 1 von Willebrand disease.

**Abbreviations:**

α Angle

ACT Activated clotting time

CL30% Clot lysis index at 30 minutes

CL60% Clot lysis index at 60 minutes

FVIII Factor VIII activity

G Global clot strength

HES Hydroxyethyl starches

K Clot formation time

LPS Lipopolysaccharide

LY30% Clot lysis rate at 30 minutes

LY60% Clot lysis rate at 60 minutes
Introduction:

Fluid therapy is a common treatment for hospitalized patients; however, debate exists regarding optimal fluid selection. Although crystalloids and colloids are both routinely recommended for use in critically ill veterinary patients, indications and side effects of these fluids have not been extensively investigated in dogs. General indications for colloid therapy include intravascular volume expansion for patients with hypovolemic or distributive shock, as well as patients with hypoalbuminemia and decreased oncotic pressure (Hughes et al. 2012). Unfortunately, adverse effects have been associated with the use of certain synthetic colloids including the induction of coagulopathies, impaired
renal function, and prolonged tissue storage resulting in pruritus (Dart et al. 2010, Kozek-Langenecker 2005, Westphal et al. 2009). Several human studies have recently compared crystalloids and colloids during fluid resuscitation, and have failed to identify a survival advantage for either type of fluid (Bayer et al. 2011, Perner et al. 2012, Myburgh et al. 2012).

Different synthetic colloids are available, but hydroxyethyl starches (HES) are the products most routinely used in North America (Bayer et al. 2011). Different generations of HES with diverse physicochemical properties exist, with varied effects noted in vivo. HES are characterized by their average molecular weight (MW) and dispersion, degree of substitution, and C2/C6 ratio (Chan 2008, Goggs et al. 2008, Westphal et al. 2009). Adverse effects associated with HES are determined by the in vivo degradation of the HES molecules (Kozek-Langenecker et al. 2007). Tetrastarch, a third generation product, has a smaller average MW and lower degree of substitution, thereby exhibiting more rapid elimination and lack of accumulation compared to other HES products (Goggs et al. 2008, Westphal et al. 2009). Conflicting reports support that tetrastarch has a lower potential impact on coagulation compared to other larger MW HES products (Kozek-Langenecker 2005, Sossdorf et al. 2009, Westphal et al. 2009). Adverse effects of HES on hemostasis in humans are characterized by decreased factor VIII (FVIII) and von Willebrand factor (vWF) activity, dilution of clotting factors, decreased expression of integrin αIIbβ3 on activated platelets leading to decreased aggregation, decreased fibrinogen polymerization, acquired fibrinogen deficiency, and impaired fibrinolysis (Kozek-Langenecker 2005, Kozek-Langenecker et al. 2007, Westphal et al. 2009).
Thromboelastography (TEG®) provides a global assessment of hemostasis using whole blood (Donahue et al. 2005). Studies in vitro and in vivo using TEG® and similar viscoelastic devices have been performed in humans to evaluate the effect of HES on hemostasis (Hartog et al. 2011, Jamnicki et al. 2000, Kozek-Langenecker et al. 2007). Progressive in vitro hemodilution with tetrastarch produces a hypocoagulable TEG® profile characterized by increased reaction (R) and clot formation (K) times, and decreased angle (α) and maximal amplitude (MA) (Hartog et al. 2011). Similar in vitro studies using higher MW HES products have been performed in dogs using platelet function analysis (PFA-100) and reveal prolonged closure time consistent with platelet dysfunction (Wieranga et al. 2007, Smart et al. 2009). To date, there are no published veterinary studies investigating the in vivo effects of HES on coagulation in dogs using TEG®.

The primary objective of this study was to compare the effects of an isotonic crystalloid (0.9% NaCl) and synthetic colloid (tetrastarch) on coagulation in healthy dogs and dogs with induced systemic inflammation. The secondary objectives were to compare two different protocols for TEG® activation and to determine the correlation between TEG® variables and traditional coagulation test results. The authors hypothesized that an IV bolus of tetrastarch would produce a hypocoagulable TEG® profile compared to 0.9% NaCl administration in both healthy dogs and dogs with systemic inflammation. The authors also hypothesized that blood samples activated with tissue factor (TF) would produce a more hypercoagulable TEG® profile when compared to blood samples activated with kaolin. Finally, the authors hypothesized that TEG® variables would be correlated with traditional coagulation test results.
Materials and Methods:

**Animals:** Sixteen healthy adult purpose-bred Beagles were included in this study. All dogs were deemed healthy on the basis of a normal physical examination, CBC, biochemistry profile, prothrombin time (PT), and partial thromboplastin time (PTT). The institution Animal Care Committee approved the study. Dogs were housed and handled according to the guidelines of the Canadian Council on Animal Care, the requirements of the Animals for Research Act Revised Statutes of Ontario, and the institution Animal Care Policy.

**Experimental design:** A randomized, placebo-controlled, double-blinded study was performed. Dogs were randomized into one of two fluid resuscitation groups for the entire study: 8 dogs received an isotonic crystalloid (0.9% NaCl) and 8 dogs received a synthetic colloid (tetrasaccharide). Dogs were randomized to first receive either 5 µg/kg, IV lipopolysaccharide (LPS, *Escherichia coli* 0127:B8) or an equal volume of placebo (0.9% NaCl) (T0) via a cephalic catheter. Thirty minutes later (T0.5), each dog received 40 mL/kg IV of the designated resuscitation fluid delivered over 30 minutes. The study was repeated with dogs receiving the opposite pre-fluid treatment (LPS or placebo) after a minimum 14-day washout period during which no treatments were administered outside of normal husbandry and care. The first author was blinded to the type of fluid given and randomization was performed using an online number generating program.

Dogs were fasted 12 hours prior to the study and allowed free access to water. At the start of the experimental period, butorphanol (0.2 mg/kg, IM) was given for
analgesia and was repeated IV at 2 (T2) and 4 (T4) hours after baseline (T0). A 20-gauge catheter was placed in the cephalic vein to enable IV administration of either placebo or LPS and butorphanol. Dogs were monitored for vomiting or diarrhea. At the completion of the experimental period (T6), the cephalic catheter was removed and the dogs were re-housed and offered food.

**Sample Collection and Tests:** Blood samples (5 mL) were collected via jugular venipuncture at baseline (T0) and 1 (T1), 2 (T2), 4 (T4), and 24 (T24) hours after LPS or placebo administration. Blood samples were collected into a max-ACT tube, EDTA tube, and two plastic Vacutainer tubes with sodium citrate (3.2% trisodium citrate and blood in a 1: 9 ratio). The max-ACT tube was used immediately to determine the activated clotting time (ACT) by the axillary method as described previously (Bateman et al. 1999). The EDTA blood was used for white blood cell and platelet count, which were performed at a commercial laboratory using an automated analyzer with laser light scatter and cytochemical staining methods. One of the two Vacutainer tubes containing sodium citrate was stored at room temperature for 30 minutes and used for TEG® analysis and the other was centrifuged immediately at 700G for 15 minutes. The plasma was then removed and stored at -70°C for batch analysis of PT, PTT, FVIII activity, von Willebrand factor antigen (vWFAg) and collagen binding activity (vWFCBA), and D-dimer concentration.

**Coagulation Testing**

PT and PTT were performed at the coagulation laboratory at the Foster Hospital for Small Animals at Tufts University. PT and PTT were both determined by means of
an automated analyzer\(^j\) and commercially available reagents,\(^k,l\) as described previously (Iazbik et al. 2001). Reference intervals were based on values obtained from 35 healthy dogs. FVIII activity, \(vWF\)Ag and \(vWF\)CBA, and D-dimer concentration were measured at the Comparative Coagulation Section of the Animal Health Diagnostic Center at Cornell University. FVIII activity was performed in a modified one-stage activated PTT technique using a semi-automated clot detection instrument\(^m\) and canine congenital FVIII deficient substrate plasma, as previously described (Stokol et al. 2000, Wardrop et al. 2001). \(vWF\)Ag was measured by ELISA with monoclonal anti-canine \(vWF\) antibodies (Benson et al. 1991). \(vWF\)CBA was measured by ELISA with bovine mixed, types I and III, collagen and the same monoclonal anti-canine \(vWF\) antibodies (Sabino et al. 2006).\(^n\) The standard curves for all factor assays were derived from dilutions of a pooled canine plasma standard, prepared from 15 healthy dogs and stored in single-use aliquots at -70\(^0\) C. Plasma D-dimer concentration was measured in a quantitative, immunoturbidimetric method using a commercial kit.\(^o\) The assay standard was generated from dilutions of the manufacturer’s human D-dimer calibrator.

**Thromboelastography**

TEG\(^®\) was performed using a commercial analyzer\(^p\) and simultaneously running kaolin\(^q\) and TF\(^r\) activated samples 30 minutes after sample collection. TF was reconstituted with sterile water per the manufacturer’s instructions. A further dilution was performed with HEPES buffer with 2% bovine serum albumin to a final dilution of 1:50,000, as described elsewhere (Wiinberg et al. 2005). In a polypropylene cryotube,\(^a\) 25 \(\mu\)L of the reconstituted TF was added to 400 \(\mu\)L of citrated whole blood. Simultaneously, 1000 \(\mu\)L of citrated whole blood was added to a kaolin vial.\(^t\) Both samples were gently
mixed by inverting each tube 5 times. Then 20 μL of CaCl$_2$ was added to 340 μL of the activator (TF or kaolin) and citrated whole blood mixture, for a total volume of 360 μL per cup. Quality control was performed following recommendations from the manufacturer. The TEG® analyses were run for a maximum of 90 minutes or minimally until R, K, $\alpha$, MA, global clot strength (G), clot lysis index at 30 minutes (CL30%), and clot lysis rate at 30 minutes (LY30%) values were obtained. When possible, clot lysis index at 60 minutes (CL60%), and clot lysis rate at 60 minutes (LY60%) values were also obtained. TEG® results were compared with a reference interval established at the same institution from analysis of 33 healthy dogs (TF-activated, unpublished data) and 40 healthy dogs (kaolin-activated) (Flint et al. 2012).

**Statistical Methods:** A Latin square design with repeated measures over time and a generalized linear mixed-model was employed to analyze the data using proc mixed. The AKAIKE information criterion (AIC) was used to determine an error structure for the auto-regression. The assumptions of the ANOVA were assessed by comprehensive residual analyses. Shapiro-Wilk, Kolmogorov-Smirnov, Cramer-von Mises, and Anderson-Darling tests were conducted to analyze for normal distribution. Residuals were plotted against predicted values and explanatory variables (treatment, fluid, time, dogs) to look for outliers or unequal variance. If residual analyses suggested a need for data transformation, logarithmic transformation was done before data analysis. If comparisons of the factors “treatment” and “time” were significant, differences between dogs receiving 0.9% NaCl or tetrastarch were compared using paired t-tests. The level of significance for the t-tests was corrected using a Tukey’s or Dunnett’s test. Significance was set at $p < 0.019$ for all variables. Correlations between ACT, platelet count, PT, PTT,
FVIII activity, vWFAg, vWF CBA, and D-dimer concentration, and individual TEG® variables were performed via a Pearson correlation for normally distributed data and a Spearman correlation for non-normally distributed data. The authors categorized correlation as follows: $r \geq 0.95$ excellent, $r \geq 0.85$ to 0.94 very good, $r \geq 0.75$ to 0.84 good, $r \geq 0.50$ to 0.74 fair, and $r < 0.50$ poor. Significance was set at $p < 0.05$ for all correlations. Commercially available software was used for the statistical analyses and graph generation.

Results:

All dogs were female (7 intact, 9 spayed) ranging in age from 16-44 months (median 18 months). The median body weight was 8.85 kg (range 7.8-11 kg).

Healthy Dogs (Placebo Phase):

Compared to dogs given a fluid bolus of 0.9% NaCl, dogs given a fluid bolus of tetrastarch had a decreased platelet count ($p = 0.019$, Figure 3.1a), increased PTT ($p < 0.001$, Figure 3.2a) at T1, decreased vWFAg at T1 ($p < 0.001$) and T2 ($p < 0.001$) (Figure 3.3a), and a decreased vWF CBA at T1 ($p = 0.003$) and T2 ($p = 0.003$) (Figure 3.4a). There was no significant difference in ACT, PT, FVIII activity, vWFAg/CBA ratio, or D-dimer concentration between either fluid group (data not shown).

Compared to dogs given 0.9% NaCl, dogs receiving tetrastarch had a decreased R ($p = 0.017$, Figure 3.5a) and increased K ($p < 0.001$, Figure 3.6a) at T1, decreased $\alpha$ between T1 ($p < 0.001$) and T2 ($p = 0.001$) (Figure 3.7a), decreased MA between T1 ($p < 0.001$) and T4 ($p = 0.016$) (Figure 3.8a), decreased G between T1 ($p < 0.001$) and T4 ($p = 0.006$) (Figure 3.9a), increased CL30% between T1 ($p < 0.001$) and T4 ($p = 0.016$)
(Figure 3.10a), increased LY30% at T1 (p < 0.001) (Figure 3.11a), increased CL60% at T2 (p = 0.002) (Figure 3.12a), and increased LY60% between T2 (p = 0.004) and T4 (p = 0.005) (Figure 3.13a).

When kaolin and TF activation protocols were compared, there were no significant differences in any TEG® variables at any time points (data not shown). When comparing kaolin-activated TEG® variables to traditional coagulation tests, vWFAg had fair correlation with MA (r = 0.52; p < 0.001) and G (r = 0.51; p < 0.001). Similarly, when comparing TF-activated TEG® variables to traditional coagulation tests, vWFAg had fair correlation with MA (r = 0.56; p < 0.001) and G (r = 0.55; p < 0.001). Other TEG® variables and traditional coagulation tests were either poorly or not correlated.

Dogs with Systemic Inflammation (LPS Phase):

All dogs demonstrated lethargy and gastrointestinal signs during the first 1-2 hours including hypersalivation and diarrhea within 30 minutes of LPS administration. All dogs administered LPS had a significant leukopenia between 1 (p < 0.001) and 4 hours (p < 0.001) and leukocytosis at 24 hours (p < 0.001) compared to baseline (data not shown).

Compared to dogs given 0.9% NaCl, dogs given tetrastarch had a decreased platelet count at T2 (p = 0.019, Figure 3.1b), increased PTT at T1 (p < 0.001) and T2 (p = 0.016) (Figure 3.2b), decreased vWFAg at T1 (p < 0.001) and T2 (p < 0.001) (Figure 3.3b), and decreased vWFCBA at T1 (p = 0.002) and T2 (p = 0.002) (Figure 3.4b). There was no significant difference in ACT, PT, FVIII, vWFAg/CBA ratio, or D-dimer concentration between dogs in either fluid group (data not shown).
Compared to dogs given 0.9% NaCl, dogs given tetrastarch had increased K (Figure 3.6b) and decreased α (Figure 3.7b), MA (Figure 3.8b), and G (Figure 3.9b) between T1 (p < 0.001) and T4 hours (p < 0.001). Dogs given tetrastarch also had increased CL30% at T1 (p = 0.014) (Figure 3.10b), increased LY30% at T1 (p < 0.001) and T2 (p = 0.005) (Figure 3.11b), increased CL60% at T2 (p = 0.006) and T4 (p = 0.005) (Figure 3.12b), and increased LY60% at T4 hours (p = 0.014) (Figure 3.13b) compared to dogs administered 0.9% NaCl. There was no significant difference in R between dogs in either fluid group (Figure 3.5b).

When kaolin and TF activation protocols were compared, there were no significant differences in any TEG® variables at all time points (data not shown). When comparing kaolin-activated TEG® variables to traditional coagulation tests, vWFAg had fair correlation with K (r = -0.56; p < 0.001), α (r = 0.58; p < 0.001), MA (r = 0.64; p < 0.001), and G (r = 0.64; p < 0.001). vWFCBA had fair correlation with MA (r = 0.51; p < 0.001). Similarly, when comparing TF-activated TEG® variables to traditional coagulation tests, vWFAg had fair correlation with K (r = -0.57; p < 0.001), α (r = 0.58; p < 0.001), MA (r = 0.66; p < 0.001), and G (r = 0.64; p < 0.001). vWFCBA had fair correlation with MA (r = 0.53; p < 0.001) and G (r = 0.51; p < 0.001). Other TEG® variables and traditional coagulation tests were either poorly or not correlated.

Discussion:

The results of the present study revealed that in both healthy dogs and dogs with LPS-induced systemic inflammation, administration of 0.9% NaCl and tetrastarch both caused similar prolongations in ACT and PT. However, administration of tetrastarch to
both healthy dogs and dogs with systemic inflammation was associated with a greater immediate decrease in platelet count and prolongation in PTT compared to 0.9% NaCl. Additionally, both healthy dogs and dogs with systemic inflammation had significantly decreased vWFAg and vWFCBA after tetrastarch administration compared to 0.9% NaCl administration, suggesting acquired type 1 von Willebrand disease. In healthy dogs, tetrastarch administration was associated with both hypo- (increased K, and decreased α, MA and G) and hypercoagulable (decreased R) TEG® variables compared to 0.9% NaCl administration. Conversely, in dogs with systemic inflammation, tetrastarch administration was only associated with hypocoagulability characterized by an increased K, and decreased α, MA and G compared to 0.9% NaCl administration. Hyperfibrinolysis characterized by increases in CL30%, LY30%, CL60%, and LY60% occurred in both healthy dogs and dogs with systemic inflammation given tetrastarch compared to dogs given 0.9% NaCl. Interestingly, there was no significant difference in TEG® variables measured using different activators and TEG® variables were only poorly to fairly correlated with traditional coagulation tests.

The similar prolongations in ACT and PT after administration of 0.9% NaCl and tetrastarch to both healthy dogs and dogs with systemic inflammation suggests similar volume expansion and hemodilution by both fluid products, with no additional effect of the tetrastarch on these traditional coagulation tests. Conversely, compared to a bolus of 0.9% NaCl, a bolus of tetrastarch to healthy dogs and dogs with systemic inflammation was associated with a greater decrease in platelet count and prolongation in PTT. Given that tetrastarch administration has no known direct effects on platelet count (Kozek-Langenecker 2005, Kozek-Langenecker et al. 2007, Westphal et al. 2009) and because
FVIII activity was not different between the two fluid groups, this suggests a greater hemodilution effect with tetrastarch compared to 0.9% NaCl administration. The overall dilutional effect of HES on platelets and clotting factors leading to prolongation of coagulation times has been demonstrated in other in vivo human studies (Eriksen et al. 2009, Ruttmann et al. 1998). However, to date there are no published veterinary studies investigating the effect of HES on traditional coagulation tests. The blood volume expansive effect of isotonic crystalloids and synthetic colloids are not equivalent as the latter results in fluid recruitment from the interstitial compartment, in addition to the administered volume (Silverstein et al. 2005). Given the paucity of literature on the volume expansive effects of tetrastarch in dogs, a dosage that would give equivalent volume expansion with the crystalloid in the present study could not be determined. An equal volume for both 0.9% NaCl and tetrastarch was therefore selected because the immediate intravascular volume expansion would be equivalent, although ultimately increased and more persistent for tetrastarch. Although FVIII activity was not statistically different between the two fluid groups, it was appreciably decreased after tetrastarch administration and increased after 0.9% NaCl administration. Therefore, the prolongation in PTT might be secondary to an effect of tetrastarch on FVIII activity, thereby affecting the intrinsic pathway of coagulation, while the extrinsic pathway measured by PT was unaffected. As ACT also evaluates the intrinsic pathway, the different results obtained compared to PTT are attributed to differences in sensitivity for both assays.

Interestingly, both vWFAg and vWFCBA were also decreased for less than 2 hours after tetrastarch administration compared to 0.9% NaCl administration and then returned to baseline, suggesting transient acquired type 1 von Willebrand disease. This is
consistent with findings from studies in humans that have observed mild acquired von Willebrand disease after administration of rapidly degradable low MW HES such as tetrastarch, with activities returning to normal 5 hours following administration (Kozek-Langenecker et al. 2007). Decreased FVIII activity has been identified in dogs with von Willebrand disease due to the stabilizing role of vWF on circulating FVIII levels (Brinkhous et al. 1985); therefore, one would expect decreased FVIII activity would be seen in conjunction with the decreased vWFAg and vWFCBA. Conversely, two human studies reveal that tetrastarch doses up to 50-70 mL/kg IV were administered without changes in FVIII concentration (Kasper et al. 2003, Neff et al. 2003). Although FVIII activity decreased after tetrastarch administration in the present study, it was not statistically different from 0.9% NaCl administration. However, it is possible that the present study was underpowered to detect significant changes in FVIII activity following tetrastarch administration.

In the present study, TEG® variables were suggestive of increased hypocoagulability following tetrastarch administration compared to 0.9% NaCl in both healthy dogs and dogs with LPS-induced systemic inflammation. This is consistent with findings from in vitro studies in healthy humans and humans undergoing liver transplant surgery that found hypocoagulability characterized by increased R and K, and decreased α and MA after tetrastarch administration (Jamnicki et al. 1998, Bang et al. 2010). Interestingly, in vivo studies in humans reveal that similar hypocoagulable effects are only seen immediately following HES administration, but do not persist after the HES bolus (Felfernig et al. 2003, Jin et al. 2010). This finding is attributed to the more rapid elimination of tetrastarch due to its lower MW and shorter half-life compared to other
HES (Felfernig et al. 2003, Jin et al. 2010). Similarly, all TEG® variables that were altered in dogs in the present study 1-4 hours after administration of tetrastarch, returned to baseline 24 hours after the fluid bolus.

There are some veterinary studies investigating the effects of HES on coagulation in dogs that also confirm the development of hypocoagulability following HES administration. In vitro and in vivo reports demonstrate that higher MW HES are associated with platelet dysfunction characterized by prolonged closure time using PFA-100 (Wieranga et al. 2007, Smart et al. 2009). In vitro dilution with a lower MW product HES 130/0.42 also causes hypocoagulability manifested by prolonged closure time using PFA-100 (Classen et al. 2012). However, a recent study investigating the effect of tetrastarch on coagulation in a canine hemorrhagic shock model found no difference in platelet function as assessed with PFA-100. Another study used TEG® to investigate the effect of HES on coagulation and found that tetrastarch had less impact on coagulation than hetastarch at higher dilutions in vitro, but still resulted in hypocoagulability characterized by changes in K, α, and MA. Similarly, in vitro dilution with tetrastarch caused hypocoagulability secondary to a dose-dependent alteration in fibrinogen concentration and decreased platelet function using thromboelastometry in a recent study (Falco et al. 2012). Therefore, while none of these studies investigate the in vivo effects of tetrastarch on TEG® in dogs, they consistently show that in vitro hemostatic testing confirms a hypocoagulable effect of HES in dogs. Additionally, serial in vitro dilution of whole blood samples obtained from healthy dogs to alter the hematocrit value was associated with hypercoagulable TEG results and hypercoagulable thromboelastometry results (Smith et al. 2012). In the present study, packed cell volume
was decreased by fluid administration, but remained within the reference interval, therefore, a significant effect of RBC mass on the measured TEG variables was unlikely.

The alterations in TEG® variables CL30%, LY30%, CL60% and LY60% observed in the present study in dogs receiving tetrastarch are suggestive of hyperfibrinolysis. This has been previously reported in vivo in humans given higher MW HES solutions and is hypothesized to be secondary to incorporation of HES molecules into the blood clot, or to increased plasminogen activator activity (Strauss et al. 2002). Despite evidence of hyperfibrinolysis, there was no difference in D-dimer concentration in dogs receiving tetrastarch compared to dogs given 0.9% NaCl in the present study. Likewise, a recent case series of dogs with disseminated intravascular coagulation revealed no association between D-dimer concentration and fibrinolytic TEG® variables (Wiinberg et al. 2008). Another phenomenon called clot retraction has been recognized and can resemble fibrinolytic patterns on TEG® and appears to be more prominent with higher platelet counts (Katori et al. 2005). Considering the platelet count decreased after fluid administration in the present study, the observed hyperfibrinolytic variables suggest true hyperfibrinolysis associated with tetrastarch administration. Other assays of fibrinolysis such as fibrin degradation products, fibrinogen, or plasminogen concentrations could be measured in future studies to further assess the effect of tetrastarch on fibrinolysis.

In the present study, unlike the proposed hypothesis, both activation protocols for TEG® generated similar results. Use of activators allows a reduction in intra-assay variability associated with TEG® (Wiinberg et al. 2005). No standard protocol for TEG® activation currently exists in veterinary medicine; however, depending on the activator
used, different results are anticipated. TEG® variables using blood samples activated with TF demonstrate coagulation abnormalities via activation of the extrinsic pathway, whereas measurements using kaolin-activated blood samples reflect abnormalities associated with the intrinsic pathway (Banerjee et al. 2011). Studies in dogs and cats have suggested that the results obtained using different activators are not directly comparable (Banerjee et al. 2011, Marschner et al. 2010, Wiinberg et al. 2010). Interestingly, TF-activated TEG® variables were not significantly different from kaolin-activated TEG® variables in the present study, which might have been because of the non-standard dilution of TF used. Guidelines for TF dilution for TEG® activation are not available; however, most studies use a dilution of 1:2,780 (Wiinberg et al. 2005). Conversely, the present study diluted TF to a final concentration of approximately 1:50,000, which likely reduced the effects of the TF and caused results comparable to those with kaolin activation.

In the present study, traditional coagulation tests (platelet count, ACT, PT, PTT, and D-dimer concentration) had weak correlation with TEG® variables (both TF and kaolin activated). Many studies have investigated the correlation between TEG® variables and traditional coagulation tests (Kol et al. 2010, Wiinberg et al. 2009, Zuckerman et al. 1981). R is typically associated with soluble clotting factors, thus representing the intrinsic pathway of coagulation (Zuckerman et al. 1981). Some studies have reported good correlation between R and PT or PTT (Alexander et al. 2010, Martini et al. 2008, Wagg et al. 2009). In the present study, administration of tetrastarch was associated with prolongation of PTT, but decreased R in healthy dogs, and no change in R in dogs with LPS-induced systemic inflammation. Similar findings were observed in a
study comparing TEG® variables in human volunteers administered either HES (200/0.5) or normal saline (Ruttmann et al. 1998). The authors hypothesized that difference in methodology between TEG® and PT or PTT explained the differences observed, since TEG® evaluates whole blood coagulation, whereas PT and PTT are platelet-poor plasma based assays (Ruttmann et al. 1998). The slightly prolonged PT and PTT following administration of HES (200/0.5) were attributed to dilution of coagulation factors in plasma, while decreases of R and K was explained by platelet activation or other cell-mediated effects not assessed by cell-depleted plasma assays (Ruttmann et al. 1998). In the present study, platelet count was poorly correlated with MA, G, and α in healthy dogs. However, vWF-Ag and vWFCBA had poor to fair correlation with hypocoagulable TEG® profiles (increased K; smaller α, MA, and G) in healthy dogs and dogs with LPS-induced systemic inflammation. K and α are generally affected by platelet count and function, fibrinogen concentration and function, and clotting factor activity, whereas MA and G are influenced by platelet count and function, and fibrinogen concentration (Katori et al. 2005). The influence of vWF disease on TEG® has not been investigated.

The present study has some limitations that warrant discussion. First, the small sample size rendered some of the analyses underpowered to detect significant differences. Likewise, some TEG® variables had significant differences at baseline between dogs receiving 0.9% NaCl and those given tetrastarch. However, the subsequent significant changes at each time point in dogs given tetrastarch were in the opposite direction compared to dogs given 0.9% NaCl and at 24 h after fluid administration there were no longer significant differences between the two fluids. All dogs were deemed healthy based on physical examination, routine blood work, and PT and PTT, but it is possible
that subclinical diseases were missed that affected our results. More likely, type I error
due to the small sample size created these differences at baseline. Second, the effect of
tetrastarch on platelet function might have been underestimated in the present study.

TEG® was previously found to be insensitive compared to flow cytometry and
aggregometry at assessing platelet function in a human study (Bowbrick et al. 2003).

Other techniques used to evaluate platelet function including platelet aggregometry, PFA-
100, flow cytometry, and TEG® platelet mapping (Jandrey 2012) should be considered in
future studies investigating the coagulation effects of HES in dogs. Third, all dogs
enrolled in this study were female and almost evenly distributed between intact and
spayed. Influence of gender on viscoelastic evaluation of hemostasis has been
demonstrated in people resulting in recommendations to report different reference
intervals for each sex (Sucker et al. 2011, Theusinger et al. 2010); however, no such
difference has been found in dogs (Armstrong et al. 2011). Similarly, pregnant women
have a more hypercoagulable profile and unique reference intervals have also been
established for that population (Bauer et al. 2009, Polak et al. 2011). To date, the
influence of reproductive hormones on TEG® in intact dogs has not been investigated.

Fourth, the washout period was selected based on the plasma terminal half life \(t_{1/2B}\) of
tetrastarch in humans, which is 12.1 hours following administration of a single dose of
500 mL (Waitzinger et al. 1998). Such information is not available for dogs and the
presence of persistent circulating tetrastarch molecules for longer than the 14-day
washout period cannot not be excluded. Fifth, it is also important to consider that while
an LPS model was used in the present study to mimic the systemic changes encountered
during naturally occurring sepsis (Aksu et al. 2012, Baum et al. 1990, Hoffmann et al.
2002, Schäper et al. 2008), it does not necessarily replicate all changes that occur in hospitalized dogs. Finally, the rapid infusion of 40 mL/kg of tetrastarch IV over 30 minutes does not necessarily reflect clinical practice. As such, the results from the present study cannot necessarily be extrapolated to all hospitalized dogs with systemic inflammation or naturally occurring sepsis receiving synthetic colloids.

Conclusions:

Healthy dogs and dogs with LPS-induced systemic inflammation administered tetrastarch had prolonged PTT, hypocoagulable and hyperfibrinolytic TEG® variables, and acquired type 1 von Willebrand disease compared to dogs given 0.9% NaCl, which resolved within 4 hours. Most coagulation variables were within the reference interval, which makes the hypocoagulable changes associated with tetrastarch administration of uncertain clinical significance and precludes any absolute contraindication to the use of tetrastarch in healthy dogs or dogs with systemic inflammation. Additionally, when using a recently published dilution protocol for TF, no significant difference in TF-activated TEG® variables was observed compared to kaolin-activated TEG® results. Future studies investigating the effect of other doses or constant rate infusions of tetrastarch on coagulation, as well as in dogs with naturally occurring sepsis, are warranted and more sensitive methods for assessing platelet dysfunction should be included.

Footnotes:

a. 0.9% NaCl, Baxter, Mississauga, ON, Canada.

b. Voluven, Fresenius Kabi, Mississauga, ON, Canada.

c. Escherichia coli serotype 0127:B8, Sigma-Aldrich, St. Louis, MO.
d. Torbugesic, Fort Dodge Animal Health, NY.

e. BD Insyte Autoguard catheter, BD Medical, Sandy, UT.

f. Max-ACT test tubes, Helena Laboratories, Beaumont, TX.

g. Capiject EDTA micro collection tubes, Terumo, Somerset, NJ.

h. BD Vacutainer Plus plastic citrate, Becton, Dickinson, and Company, Franklin Lakes, NJ.

i. Advia 2120, Siemens Medical Solutions Diagnostics, Tarrytown, NY.

j. ACL Elite, Instrumentation Laboratory, Bedford, MA.

k. RecombiPlasTin 2G, Instrumentation Laboratory, Bedford, MA.

l. SynthASil, Instrumentation Laboratory, Bedford, MA.

m. ST4, Diagnostica Stago Inc., Parsippany, NJ.


o. HemosIL D-dimer, Instrumentation Laboratory, Bedford, MA.

p. Kaolin, Haemoscope Corporation, Niles, IL.

q. Innovin, Siemens Healthcare Diagnostics Inc., Newark, DE.

r. TEG®, Haemoscope Corporation, Niles, IL.

s. Cryogenic vial, Corning Inc. Corning, NY.

l. Kaolin, Haemoscope Corporation, Niles, IL.

u. Calcium chloride, Haemoscope Corporation, Niles, IL.

v. Plain cups and pins, Haemoscope Corporation, Niles, IL.

w. SAS Online v.9.1.3, SAS Institute, Cary, NC

x. Prism 5, GraphPad Software, La Jolla, CA.


References:


36. Martini WZ, Cortez DS, Dubick MA, et al. Thromboelastography is better than PT, aPTT, and activated clotting time in detecting relevant clotting abnormalities


60. Wiinberg B, Kristensen AT. Thromboelastography in veterinary medicine. Semin

Figure 3.1 – Comparison of platelet count in 16 dogs receiving 0.9% NaCl or tetrastarch after placebo or LPS administration. a) Placebo b) LPS. Time of administration of 0.9% NaCl or tetrastarch administration represented as ↑. Significant differences between fluids (p < 0.019) are indicated with *. All values are expressed as mean ± SD. Reference interval: 117-418 x10^9/L.
Figure 3.2 – Comparison of PTT in 16 dogs receiving 0.9% NaCl or tetrastarch after placebo or LPS administration. a) Placebo b) LPS. Time of administration of 0.9% NaCl or tetrastarch administration represented as ↑. Significant differences between fluids (p < 0.019) are indicated with *. All values are expressed as mean ± SD. Reference interval: 10-20 seconds.
Figure 3.3 – Comparison of kaolin-activated vWFAg in 16 dogs receiving 0.9% NaCl or tetrastarch after placebo or LPS administration. a) Placebo b) LPS. Time of administration of 0.9% NaCl or tetrastarch administration represented as ↑. Significant differences between fluids (p < 0.019) are indicated with *.

All values are expressed as mean ± SD. Reference interval: 50-108%.
Figure 3.4 – Comparison of kaolin-activated vWFcBA in 16 dogs receiving 0.9% NaCl or tetrastarch after placebo or LPS administration. a) Placebo b) LPS. Time of administration of 0.9% NaCl or tetrastarch administration represented as ↑. Significant differences between fluids (p < 0.019) are indicated with *. All values are expressed as mean ± SD. Reference interval: > 50%.
Figure 3.5 – Comparison of kaolin-activated R in 16 dogs receiving 0.9% NaCl or tetrastarch after placebo or LPS administration. a) Placebo b) LPS. Time of administration of 0.9% NaCl or tetrastarch administration represented as ↑. Significant differences between fluids (p < 0.019) are indicated with *. All values are expressed as mean ± SD. Reference interval: 2-6 minutes.
Figure 3.6 – Comparison of kaolin-activated K in 16 dogs receiving 0.9% NaCl or tetrastarch after placebo or LPS administration. a) Placebo b) LPS. Time of administration of 0.9% NaCl or tetrastarch administration represented as ↑. Significant differences between fluids (p < 0.019) are indicated with *. All values are expressed as mean ± SD. Reference interval: 1-3 minutes.
Figure 3.7 – Comparison of kaolin-activated $\alpha$ in 16 dogs receiving 0.9% NaCl or tetrastarch after placebo or LPS administration. a) Placebo b) LPS. Time of administration of 0.9% NaCl or tetrastarch administration represented as ↑. Significant differences between fluids ($p < 0.019$) are indicated with *. All values are expressed as mean ± SD. Reference interval: 48-75.
Figure 3.8 – Comparison of kaolin-activated MA in 16 dogs receiving 0.9% NaCl or tetrastarch after placebo or LPS administration. a) Placebo b) LPS. Time of administration of 0.9% NaCl or tetrastarch administration represented as ↑. Significant differences between fluids (p < 0.019) are indicated with *. All values are expressed as mean ± SD. Reference interval: 46-64 mm.
Figure 3.9 – Comparison of kaolin-activated G in 16 dogs receiving 0.9% NaCl or tetrastarch after placebo or LPS administration. a) Placebo b) LPS. Time of administration of 0.9% NaCl or tetrastarch administration represented as ↑. Significant differences between fluids (p < 0.019) are indicated with *. All values are expressed as mean ± SD. Reference interval: 4.2K-8.0K d/sc.
Figure 3.10 – Comparison of kaolin-activated CL30% in 16 dogs receiving 0.9% NaCl or tetrastarch after placebo or LPS administration. a) Placebo b) LPS. Time of administration of 0.9% NaCl or tetrastarch administration represented as ↑. Significant differences between fluids (p < 0.019) are indicated with *. All values are expressed as mean ± SD. Reference interval: 92-100%.
Figure 3.11 – Comparison of kaolin-activated LY30% in 16 dogs receiving 0.9% NaCl or tetrastarch after placebo or LPS administration. a) Placebo b) LPS. Time of administration of 0.9% NaCl or tetrastarch administration represented as ↑. Significant differences between fluids (p < 0.019) indicated with *. All values are expressed as mean ± SD. Reference interval: 0-2%.
Figure 3.12 – Comparison of kaolin-activated CL60% in 16 dogs receiving 0.9% NaCl or tetrastarch after placebo or LPS administration. a) Placebo b) LPS. Time of administration of 0.9% NaCl or tetrastarch administration represented as ↑. Significant differences between fluids (p < 0.019) are indicated with *. All values are expressed as mean ± SD. Reference interval: 85-100%.
Figure 3.13 – Comparison of kaolin-activated LY60% in 16 dogs receiving 0.9% NaCl or tetrastarch after placebo or LPS administration. a) Placebo b) LPS. Time of administration of 0.9% NaCl or tetrastarch administration represented as ↑. Significant differences between fluids (p < 0.019) indicated with *. All values are expressed as mean ± SD. Reference interval: 0-15%.
CHAPTER 4
SUMMARY AND CONCLUSIONS

The effects of tetrastarch administration on hemodynamic, laboratory, and hemostatic variables in healthy dogs and dogs with systemic inflammation have been investigated in this thesis. Additionally, two different protocols for TEG® activation were compared and the correlation between TEG® variables and traditional coagulation test results was investigated.

First, the effects of tetrastarch administration on hemodynamic and laboratory variables in healthy dogs and dogs with systemic inflammation were compared to those after 0.9% NaCl administration. It was hypothesized that an IV bolus of tetrastarch would cause more measurable effects on vital parameters and COP compared to 0.9% NaCl in both healthy dogs and dogs with systemic inflammation. This hypothesis was validated as a 40 mL/kg IV bolus of tetrastarch caused higher increases in HR and blood pressure compared to 0.9% NaCl administration in healthy dogs; however, the effects on hemodynamic parameters were transient and lasted less than 2 hours. In dogs with LPS-induced systemic inflammation, resuscitation with tetrastarch caused a greater immediate increase in HR and blood pressure with similar changes in other hemodynamic and laboratory variables. Overall, tetrastarch increased COP in both healthy dogs and dogs with systemic inflammation; however, measurement of TS did not coincide with the change in COP. An LPS model was used in this study to mimic the systemic changes encountered during naturally occurring sepsis. Dogs did develop clinical and laboratory manifestations of systemic inflammation, but did not show evidence of circulatory collapse. The LPS model does not replicate all changes associated with naturally
occurring sepsis. As such, results from the present study cannot necessarily be extrapolated to all dogs with systemic inflammation or naturally occurring sepsis. Considering the demonstrated adverse hemostatic effects in the second study, the reported renal effects of tetrastarch found in human studies, and the limited additional benefit of tetrastarch administration on hemodynamic variables demonstrated in the present study, the increased cost associated with the use of tetrastarch likely negates its use as a first line treatment during fluid resuscitation in dogs. However, further evaluation in clinical patients with more pronounced hemodynamic changes may be warranted.

Second, the effect of tetrastarch administration on coagulation in healthy dogs and dogs with systemic inflammation was compared with that of 0.9% NaCl administration. Secondary objectives were to compare two different protocols for TEG® activation and to determine the correlation between TEG® variables and traditional coagulation test results. It was hypothesized that an IV bolus of tetrastarch would produce a hypocoagulable TEG® profile compared to 0.9% NaCl administration in both healthy dogs and dogs with systemic inflammation. It was further hypothesized that blood samples activated with TF would produce a more hypercoagulable TEG® profile when compared to blood samples activated with kaolin. Finally, the authors hypothesized that TEG® variables would be correlated with traditional coagulation test results. The first hypothesis was accepted in dogs with systemic inflammation as tetrastarch administration produced an increased K, and decreased α, MA and G compared to 0.9% NaCl administration. Conversely, in healthy dogs, tetrastarch administration produced both hypo- (increased K, and decreased α, MA and G) and hypercoagulable (decreased R)
TEG® variables compared to 0.9% NaCl administration. This was consistent with two recent *in vitro* studies that found hypocoagulability associated with tetrastarch dilution using TEG® and thromboelastometry. Hypercoagulability as evidence by decreased R was identified similarly in a study comparing administration HES (200/0.5) with that of 0.9% NaCl in healthy humans. The authors suggested the hypercoagulability was a result of platelet activation by HES molecules. In the present study, both healthy dogs and dogs with systemic inflammation also had significantly decreased vWFAg and vWFCBA for less than two hours after tetrastarch administration compared to 0.9% NaCl administration, suggesting a transient acquired type 1 von Willebrand disease. This is consistent with findings from studies in humans that have observed mild acquired von Willebrand disease after administration of rapidly degradable low MW HES such as tetrastarch, with vWF activities returning to normal 5 hours after administration. Although FVIII activity decreased after tetrastarch administration in the present study, it was not statistically different from 0.9% NaCl administration. However, it is possible that the study was underpowered to detect significant changes in FVIII activity following tetrastarch administration. Both groups in the study also had evidence of hyperfibrinolysis characterized by increases in CL30%, LY30%, CL60%, and LY60%. Similarly, this has been previously reported *in vivo* in humans given higher MW HES solutions.

The second and third hypotheses regarding TEG® activation and correlations of TEG® variables with traditional coagulation tests, respectively, were rejected, as there was no significant difference in TEG® variables measured using different activators and TEG® variables were only poorly to fairly correlated with traditional coagulation tests.
No standard protocol for TEG® activation currently exists in veterinary medicine; however, depending on the activator used, different results are anticipated. TF was diluted to a final non-standard concentration of 1:50,000, which likely reduced the effects of the TF and caused results comparable to those seen with kaolin activation. This TF concentration was chosen based on recently published studies that intended to produce less robust activation of the TF activated TEG® compared to the traditional protocols.

Limitations of the study included the small sample size, which rendered some of the analyses underpowered to detect significant differences. Likewise, some TEG® variables had significant differences at baseline between dogs receiving 0.9% NaCl and those given tetrastarch. However, the subsequent significant changes at each time point in dogs given tetrastarch were in the opposite direction compared to dogs given 0.9% NaCl and at 24 h after fluid administration there were no longer significant differences between the two fluid groups. Also, considering the insensitivity of TEG® in assessing platelet function, other techniques used to evaluate platelet function including platelet aggregometry, PFA-100, flow cytometry, and TEG® platelet mapping should be considered in future studies.

In conclusion, resuscitation with equal volumes of 0.9% NaCl and tetrastarch caused similar changes in hemodynamic and laboratory variables in dogs with systemic inflammation. However, larger increases in HR and blood pressure were seen within the first 2 hours following tetrastarch administration and tetrastarch administration increased COP in all dogs. In addition, tetrastarch bolus administration to dogs with systemic inflammation resulted in a transient hypocoagulability characterized by a prolonged PTT, decreased clot formation speed and clot strength, and acquired type 1 von Willebrand
disease. Considering the demonstrated adverse hemostatic effects of tetrastarch administration and the limited additional benefit of tetrastarch administration on hemodynamic variables demonstrated, the increased cost associated with the use of tetrastarch likely negates its use as a first line treatment during fluid resuscitation in dogs. However, the observed positive effect on COP and the transient increase in blood pressure following tetrastarch administration suggest that it might be useful as an adjunctive treatment to crystalloid therapy in patients with low oncotic pressure or refractory shock. Future studies investigating the effect of other doses or constant rate infusions of tetrastarch on hemodynamic, laboratory, and hemostatic variables in dogs with naturally occurring sepsis are warranted. Finally, more sensitive methods for assessing platelet dysfunction should be included in future studies.
CHAPTER 5: APPENDICES

CHAPTER 3: Effect of synthetic colloid administration on coagulation in healthy dogs and dogs with systemic inflammation

Figure 3.14* - Comparison of ACT in 16 dogs receiving 0.9% NaCl or tetrastarch after placebo or LPS administration. a) Placebo b) LPS. Time of administration of 0.9% NaCl or tetrastarch administration represented as ↑. Significant differences between fluids (p < 0.019) are indicated with *. All values are expressed as mean ± SD.
Figure 3.15* - Comparison of PT in 16 dogs receiving 0.9% NaCl or tetrastarch after placebo or LPS administration. a) Placebo b) LPS. Time of administration of 0.9% NaCl or tetrastarch administration represented as ↑. Significant differences between fluids (p < 0.019) are indicated with *. All values are expressed as mean ± SD.
Figure 3.16* - Comparison of FVIII in 16 dogs receiving 0.9% NaCl or tetrastarch after placebo or LPS administration. a) Placebo b) LPS. Time of administration of 0.9% NaCl or tetrastarch administration represented as ↑. Significant differences between fluids (p < 0.019) are indicated with *. All values are expressed as mean ± SD.
Figure 3.17* - Comparison of D-dimer concentration in 16 dogs receiving 0.9% NaCl or tetrastarch after placebo or LPS administration. a) Placebo b) LPS. Time of administration of 0.9% NaCl or tetrastarch administration represented as ↑. Significant differences between fluids (p < 0.019) are indicated with *. All values are expressed as mean ± SD.
Figure 3.18* - Comparison of WBC in 16 dogs receiving 0.9% NaCl or tetrastarch after LPS administration. a) Placebo b) LPS. Time of administration of 0.9% NaCl or tetrastarch administration represented as ↑. Significant differences between fluids (p < 0.019) are indicated with *. All values are expressed as mean ± SD.
Table 3.1* - Placebo phase. Comparison of TEG variables using kaolin or tissue factor in 16 dogs receiving NaCl 0.9% or tetrastarch after placebo and LPS administration. Significant differences (p < 0.019) indicated in bold. All values are expressed as mean ± SD.
<table>
<thead>
<tr>
<th>Time (h)</th>
<th>R (min)</th>
<th>K (min)</th>
<th>α (°)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>NaCl 0.9%</td>
<td>Tetrastarch</td>
<td>NaCl 0.9%</td>
</tr>
<tr>
<td></td>
<td>Kaolin</td>
<td>TF</td>
<td>Kaolin</td>
</tr>
<tr>
<td>0-2</td>
<td>4.5 ± 1.1</td>
<td>4.7 ± 0.7</td>
<td>1.6 ± 0.3</td>
</tr>
<tr>
<td>1-3</td>
<td>3.4 ± 0.8</td>
<td>3.4 ± 0.8</td>
<td>1.4 ± 0.3</td>
</tr>
<tr>
<td>2-4</td>
<td>3.8 ± 1.1</td>
<td>3.2 ± 0.9</td>
<td>1.6 ± 0.3</td>
</tr>
<tr>
<td>4-24</td>
<td>4.3 ± 0.9</td>
<td>4.0 ± 0.8</td>
<td>1.6 ± 0.2</td>
</tr>
</tbody>
</table>

| Time (h) | MA (mm) | G (d/sec) | CL30% (%) | LY30% (%) | |
|---------|---------|----------|------------|----------|
|         | NaCl 0.9% | Tetrastarch | NaCl 0.9% | Tetrastarch | NaCl 0.9% | Tetrastarch |
|         | Kaolin | TF | Kaolin | TF | Kaolin | TF | Kaolin | TF | Kaolin | TF |
| 0       | 61.9 ± 5.8 | 52.1 ± 2.5 | 3.5 ± 0.9 | 8.4 ± 2.0 | 5.6 ± 1.5 | 6.6 ± 1.2 | 93.7 ± 14.0 | 94.1 ± 12.3 | 84.5 ± 23.0 | 88.6 ± 9.6 |
| 1       | 58.1 ± 6.8 | 41.7 ± 2.9 | 7.2 ± 2.1 | 5.6 ± 1.8 | 3.7 ± 0.4 | 4.0 ± 0.6 | 91.6 ± 10.8 | 87.5 ± 16.0 | 68.0 ± 30.1 | 68.4 ± 17.6 |
| 2       | 58.2 ± 7.8 | 46.9 ± 3.7 | 7.3 ± 2.2 | 6.8 ± 2.3 | 4.7 ± 0.9 | 5.0 ± 2.5 | 91.7 ± 12.1 | 70.0 ± 28.0 | 67.0 ± 17.5 | 82.0 ± 17.6 |
| 4       | 60.4 ± 5.1 | 49.7 ± 3.6 | 7.8 ± 1.8 | 7.1 ± 2.0 | 5.2 ± 0.6 | 5.0 ± 0.7 | 93.2 ± 12.6 | 93.6 ± 31.6 | 79.3 ± 44.7 | 84.7 ± 12.1 |
| 24      | 71.5 ± 2.4 | 68.7 ± 2.6 | 12.6 ± 1.5 | 12.6 ± 1.2 | 11.1 ± 2.0 | 11.2 ± 2.0 | 99.7 ± 0.7 | 99.8 ± 0.6 | 99.5 ± 0.8 | 99.5 ± 0.7 |

Table 3.2* - LPS phase. Comparison of TEG variables using kaolin or tissue factor in 16 dogs receiving NaCl 0.9% or tetrastarch after placebo and LPS administration. Significant differences (p < 0.019) indicated in bold. All values are expressed as mean ± SD.