CARDIOVASCULAR AND HEMATOLOGICAL EFFECTS OF HETASTARCH AND HYPERTONIC SALINE SOLUTIONS DURING EXPERIMENTAL ENDOTOXEMIA IN HORSES

By

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Chairman: Martin O. Furr
Veterinary Medical Sciences

(ABSTRACT)

Justification: Endotoxemia and sepsis are major causes of mortality in horses, resulting in significant economic losses for the equine industry.

Objective: To determine the effects of the combination of Hypertonic Saline Solution and Hetastarch in endotoxemic horses.

Animals: Eighteen horses divided into three groups of six.

Procedure: All horses received a total dose of intravenous E. coli endotoxin infused at 50 ug/kg; divided into a bolus infusion of 20 ug/kg followed by 30 ug/kg given over 30 minutes. After induction of endotoxic shock; group I (control) received a bolus (15 ml/kg) of isotonic solution, group II (isotonic solution) received a bolus (60 ml/kg) of
balanced polyionic crystalloid solution and group III (Hypertonic saline plus Hetastarch) received a bolus of 5 ml/kg of hypertonic saline, followed by a bolus of 10 ml/kg of Hetastarch. Hemodynamic and hematological parameters were measure at different time points.

**Results:** Hemodynamic, biochemical and hematological differences were observed among the three groups.

**Conclusions and Relevance:** the use of large volume crystalloid fluid resuscitation causes volume overload, exerting deleterious effects on the cardiovascular and pulmonary systems. The use of small volume resuscitation (HSS-HES) showed a trend towards better cardiovascular and pulmonary function, without the deleterious effects of volume overload. Abnormalities with regard to coagulation were not seen for the time period of the experimental protocol and the dose regimen used for HSS-HES. Small volume resuscitation in critically ill horses shows promise for it’s beneficial effects in cardiovascular and pulmonary functions.
Dedication

Dedicated with thanks to my family for their encouragement through this period in my life. And to Ruth for her patience, love and unconditional support.
Acknowledgements

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Ms Ruth Thomson for the tables and graphs design.

Mrs Elaine Meilahn for the assistance with the statistical analysis.
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<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Full Form</th>
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<tbody>
<tr>
<td>ADP</td>
<td>adenosine diphosphate</td>
</tr>
<tr>
<td>AG-II</td>
<td>angiotenin II</td>
</tr>
<tr>
<td>ANP</td>
<td>atrial natriuretic peptide</td>
</tr>
<tr>
<td>aPC</td>
<td>activated protein C</td>
</tr>
<tr>
<td>ANOVA</td>
<td>analysis of variance</td>
</tr>
<tr>
<td>aPTT</td>
<td>activated partial thromboplastin time</td>
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<tr>
<td>ARDS</td>
<td>acute respiratory distress syndrome</td>
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<tr>
<td>ATP</td>
<td>adenosine triphosphate</td>
</tr>
<tr>
<td>cAMP</td>
<td>cyclic adenosine monophosphate</td>
</tr>
<tr>
<td>CD</td>
<td>cluster of differentiation</td>
</tr>
<tr>
<td>cGMP</td>
<td>cyclic Guanosine Monophosphate</td>
</tr>
<tr>
<td>CI</td>
<td>cardiac index</td>
</tr>
<tr>
<td>CO</td>
<td>cardiac output</td>
</tr>
<tr>
<td>COX</td>
<td>cyclooxgenase</td>
</tr>
<tr>
<td>CVP</td>
<td>central venous pressure</td>
</tr>
<tr>
<td>DIC</td>
<td>disseminated intravascular coagulation</td>
</tr>
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<td>DMSO</td>
<td>dimethyl sulfoxide</td>
</tr>
<tr>
<td>EDVI</td>
<td>end diastolic volume index</td>
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<tr>
<td>ELAM-1</td>
<td>endothelial leukocyte adhesion molecule 1</td>
</tr>
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<td>ERO₂</td>
<td>oxygen extraction ratio</td>
</tr>
<tr>
<td>ESVI</td>
<td>end systolic volume index</td>
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<tr>
<td>Acronym</td>
<td>Description</td>
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<tr>
<td>-----------</td>
<td>---------------------------------------</td>
</tr>
<tr>
<td>ET-1</td>
<td>endothelin-1</td>
</tr>
<tr>
<td>FDPs</td>
<td>fibrin degradation products</td>
</tr>
<tr>
<td>HES</td>
<td>hydroxyethyl starch solutions</td>
</tr>
<tr>
<td>ICAM-1</td>
<td>intercellular adhesion molecule-1</td>
</tr>
<tr>
<td>IL</td>
<td>Interleukin</td>
</tr>
<tr>
<td>iNOS</td>
<td>inducible nitric oxide synthase</td>
</tr>
<tr>
<td>LBP</td>
<td>lipopolysaccharide binding protein</td>
</tr>
<tr>
<td>LPS</td>
<td>lipopolysaccharide</td>
</tr>
<tr>
<td>LVEDVI</td>
<td>left ventricular end diastolic volume index</td>
</tr>
<tr>
<td>LVEF</td>
<td>left ventricular ejection fraction</td>
</tr>
<tr>
<td>LVSVI</td>
<td>left ventricular stroke volume index</td>
</tr>
<tr>
<td>LVSWI</td>
<td>left ventricular stroke work index</td>
</tr>
<tr>
<td>MODS</td>
<td>multiple organ dysfunction syndrome</td>
</tr>
<tr>
<td>MOF</td>
<td>multiple organ failure</td>
</tr>
<tr>
<td>MW</td>
<td>molecular weight</td>
</tr>
<tr>
<td>NF-κB</td>
<td>nuclear factor B</td>
</tr>
<tr>
<td>NO</td>
<td>nitric oxide</td>
</tr>
<tr>
<td>NOS</td>
<td>nitric oxide synthase</td>
</tr>
<tr>
<td>NSAIDs</td>
<td>non-steroidal anti-inflammatory drugs</td>
</tr>
<tr>
<td>PAF</td>
<td>platelet activator factor</td>
</tr>
<tr>
<td>PAI-1</td>
<td>plasminogen activator inhibitor type-1</td>
</tr>
<tr>
<td>PAP</td>
<td>pulmonary arterial pressure</td>
</tr>
<tr>
<td>Acronym</td>
<td>Description</td>
</tr>
<tr>
<td>-------------</td>
<td>------------------------------------------------------</td>
</tr>
<tr>
<td>PARP-1</td>
<td>poly (ADP-ribose) polymerase family, member 1</td>
</tr>
<tr>
<td>PDH</td>
<td>pyruvate dehydrogenase enzyme complex</td>
</tr>
<tr>
<td>PECAM</td>
<td>platelet endothelial adhesion molecule</td>
</tr>
<tr>
<td>PG</td>
<td>prostaglandin</td>
</tr>
<tr>
<td>PLA-2</td>
<td>phospholipase A₂</td>
</tr>
<tr>
<td>PO₂</td>
<td>partial pressure of oxygen</td>
</tr>
<tr>
<td>PT</td>
<td>prothrombin time</td>
</tr>
<tr>
<td>PTH</td>
<td>parathyroid hormone</td>
</tr>
<tr>
<td>RVEF</td>
<td>right ventricular ejection fraction</td>
</tr>
<tr>
<td>SIRS</td>
<td>systemic inflammation response syndrome</td>
</tr>
<tr>
<td>SVI</td>
<td>stroke volume index</td>
</tr>
<tr>
<td>SVR</td>
<td>systemic vascular resistance</td>
</tr>
<tr>
<td>TGFβ</td>
<td>transforming growth factor beta</td>
</tr>
<tr>
<td>TLR4</td>
<td>toll like receptor four</td>
</tr>
<tr>
<td>TNF</td>
<td>tumor necrosis factor</td>
</tr>
<tr>
<td>TNFα</td>
<td>tumor necrosis factor alpha</td>
</tr>
<tr>
<td>t-PA</td>
<td>tissue plasminogen activator</td>
</tr>
<tr>
<td>TxA₂</td>
<td>thromboxane A₂</td>
</tr>
<tr>
<td>TxB₂</td>
<td>thromboxane B₂</td>
</tr>
<tr>
<td>VCAM-1</td>
<td>vascular cell adhesion molecule-1</td>
</tr>
<tr>
<td>VO₂</td>
<td>oxygen consumption</td>
</tr>
<tr>
<td>vWf</td>
<td>von Willebrand factor</td>
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Review of Literature

Human sepsis and septic shock are two of the major causes of mortality and morbidity in the developing world.\textsuperscript{1,2} Within the human population severe sepsis is very common, consumes considerable health care resources and is associated with high mortality.\textsuperscript{3} The majority of septic human patients die of refractory hypotension and cardiovascular collapse.\textsuperscript{4} Despite major advances in the understanding of sepsis, the mortality in human medicine has changed little in the past twenty years.\textsuperscript{1,5} In human sepsis the overall mortality is approximately 30\%, rising to 40\% in elderly and it is 50\% or greater in patients with septic shock.\textsuperscript{3,6} However, a great deal of variability exists among studies that describe the epidemiology of sepsis, in regard to outcomes assessed, populations studied and treatments applied.\textsuperscript{7} Gram negative bacteria accounts for 60\% of the human sepsis cases.\textsuperscript{6}

Common causes of endotoxemia in the horse include neonatal gram negative sepsis, bacterial pneumonia and pleuropneumonia, endometritis, peritonitis, strangulating intestinal obstruction and infectious colitis.\textsuperscript{8-13} Endotoxemia is an important problem within the horse population due to the high frequency of complications and poor prognosis.\textsuperscript{12,14} In equine medicine, no epidemiologic data pertaining to the incidence and mortality associated with severe sepsis per se exists.\textsuperscript{15} Colic is the leading cause of death in adult horses and bacterial septicemia is the major killer in foals younger than seven days of age.\textsuperscript{9,11,14-17} Twenty five to 45\% of horses with gastrointestinal disease presented
to referral institutions, and 40 % to 50 % of foals that are presented with suspected septicemia, are endotoxemic. In adult horses sepsis is more commonly found during gastrointestinal disturbances (colic, proximal enteritis or colitis) and is probably most often caused by endotoxemia, rather than true bacterial infection. The mortality rate of horses with strangulating colic is high; absorption of endotoxin or lipopolysaccharide (LPS) from the gut is believed to be responsible for many of the associated hemodynamic and pathologic changes. Despite these unfavorable characteristics, endotoxemia is not uniformly fatal, and horses can recover if early and aggressive therapy is instituted.

**Definitions**

*Endotoxemia:* is the presence of endotoxin in the blood stream. The term is used clinically to define the uncontrolled inflammatory reaction caused by endotoxin.

*Systemic inflammatory response syndrome (SIRS):* is the cascade of inflammatory mediators and their sequella in response to a variety of severe clinical insults, independent of the cause. These insults can be infectious (sepsis) or non infectious in origin.

*Sepsis:* a form of systemic inflammatory response triggered by infectious (Gram negative or Gram positive bacteria or fungi) disorders. Sepsis is a clinical syndrome that results from the systemic response of the body to infection and is characterized and modulated by various proinflammatory and antiinflammatory pathways.
Severe sepsis: is sepsis associated with organ dysfunction, hypoperfusion abnormality or sepsis induced hypotension. Hypoperfusion abnormalities include lactic acidosis, oliguria or altered mental status.\textsuperscript{20}

Septic shock: is a subset of severe sepsis. Defined as sepsis induced hypotension, persisting despite adequate fluid resuscitation, along with the presence of hypoperfusion or organ dysfunction.\textsuperscript{20,21}

Multiple organ dysfunction syndrome (MODS): is the presence of altered organ function in an acutely ill patient such that homeostasis cannot be maintained without intervention.\textsuperscript{20}

Sepsis is the systemic inflammatory response to infection; therefore proliferation of microorganisms at a nidus of infection initiates the pathogenic cascade of sepsis.\textsuperscript{4,21} The organisms may invade the bloodstream or proliferate locally and release various substances to the circulation.\textsuperscript{21} Substances released by bacteria can be a structural part of the microorganism (endotoxin) or an exotoxin that is synthesized and released by the microorganism.\textsuperscript{2,4,21} After initial exposure to an infectious stimulus the inflammatory cascade is initiated, with the release of local and systemic inflammatory mediators.\textsuperscript{1,4}

Several studies have demonstrated that gram negative bacteria (containing endotoxin), gram positive bacteria, fungi or protozoan organisms are capable of triggering a common pathway that results is similar cardiovascular abnormalities that characterized septic syndrome.\textsuperscript{21,22,23,24} Whether infection is necessary to initiate sepsis has been subject of debate.\textsuperscript{25} Some investigators propose that infection is central in the role of sepsis and its progression to SIRS and MODS.\textsuperscript{25} They propose that the gut
bacterial reservoir acts as the “motor” that initiates the sepsis cascade. However, bacteria might be sufficient to initiate the syndrome but unnecessary to sustain it.

**Endotoxin structure**

LPS is a structural component of the outer wall of Gram negative bacteria and is primarily composed of lipids and polysaccharides. LPS is very important for the well being of the bacteria and aids in the bacterial resistance to host defense mechanisms. LPS is released upon bacterial lysis or during rapid bacterial growth. LPS can be divided into three structural units, the O-specific chain, the core region and the lipid-A component (Figure 1).

The outer portion of endotoxin, termed the O-antigen, is composed of a chain of repeating polysaccharides. The different O-specific polysaccharides are highly variable between bacterial species and act as an antigen, thus conferring antigenic specificity to Gram negative bacteria.

The core portion is structurally less variable and is composed of segments, the O-chain proximal outer core and the lipid-A-inner proximal core. The core oligosaccharide region links the O-polysaccharide with the inner lipid A region. The core oligosaccharide portion is composed of specific arrangements of several monosaccharides, N-acetylglucosamine, phosphate and ethanolamine linked to lipid A by a 3-deoxy-D-manno-2-octulosonic acid. This region as well as the lipid A region are well conserved among different bacteria.
The lipid (hydrophobic) portion is termed lipid A.\textsuperscript{5,27} This is a particularly important component of endotoxin because it is a directly responsible for the toxic effects that result from the interaction of lipid A with different biologic systems.\textsuperscript{18,26} The chemical structure of lipid A is well conserved between different species of Gram negative bacteria.\textsuperscript{5,18,26}

**The physical barriers to endotoxin are breached**

Some organisms can cause septic shock by disrupting the intestinal mucosal barrier; therefore Gram negative bacteria can leak into the central circulation. Although controversial, proponents of this theory suggest that this route is the final common pathway of irreversible shock produced from any etiology.\textsuperscript{23}

The gastrointestinal tract is a reservoir for Gram negative bacteria that release endotoxin when they die or multiply, thus the large intestine contains a large amount of endotoxin.\textsuperscript{12,14,18,26} The intestinal mucosal barrier, composed of epithelial cells, tight junctions, cellular secretions and the lamina propria, efficiently restricts the transmural movement of endotoxin and bacteria.\textsuperscript{9,11,12,18,26} Hepatic Kupffer cells are effective in clearing small amounts of endotoxin, therefore limiting its access to the systemic circulation.\textsuperscript{9,12,18,26} Inflammation, ischemia or trauma to the gut, or bacterial overgrowth, however cause a disruption of the integrity of the intestinal mucosal barrier allowing translocation of endotoxins or bacteria into the portal blood, lymph nodes and/or peritoneal cavity.\textsuperscript{5,8,9,11,12,14,18,26,28,29} This massive endotoxin translocation overwhelms the hepatic clearance capacity and allows endotoxin to gain access to the peripheral
circulation. 9,18 By these two means large amount of endotoxin reach the peripheral circulation. 9 There is substantial evidence supporting the role of LPS as an important trigger in the pathological derangements of equine acute abdominal disease. 30 Horses are more sensitive to endotoxin than other mammalian species. 11,13,15,31 The lethal LPS dose in ponies is 200 to 400 ug/kg, whereas the lethal dose for rabbits and guinea pigs is 3 and 10 mg/kg respectively. 31 The cause of this unique susceptibility of horses to endotoxemia has not been determined. 31 Bacteria can also gain access to the blood when there is severe localized or disseminated Gram negative infection of soft tissue. 9

The pathogenecity of the bacteria, number of infective microorganisms and the innate host immunocompetence will determine whether septicemia, metastatic infection or both will result. 14 The phenomenon of translocation is an indicator of failure of the host’s immune and mechanical defense systems during SIRS and sepsis. 5

**Interaction of endotoxin with cells and proteins of blood to produce inflammation**

In humans LPS is responsible for approximately 50 % of the septic episodes. 5 Once endotoxin enters the circulation it can interact with plasma proteins or blood cells or be removed by macrophages in the liver, spleen or pulmonary vasculature. 12 The primary target cells of LPS in mammalian species are the professional phagocytes of innate immunity (peripheral monocytes, tissue macrophages and neutrophils), which constitutively express cluster of differentiation (CD14) antigen and the Toll-Like Receptor – 4 (TLR4) in their cell membranes. 27 LPS also has been documented to induce complement. 27 The aim of these responses is to eliminate the invading microbial
pathogen. 27 There is also the recruitment of adaptative, highly specific immune response via the selection and clonal expansion of pathogen specific T and B lymphocytes. 27 The objective of this latter response is the generation of specific antibodies directed against epitopes expressed by the polysaccharide portion of LPS and generation of protective immunological memory. 27

The lipid A portion of LPS, which is hydrophobic, forms aggregates in the plasma. These aggregates are dispersed into monomers by a plasma protein called lipopolysaccharide binding protein (LBP). 12,18,19,27 LBP is an acute phase protein, synthesized by hepatocytes in response to inflammation. 18 LBP has a strong affinity and specificity for the lipid A portion of LPS and the CD14 receptor in the cell membrane of monocytes, macrophages and Kupffer cells. 5,12,16,18,19 LBP facilitates the cellular interaction with endotoxin; however it is not essential for such interaction. 12 TNF-a is released from mononuclear cells after exposure to LPS, the interaction of LPS and CD-14 is necessary for TNF-a production. 16 When LBP is absent much greater concentrations of LPS are required to stimulate TNF-a synthesis. 16

In addition to the membrane bound CD-14 present on myeloid cells, a soluble form is released into the bloodstream and has been shown to bind LPS in an LPB dependent manner. 5,6,32 These complexes were shown to stimulate membrane CD-14 negative cells, such as dendritic cells, endothelial, epithelial and smooth muscle cells, thus these cells can also respond to LPS. 5,6

CD14 has an important role in the response to endotoxin, however it lacks a transmembrane domain. 5,6,32 Thus, by itself it cannot transmit the signal across the
membrane and stimulate second messenger systems or signal transduction pathways. The second receptor TLR-4, has transmembrane and intracellular components, thus this molecule is responsible for intracellular signaling. The action of TLR-4 is facilitated by a protein called MD-2. MD-2 is a molecule that is physically associated with TLR-4 on the cell surface and confers responsiveness to LPS. Therefore MD-2 is a link between LPS and TLR-4 signaling. The role of MD-2 seems to be the correct positioning of TLR-4 on the cell surface.

The biologic effects of endotoxin are mediated effects and thus are the result of the interaction of LPS with host target cells and the production of endogenous mediators. Activation of a TLR-4/MD-2 complex on the cellular surface produces the phosphorylation of an intracellular inhibitory protein called IkB. The result being the release of the associated transcription factor called nuclear factor kB (NF-kB). NF-kB enters the cell nucleus where it binds to the promoter region of genes encoding for inflammatory mediators, such as TNF-a, eicosanoids, IL-1, IL-6 and tissue factor; there is also stimulation of antiinflammatory mediators such as IL10.

Following the host microbial interaction there is widespread activation of the innate immune response, the purpose of which is to coordinate a defensive response involving both humoral and cellular components. Mononuclear cells play a key role, releasing the classic pro-inflammatory cytokines TNF-a, IL-1 and IL-6 and an array of other cytokines. TNF-a is elevated in approximately 20 % of the horses with colic and it has been associated with high mortality.
TNF-a and IL-1 are the prototypic inflammatory cytokines that mediate many of the immunopathological features of LPS induced shock. These cytokines are released during the first 30 – 90 minutes after exposure to LPS and in turn activate a second level of inflammatory cascade including additional cytokines (IL-12, IL-15 and IL-18), lipid mediators and reactive oxygen species, as well as up-regulating cell adhesion molecules that results in the initiation of inflammatory cell migration into tissues. These cytokines stimulate polymorphonuclear leukocytes, macrophages and endothelial cells, to release a number of downstream inflammatory mediators, including platelet activator factor (PAF) and nitric oxide (NO), further amplifying the inflammatory cascade. These cells also release a variety of biologically active mediators with proinflammatory and antiinflammatory properties. Activation of neutrophils includes their adhesion to the endothelium, and their emigration to injured tissues, with the final outcome being inflammation, tissue destruction and loss of function.

IL-6 is produced during sepsis by several cell types exposed to TNF-a and IL-1. Although IL-6 is an important cytokine released during sepsis it is not a proinflammatory cytokines in itself and it does not have a causal role in septic shock. In humans IL-6 levels are correlated better with mortality than TNF-a and IL-1. In horses IL-6 also seems to be a better marker of disease severity and mortality.

Antiinflammatory cytokines include IL-4, IL-10, IL-11, IL-13, Transforming Growth Factor beta (TGF-ß), soluble TF receptors and IL-1 receptor antagonist. The effects of these mediators on different organ systems are responsible for the
pathophysiologic changes associated with SIRS and sepsis.\textsuperscript{1,4,15,21} If homeostasis cannot be maintained MODS will ensue. \textsuperscript{1}

**Neutrophils become activated and bind to endothelial cells**

Neutrophils, platelets, vascular endothelium and macrophages are considered to be the major cells involved in endotoxic injury.\textsuperscript{36} The interaction that initiates endotoxic shock probably occurs between macrophages and LPS.\textsuperscript{36} Amplification of injury is associated with sequestration of neutrophils and platelets in target organs with vascular endothelium becoming “sticky and leaky”.\textsuperscript{36}

Neutrophils follow an orderly passage from the vasculature to appropriate sites of action where an injury or infection has occurred.\textsuperscript{37} With the appropriate stimulus neutrophils marginate to the vessel periphery and roll along the surface of the vessel wall, interacting with endothelial cells through surface selectins (L, E and P); this is a weak and transient interaction.\textsuperscript{38,37-39} L-selectin is constitutively expressed on the surface of neutrophils and plays a major role in the initial endothelial-neutrophil interaction (“neutrophil rolling”).\textsuperscript{40,41} As a result of this interaction neutrophils gradually slow down rolling on the surface of the endothelium until they come to a complete stop.\textsuperscript{38,39}

Platelet Activator Factor (PAF) secreted by endothelial cells stimulate neutrophils to increased the expression of surface β\textsubscript{2}-integrins (CD18/CD11).\textsuperscript{37,38} Neutrophils come to a complete stop due to the strong interaction between integrins (CD18/CD11) expressed on the neutrophils surface and the endothelial receptor of the immunoglobulin superfamily (ICAM-1 and ICAM-2).\textsuperscript{37-39,42} Through diapedesis neutrophils pass into the interstitial
space, migrating down a chemotactic gradient generated by chemoattractants such as C5a and chemokines; by this means neutrophils reach the source of tissue injury, infection or inflammation.\textsuperscript{37-39,42}

During inflammation adhesion molecules on the endothelial surface are up regulated to interact with activated circulating leukocytes.\textsuperscript{43} This second stage of increased leukocyte endothelial cell adhesion takes hours to develop and is mediated by cytokines.\textsuperscript{38,43} Endothelial cells activated by IL-1β and TNF-α express E-selectin, which further enhances neutrophil adhesiveness.\textsuperscript{35,38,42,43} Once the neutrophils arrive at the septic foci phagocytosis and microbial killing ensue. The latter is the result of neutrophil degranulation with the release of proteolytic enzymes and generation of reactive oxygen species.\textsuperscript{42}

Neutrophil activation and transmigration are important for the host to withstand and combat a septic challenge, and are integral to the successful functioning of the innate immune system.\textsuperscript{42} Neutrophils are essential for bacterial killing, however can also produce injury to the host tissues.\textsuperscript{43} In the inflammatory response to infection the interaction of neutrophils and endothelial cells plays a pivotal role.\textsuperscript{43} Vascular damage and increased vascular permeability occur in two stages.\textsuperscript{38} There is first an immediate increase in vascular permeability, mediated by vasoactive molecules released by damaged tissues.\textsuperscript{38} The second phase occurs several hours after the onset of inflammation, at the time when leukocytes are beginning to emigrate.\textsuperscript{38} Neutrophil rolling along the epithelium initiate a cascade of cellular interactions that result in
endothelial damage (capillary leak syndrome) and subsequent development of multiple organ failure (MOF). \(^{43}\)

Neutrophils are major players in organ injury during sepsis and endotoxemia. Studies have demonstrated that neutrophils activated intravascularly are rigid, have increased expression of integrin adhesion molecules (CD-11/CD-18) and are primed to release oxygen radicals; however have a decreased capacity to migrate into tissues. \(^{17,31,39}\) Sepsis causes an increase in neutrophil stiffness, thus neutrophils are lodged in capillary beds independently of adhesions molecules. \(^{17,31,39}\) Thus studies suggest that the activation of the pulmonary vascular endothelium, rather than the activation of leukocytes, is the predominant event that induces sepsis and mediates neutrophil accumulation in the lung during endotoxemia. \(^{39}\) However the increase in neutrophil stiffness only lasts 5 to 30 minutes; therefore, adhesive mechanisms may be required to retain neutrophils in the lungs during sepsis. \(^{39}\) This LPS induced stiffening may be a mechanism by which bacteria “send” neutrophils to the lungs to avoid bacterial clearance at peripheral tissues. \(^{39}\) Therefore, activated neutrophils that are lodged in small vessels caused an increase in vascular permeability, acute respiratory distress syndrome (ARDS) and MOF. \(^{17,31,39}\) In horses, repeated exposure to LPS produces damage to the pulmonary arteries, in the areas of leukocyte margination. \(^{44}\)

Membrane-bound adhesion molecules and soluble circulating iso-forms of adhesion molecules increase during sepsis. \(^{43}\) Increase circulating adhesion molecules result either from an increased expression (secondary to cytokine stimulation) or from proteolytic cleavage (secondary to endothelial damage). \(^{43}\) These adhesion molecules can serve as
markers of endothelial activation and damage.  

Soluble adhesion molecules may also act as modulators of the inflammatory process by either acting as chemotaxins, blocking neutrophil activation or competing with membrane bound forms of cell-cell adhesion.

Although neutrophils are indispensable for antimicrobial host defense, over stimulation or disruption of the orderly passage of neutrophils out of the circulation can cause unnecessary tissue damage. This has been implicated in the pathophysiology of organ dysfunction promoting the progression of sepsis to SIRS, MOF and death. The indiscriminate liberation of reactive oxygen species, enzymes, vascular endothelial growth factor and other metabolites by neutrophils causes capillary leak, edema and end organ damage. Neutrophil mediated tissue damage is a key pathologic factor in overwhelming infection. The regulation of the potent destructive effects of neutrophils is realized via complex intracellular signal transduction pathways. The activation of p38 MAP kinase and NF-κB results in potentiation of cytotoxic effects with liberation of proinflammatory cytokines.

**Cardiovascular physiology and pathophysiology**

Stroke volume is defined as the amount of blood pumped out of each ventricle per beat. The output of the heart per unit of time is the cardiac output; it is calculated as the product of stroke volume (milliliters per beat) multiplied by the heart rate (beats per minute). In humans there is a correlation between resting cardiac output and body surface area, the cardiac output per minute per square meter of body surface is called the cardiac index.
Cardiac output is controlled by the venous return, thus the heart itself is not the primary controller of CO. Venous return is controlled by the tissue oxygen requirements. The main reasons peripheral factors are more important in controlling the CO than the heart itself is because the heart has two reflexes mechanisms (Frank-Starling law and Bainbridge reflex) that allow it to automatically pump whatever amount of blood is received in the right atria from the veins. This in turn makes the heart contract with more force, ie. The Frank-Starling law. The Bainbridge reflex originates from the stretching of the sinus node in the right atria, in turn the increased heart rate helps to pump the extra blood. However they are limits to the amount of blood that the heart can pump, this is expressed by the cardiac output curves. In humans functioning under no stimulation the heart can pump up to 2.5 times the normal venous return before it becomes a limiting factor in the control of CO.

Techniques used in horses to measure the cardiac output are the Fick technique, dye dilution technique (indocyanine dye), thermodilution technique, more recently lithium dilution and Doppler techniques. Cardiac output values for healthy non tranquilized horses at rest were similar when using dye technique or thermodilution (40 ml of 5 % dextrose at 0° C), with a mean value between 72 to 76 ml/kg/min (cardiac index) or 32-40 L/minute. Halothane anesthesia in horses results in myocardial depression (reduction in cardiac output), therefore the mean cardiac output measured under this situation with thermodilution was 21.8 L/min.

Preload is defined as the degree of tension on the cardiac muscle when it begins to contract. Preload is considered to be the end diastolic pressure when the ventricle has
become full. Preload is a positive determinant of ventricular systolic function that depends on venous return and ventricular size and distensibility. Preload can be estimated by determining ventricular end diastolic volume by echocardiography or by measuring venous filling pressures. Assessment of venous filling pressure by means of central venous pressure, pulmonary diastolic or wedge pressure; provides an accurate estimation of preload if the ventricular compliance (distensibility) is normal and ventilation is stable.

Central venous pressure (CVP) or right atrial pressure is regulated by a balance between the ability of the right heart to pump blood into the lungs and the tendency for blood to flow from the peripheral veins into the right atrium. Factors that increase venous return or depress the right cardiac function will increased the CVP. Some of the factors responsible for increasing the venous return are: increased blood volume, increased large vessel tone that result in the increase of peripheral venous pressure, and arteriolar dilatation which decreases the peripheral resistance and allows rapid flow of blood from the arteries into the veins. In addition sepsis results in decrease arterial vascular tone and increase in venous capacitance (venous dilation) which results in a decline of the effective circulating volume.

Central venous pressure is a pressure estimate of right ventricular preload as manifested by right ventricular end diastolic volume. The use of central venous pressure as an estimate of left ventricular preload is not accurate particularly in septic patients. Ventricular afterload is the load against which the myocardium exerts its contractile force. The afterload of the ventricles is the pressure in the artery leading from the
ventricle, corresponding to the systolic pressure. Increases in afterload decrease stroke volume, unless ventricular force of contraction increases to compensate for the load. Afterload is a difficult parameter to measure in the clinical situation.

Several methods are used for evaluation of ventricular performance: left ventricular ejection fraction (LVEF), Frank Starling plots and end systolic volume pressure plots. The function of the myocardium in critically ill patients may be assessed by the Frank-Starling curve, in which an index of heart work is related to the preload of the left ventricle [i.e. left ventricular stroke work index (LVSWI) vs. left ventricular end diastolic volume index (LVEDVI)].

Ejection fraction is defined as the percentage of the end diastolic volume ejected with each beat, in humans is approximately 65%. This variable has been proven to be an extremely valuable measure of ventricular performance in health and during sepsis or septic shock. A way of calculating this parameter is by injection of radionuclide-labeled red blood cells, imaging the cardiac blood pool at the end of diastole and the end of systole (equilibrium radionuclide angiocardiography), and then calculating the ejection fraction.

**Effects of endotoxin on vascular tone and on the cardiopulmonary system**

Shock is classically defined as inadequate tissue perfusion resulting in cell dysfunction and cell death. This definition describes shock of cardiogenic, vascular, obstructive and hypovolemic mechanisms that result in poor tissue perfusion. These types of shock are characterized by an elevated systemic vascular resistance, as a
compensatory mechanism to maintain blood pressure and decreased pulmonary artery oxygenation, reflecting an increased tissue extraction of oxygen by hypoperfused tissues.²¹

Septic shock is much more complex. The onset of sepsis is usually accompanied by hypovolemia due to both arterial and venous dilatation and leakage of plasma into the extravascular space.²¹ If the hypovolemia is corrected by the aggressive administration of intravenous fluid therapy, it will result in low systemic vascular resistance, normal or increased cardiac output or cardiac index, tachycardia, and elevated oxygen concentration in pulmonary arterial blood, thus a hyperdynamic shock syndrome.¹,⁴,²¹,²⁴ This constellation of abnormalities have been categorized as distributive shock, due to the maldistribution of blood flow to various tissues.²¹ Therefore septic shock has characteristics of distributive and hypovolemic shock.⁵⁸

Endotoxin injected into healthy animals causes a shock state characterized by hypotension and reduced systemic vascular resistance, tachycardia, increased cardiac index, normal stroke volume and organ dysfunction.²¹,²⁴ If endotoxin infusion is followed by fluid administration (to simulate the management of sepsis) the left ventricular ejection fraction decreases and the ventricular volume increases.²¹,²⁴ The ratio of peak systolic pressure to end systolic volume, an index independent of load, is reduced. Therefore the administration of endotoxin produces cardiovascular changes similar to those caused by spontaneous sepsis, with a hyperdynamic response accompanied by reversible myocardial depression and ventricular dilatation.²¹
Even though the cardiac output is normal or increased during septic shock, the ventricular function is abnormal.\textsuperscript{21} Measurement of ejection fraction has been proven to be a useful measure of ventricular performance during sepsis and septic shock.\textsuperscript{21} In a septic peritonitis canine model, septic animals had a significantly lower left ventricular ejection fraction when compared with controls.\textsuperscript{24} In the same experiment after correction of hypotension by fluid administration, septic dogs had a high to normal cardiac output or cardiac index and low systemic vascular resistance.\textsuperscript{24} These changes were compatible with hyperdynamic sepsis.\textsuperscript{24}

The characteristic pattern of cardiovascular performance during septic shock has been proven to be as follows: \textsuperscript{1,21,23,24}

- Reduced left and right ventricular ejection fractions.
- Increased end diastolic and end systolic volumes of both ventricles.
- Normal stroke volume.
- Elevated heart rate and cardiac output.
- Reduced systemic vascular resistance.

The reduction in ejection fractions and biventricular dilatation occurs 24 to 48 hours after the onset of sepsis, and in patients that survive this is a reversible change that normalizes five to ten days after the onset.\textsuperscript{1,21,24} Human patients with septic shock that develop a more severe reduction in LVEF are more likely to survive.\textsuperscript{1,2,21} Patients that survive from septic shock have an acute dilation of the left ventricle with an increased end diastolic volume index (EDVI) and end systolic volume index (ESVI).\textsuperscript{1} Therefore patients with sepsis or septic shock have marked left ventricular systolic dysfunction.\textsuperscript{1}
Survivors and non survivors maintained normal or elevated cardiac index (CI) and stroke volume index (SVI). The cause of this seeming paradox, in which patients that survive developed ventricular dilatation, remains not well understood.

Abnormalities in diastolic pressure in septic patients is less clear. Human and animal investigations support the occurrence of both systolic (reduced ejection fraction) and diastolic (reduced compliance) abnormalities of ventricular function during septic shock.

The manifestations of myocardial depression have also been studied in the context of decreased ventricular response to fluid resuscitation (Figure 2). This figure represents the left ventricular stroke work index (a measure of cardiac performance) versus left ventricular EDVI (a measure of left ventricular preload) and demonstrates Frank-Starling ventricular function curves. Patients with septic shock treated with fluid resuscitation, despite increase pulmonary wedge pressure and left ventricular EDVI, showed no increase in the LVSWI. Non septic critically ill patients and septic patients without shock showed a more normal response to fluid resuscitation with parallel increases in LVEDVI and left ventricular stroke volume index (LVSVI). The importance of this study is that it demonstrated that septic shock patients have a decreased augmentation of ventricular performance. Reduced ventricular contractility (systolic dysfunction) was a major cause of myocardial depression in early septic shock.

The right ventricle is subject to different influences than the left ventricle, particularly in shock. In the systemic circulation septic shock is associated with decreased vascular resistance and blood pressure. This results in a reduced left ventricular afterload, which
in turn tends to maintain or elevate cardiac output, despite the presence of depressed left ventricular contractility. 1,2 During sepsis and septic shock the right ventricular afterload is increased due to the increased pulmonary vascular resistance associated with acute lung injury or respiratory distress syndrome. 1,2,4 This tends to decrease the right ventricular cardiac output. 1 However, due to the marked species variability in sensitivity to endotoxin, pulmonary hypertension is not always a major determinant of right ventricular function in septic shock. 59 Right ventricular dysfunction is common in sepsis and can be attributed to either increase in afterload or myocardial depression. 1 Despite the difference between ventricles, right ventricular dysfunction in septic shock closely parallels left ventricular dysfunction. 1,2,59 Decreased right ventricular ejection fraction (RVEF) and ventricular dilatation characterize right ventricular dysfunction in sepsis and septic shock. 1,4

In human critical care the use of sophisticated imaging techniques has resulted in a better understanding of septic myocardial dysfunction. 1,2 Clear evidence supports the existence of ventricular dysfunction in normotensive sepsis and septic shock patients. 1,4 Despite the presence of hyperdynamic circulation, myocardial depression in septic shock is reflected by reduced post resuscitation left ventricular ejection fraction (LVEF), ventricular dilatation and flattening of the Frank-Starling relation. 1,2 Also ventricular dilatation and decreased LVEF is seen in normotensive septic patients. 1 Decreased contractility is the major factor causing septic myocardial depression. 1 Studies have also suggested that impaired ventricular compliance may significantly contribute to myocardial depression in sepsis. 4,60
Myocardial dysfunction is a consistent and important feature of septic shock and is a significant contributing factor to the high mortality rate associated with sepsis.\textsuperscript{61} Even after correction of hypovolemia, hypoxia and acidosis, most patients with septic shock have evidence of myocardial dysfunction, characterized by biventricular dilatation, reduced ejection fraction, and requirement of inotropes to maintain cardiac index within normal ranges.\textsuperscript{4,61} Two major theories that intend to explain the nature of septic myocardial depression in human and animals exist.\textsuperscript{1,4,21,62}

1. Myocardial depression in sepsis results from global myocardial ischemia caused by reduced coronary blood flow.

2. Myocardial depression results from the direct effect of one or more circulating myocardial depressant substance.

For many years the leading theory of myocardial depression during sepsis was associated with a global myocardial hypoperfusion, leading to ischemia and myocardial depression.\textsuperscript{4} However this theory was disproved by different human and animal studies.\textsuperscript{4}

In human septic shock it has been shown that septic patients have normal to increased coronary blood flow, when compared with normal subjects.\textsuperscript{21,22,63,64} The septic subjects did not exhibit an increase in lactate production when compared with non septic individuals.\textsuperscript{21,22,63} An increased oxygen availability to the myocardium in septic patients was also noticed.\textsuperscript{21,22,63} All these findings argue against the hypothesis that global myocardial ischemia accounts for myocardial depression in septic shock.\textsuperscript{1,21,22,63,64} In a canine septic model that induced depression of myocardial contraction, the dysfunctional hearts of septic animals showed no alteration in the relationship between oxygen delivery
and consumption, no net lactate production and no significant decrease in the amount of high energy phosphates available for work. When myocardial work was increased by the administration of catecholamines, the myocardium preserved aerobic metabolism and maintained high energy phosphates. Thus further supporting the unimpaired coronary blood flow across coronary circulation during sepsis.1,24

When serum obtained from human patients in the acute stages of septic shock was incubated with myocardial cells from newborn rats; the extent and velocity of myocyte shortening were reduced, as compared with those produced by serum obtained from normal subjects, patients with structural heart disease or non septic critically ill patients.21,22 This was the first study that clearly supported the theory of a myocardial depressant substance.21,22 Patients in the acute stage of septic shock, whose serum contained this depressant substance, had lower ejection factions, larger end-diastolic volumes, higher pulmonary artery wedge pressure and higher peak blood lactate.21

There are several studies with conflicting data on the identity of the myocardial depressant factors; this is likely due to the heterogeneity of the patients studied and the diversity of their underlying conditions.61 An important model for the study of Gram negative sepsis, in humans, is meningococcal sepsis, because it is caused by a single bacteria and generally affects previously healthy children.61 In a recent study, serum from children affected by meningococcal septic shock depressed myocardial contractility of rat myocytes when compared to healthy volunteers serum.61 This study did not clearly identify the nature of the depressant substance.
Different mediators had been postulated as myocardial depressor factors, however two cytokines, TNFa and IL–1ß, are of particular importance in this respect.\textsuperscript{1,4} TNFa and IL–1ß have been shown to produce synergistic deleterious hemodynamic effects in a variety of in vivo models of shock.\textsuperscript{1} The myocardial depression produced by TNFa and IL–1ß appears to occur in two different time frames.\textsuperscript{1,4} There is early myocardial depression that occurs within 10 minutes of exposure either to TNFa, to IL–1ß, to TNFa and IL–1ß given together and to septic serum.\textsuperscript{1,4} A second mechanism is characterized by a delayed depressant effect, which begins hours after exposure to TNFa and IL–1ß and persists for days.\textsuperscript{1,4} The latter mechanism appears to involve de novo protein synthesis.\textsuperscript{1,4} The generation of NO may have a central role in the early and delayed myocardial depression responses.\textsuperscript{1,4} NO is produced by the conversion of l-arginine to l-citrulline by nitric oxide synthase (NOS), which exists in inducible (iNOS) and constitutive (cNOS) forms.\textsuperscript{1,4,65} One of the actions of NO formed in the endothelial cells is to diffuse to the smooth muscle cells, where it activates soluble guanylyl cyclase, with the generation of cyclic Guanosine Monophosphate (cGMP).\textsuperscript{1,65} cGMP, in turn, produces smooth muscle and myocardial relaxation.\textsuperscript{1,65} It has been demonstrated that sepsis induced cytokines could stimulate the myocardium to produce NO via both iNOS and cNOS.\textsuperscript{1}

The initial process is thought to occur from sequential NO and cGMP generation via cNOS activation, rather than by de novo synthesis of iNOS.\textsuperscript{4} This is consistent with the short time frame to the onset of myocardial depression.\textsuperscript{4} Other work has shown that the
generation of iNOS, NO and cGMP may be responsible for the late onset of myocardial depression. 4

As mentioned above, the pathologic generation of NO and cGMP appears to contribute to cardiovascular dysfunction in sepsis and septic shock. 1 Although NO may be central, the biochemical mechanisms underlying septic myocardial depression are clearly complex with redundant, branching pathways. 1 In humans hyperdynamic shock is linked to the presence of circulating myocardial depressant factors, which probably represents low concentrations of TNFa and IL–1ß acting in synergy. These effects are mediated by mechanisms that include NO and cGMP generation. 1

In conclusion human patients with sepsis and septic shock show biventricular dilatation and decreased ejection fraction. 2 Patients with septic shock exhibit a depressed response to fluid resuscitation (depressed Frank-Starling curve) and depressed inotropic response to catecholamines. 2 Data suggests that there is a significant alteration in diastolic function. 2 Several studies suggest that ventricular dilatation and decreased ejection fraction are associated with survival. 2 Therefore septic myocardial depression is believed to be an adaptation to septic stress, rather than a direct manifestation of septic pathophysiology. 2 The fact that septic shock causes biventricular dilatation has important clinical applications. Both right and left heart filling pressures and or end diastolic volume may be important in determining the amount of fluid resuscitation and necessity for inotropic and or vasopressor agents, in order to optimized cardiac function. 59 For example, in volume unresponsive patients with a persistently low systemic vascular
resistance, vasopressor therapy may be appropriate while patients with a failing cardiac output due to persistent myocardial depression may benefit from inotropic drugs. 59

Microcirculation

The endothelium is involved in the regulation of microcirculatory blood flow by producing a series of vascular tone regulators, such as: prostaglandin, NO, endothelin-1 (ET-1) and angiotenin II (AG-II). 66,67 Local regulation of blood flow is a balance between central mechanism (autonomic nervous system) and local regulators. 67 ET-1 is a very potent vasoconstrictor that plays an important role in local blood flow regulation. 67,68 ET-1 is released in response to hypotension, low cardiac output and endotoxin. 67 In septic patients the severity of disease parallels the concentration of ET-1. 67,69 The sympathetic nervous system becomes activated in critically ill patients. 67,70 The atrial natriuretic peptide (ANP) plays an important role in the regulation of arterial blood pressure and volume homeostasis. 67 It has been shown that the concentration of ANP increased during sepsis. 67,71 It has been shown in animal and human studies that adrenaline stimulates the secretion of ANP, by which the negative effect of adrenaline on the microcirculation may be compensated. 67,72 ET-1 stimulates the release of catecholamines and renin, contributing to vasoconstriction of the microcirculation. 67,73 In contrast, ET-1 increases the circulating levels of ANP, and ANP is a potent inhibitor of ET-1. 67 Thus ANP may function as a potent feedback regulator of ET-1. 66,67 Also prostaglandin synthesis is stimulated by adrenaline, by which counter regulatory vasodilatation may be induced. 67
Sepsis produces microcirculatory blood flow alteration by impairing vasoregulation.\textsuperscript{67} Thus the distribution of the cardiac output becomes inadequate and flow to the tissues is negatively affected.\textsuperscript{67} Various vasoactive and inflammatory mediators, systemic or endothelial derived, are important in reducing perfusion during sepsis. \textsuperscript{67} The activation of the sympathetic nervous system is a compensatory mechanism to maintain peripheral perfusion.\textsuperscript{67} Although the activation of this neurohumoral mechanism is initially beneficial, it becomes deleterious.\textsuperscript{67} Microcirculatory disturbances appears to be of major importance for the development of, and progression in the pathophysiology of shock or the evolution of MOF.\textsuperscript{67} In septic patients’ plasma, concentrations of vasopressin, ET-1, adrenaline and noradrenaline are elevated, thus the elevation of these potent vasoconstrictors may cause alterations in the microcirculation, inadequate perfusion and tissue damage.\textsuperscript{67}

**Capillary leak syndrome**

Capillary leak is the excess loss of fluid alone, or in conjunction with protein, into the interstitial space and ultimately the lymphatic space, resulting in edema formation, hypovolemia, hypoproteinemia, decreased capillary blood flow and MOF.\textsuperscript{74,75} Capillary permeability increases if alteration, damage or death of the endothelial cell or a change in the intercellular space, which renders the endothelial cell and basement membrane a less effective barrier to the passage of large molecules.\textsuperscript{74} These changes can be initiated by a variety of insults, sepsis and SIRS being the most common.\textsuperscript{74,75}
Activated neutrophils release proteases, oxygen radicals and other molecules that are toxic to the endothelial cells. IL-2 activates neutrophils by increasing the surface expression of CD-11b/CD-18, therefore contributing to leukoaggregation and endothelial cell adhesion. Neutrophils play an important role in microvascular dysfunction during septic shock.

The release of endotoxin from the bacterial cell wall initiates a cascade of events resulting in the release of cytokines (IL-1, IL-6, TNF-α), which in turn causes endothelial cell injury with capillary leak and loss of vasomotor tone. Endotoxin and TNF stimulate endothelial cell production of NO, resulting in vascular smooth muscle relaxation.

Inflammatory mediators produced during sepsis, including: prostaglandins, leukotrienes, PAF, histamine, serotonin and bradykinin trigger microvascular endothelial contraction. Contracture of these cells creates separation at the intercellular junctions, allowing leakage of fluid and small proteins into the interstitium.

Activation of the complement pathway promotes the release of thromboxanes and C5a, which stimulates neutrophils to release oxygen radicals and enzymes that are toxic to the epithelial cells.

Plasma proteins, including albumin, and water form the intravascular compartment leak into the interstitium, resulting in hypovolemia and hypotension. In septic patients there is a 300% increased in the albumin escape rate from the vascular to the interstitial space. Hypoalbuminemia in septic patients has been associated with increased mortality.
Clinical recovery from capillary leak syndrome is heralded by cessation of the need of ongoing volume replacement and exogenous catecholamines dependence and by mobilization of previously administered fluids. Recovery of capillary function likely involves repair of damaged capillaries and regeneration of new capillaries.

**Endotoxemia in horses**

The species specific clinical syndromes associated with endotoxemia result from activation of inflammatory mediator system, and to a lesser extent direct cellular toxic effects of LPS.

In the horse, most of the clinical signs associated with endotoxemia are due to the effect of proinflammatory substances synthesized by circulating and tissue fixed mononuclear phagocytes. The most widely recognized mediators are cytokines, lipid derived mediators and coagulation fibrinolytic factors.

Arachidonic acid is a 20 carbon fatty acid, located in the phospholipids of cell membranes. Arachidonic acid is released from its position in the phospholipids by phospholipase A$_2$. Once released the free arachidonic acid can be further metabolized by either cyclooxygenase or lipooxygenase. The origin of these arachidonic acid metabolites is thought to be the widespread endothelial activation which occurs during sepsis. Leukopenia due to neutrophil sequestration within the pulmonary microcirculation, induced by LPS, is a major player in endothelial damage during sepsis.
In equine endotoxic shock the increased plasma concentrations of cyclooxygenase derived metabolites include: thromboxane \( A_2 \) (TxA\(_2\)), prostaglandin I\(_2\) (PGI\(_2\)), prostaglandin F\(_{2a}\) (PGF\(_{2a}\)) and prostaglandin E\(_2\) (PGE\(_2\)); all associated with hemodynamic changes.\(^9,12,18,26,80,81\) PGI\(_2\) and TxA\(_2\) are potent vasoactive compounds that mediate the early hemodynamic dysfunction during endotoxemia.\(^9\) Evidence suggests that pulmonary macrophages, endothelial cells and platelets are the primary sources of TxA\(_2\).\(^82\) TxA\(_2\) induces platelet aggregation and vasoconstriction.\(^9,16,18,44,80\) PGI\(_2\), synthesized by endothelial cells, it is an inhibitor of platelet aggregation and a vasodilator.\(^9,16,18,44,80\) PGF\(_{2a}\) causes vasoconstriction and PGE\(_2\) causes vasodilatation.\(^18,36\) The plasma half life of TxA\(_2\) and PGI\(_2\) is very short (less than three minutes).\(^80,81\) Therefore the concentration of stable hydrolysis products, thromboxane B\(_2\) (TxB\(_2\)) and 6-keto-PGF\(_{1a}\) are used to measure the concentration of TxA\(_2\) and PGI\(_2\) in plasma respectively.\(^16,80,81\)

Marked species variability in sensitivity to endotoxin exists.\(^59\) In humans pulmonary hypertension does not seem to be an essential part of the pathophysiology of septic shock.\(^59\) Thus in septic human pulmonary hypertension is not always a major determinant of right ventricular function.\(^59\) LPS infusion in sheep is a well described animal model of the pulmonary effects of sepsis.\(^83\) LPS administration to sheep causes a peak in pulmonary arterial pressure of nearly three fold above baseline immediately after infusion and remaining elevated for twelve hours.\(^83\) Pulmonary wedge pressure following LPS infusion results in two fold increase above base line, which remains elevated for twelve hours.\(^83\)
Experimental induced endotoxic shock, in horses, is associated with a rapid increase in TxB\(_2\) concentrations, which occurs five to thirty minutes after intravenous endotoxin administration and remains elevated for at least four hours.\(^{10,16,80,81,84}\) The increase in TxA\(_2\), a potent vasoconstrictor, is associated with an elevation in pulmonary arterial pressure (PAP), hypoxemia and increased central venous pressure (CVP).\(^{9,16,18,26,36,80-82}\) Systemic hypotension was observed during the rapid increased in TxB\(_2\).\(^{81}\) Thromboxane is not involved in the pathogenesis of altered cardiovascular function in late endotoxic induced shock.\(^{10}\) By inducing platelet aggregation, TxA\(_2\) contributes to the coagulopathy seen in sepsis.\(^{9}\) Plasma concentrations of the stable metabolite of PGF\(_{2\alpha}\) also increase very rapidly (thirty minutes) after initiation of endotoxemia.\(^{18}\) Plasma 6-keto-PGF\(_{1\alpha}\) concentrations increase more slowly reaching a peak between one to two hours and return to base line in six hours.\(^{10,16,80,81}\) PGI\(_2\) is associated with hemodynamic changes that occur later in endotoxic shock (one to two hours), and are characterized by hypotension and inadequate tissue perfusion.\(^{9,18,26,36,80,81}\) PGE\(_2\) also increases later and is responsible for the development of fever.\(^{18}\)

The lipooxygenase pathway yields leukotrienes (B\(_4\), C\(_4\), D\(_4\) and E\(_4\)) which have profound effects at the microcirculatory level causing vasoconstriction and promoting capillary leakage.\(^{9,36}\) Leukotriene B\(_4\) is a potent neutrophil chemoattractant and promotes neutrophil – endothelial adhesion, further affecting the microcirculation.\(^{9,36}\) Leukotrienes cause pulmonary and coronary vasoconstriction and increased precapillary venules permeability.\(^{36}\) Leukotrienes are rapidly metabolized and eliminated by the liver, thus they are not reliably detected in blood during endotoxemia.\(^{9}\)
Another lipid derived lipid mediator involved in the pathogenesis of septic shock has been named PAF. PAF is synthesized by activated leukocytes, platelets and endothelial cells. Increased levels of PAF as occurs in experimental and natural sepsis, are responsible for thrombus formation, thrombocytopenia, increased vascular permeability, vasodilatation with resulting severe hypotension and pulmonary vasoconstriction. Administration of PAF receptor antagonists before endotoxic shock results in prevention of immediate hypotension.

Cytokines are synthesized and released by mononuclear phagocytes in response to LPS and then have effects on other cells or mediators. TNF, also known as cachectin, and IL-1 and IL-6 are key cytokines released during sepsis. Proinflammatory cytokines (like TNF, IL-1 and IL-6) are important in the onset and development of septic shock and a relation between blood levels of TNF, IL-6 and mortality has been described. TNF is important in the pathogenesis of sepsis, as indicated by its increased concentration during experimental and natural sepsis and by the fact that administration of exogenous TNF mimics the clinical and pathological changes of sepsis. On the other hand blocking TNF prevents the production of IL-1 and IL-6. TNF is an early acting intermediate, rather than a proximate effector, of the effects of endotoxemia. Horses with gastrointestinal disease exhibit an increased concentration of TNF in serum and peritoneal fluid. The plasma half life of TNF is very short (approximately six minutes). In horses TNF increases rapidly, during experimental endotoxemia, peaking within two hours and returning to base line in four to six hours. TNF is associated with hypotension, hemoconcentration,
acidosis and disseminated intravascular coagulation (DIC). Increase serum concentrations of TNF have been correlated with poor prognosis in humans and horses. In horses IL-6 has received great attention in endotoxemia studies. IL-6 is the only substance reported to be able to evoke secretion of a full spectrum of acute phase reactants by the liver. Because IL-6 has a role as a general alarm protein, it is produced by different cells including mononuclear phagocytes, fibroblasts and endothelial cells in response to a wide array of noxious stimuli including LPS. The peak plasma concentrations of IL-6 are achieved between three to four hours after onset of endotoxemia. IL-6 is associated with the febrile response. IL-6 may be an important secondary mediator to TNF and IL-1.

ET-1 is a potent vasoconstrictor released from endothelial cells that are stimulated by endotoxin. ET-1 was suggested to be one of the mediators of hemodynamic derangements during endotoxin shock and to contribute to pulmonary hypertension and peripheral vasomotor derangements. However in an equine endotoxemia model, where low endotoxin doses were used, ET-1 levels did not increase. In summary the cardiopulmonary effects of experimental endotoxemia, in horses, have included early increased cardiac output, systemic vasoconstriction, increased total peripheral vascular resistance, tachycardia, arterial hypoxemia, hyperpnea, respiratory alkalosis and pulmonary hypertension. These changes are followed by hypotension, decreased cardiac output and increased vascular permeability. Cardiac output decreased soon after endotoxin infusion in
some endotoxin trials. The decreased in cardiac output has been attributed to either depressed cardiac function, decreased venous return or increased pulmonary vascular resistance. In studies where a low dose of endotoxin was infused to horses no changes in cardiac output were seen. Therefore, cardiovascular effects of sepsis vary according to the time course and the severity of septic shock, and in the case of experimentally induced sepsis vary with the animal model, route of administration and dosage.

**Lactate and endotoxemia**

Lactate is a normal product of glucose metabolism in all cells. In anaerobic conditions pyruvate is converted to lactate via the glycolytic pathway, by this means there is production of only two moles of adenosine triphosphate (ATP) per mole of glucose. This is the primary source of energy for all cells functioning in low oxygen environment. In this case the lactate to pyruvate ratio (normal 10/1) increases. However, once oxygen becomes available again and assuming that mitochondrial function is intact, the excess lactate is metabolized back to pyruvate and processed by the Krebs cycle. Lactate formation depends heavily on pyruvate metabolism and it is metabolized exclusively from pyruvate. Pyruvate can be metabolized by three pathways. The first and most important pathway is the conversion of pyruvate to acetyl coenzyme A, mediated by pyruvate dehydrogenase enzyme complex (PDH) that requires the presence of oxygen. Pyruvate enters the Krebs cycle for production of energy in the form of ATP. Thirty eight moles of ATP are produced per mole of glucose oxidized via Krebs cycle. Secondly, pyruvate can be metabolized to glucose in
the Cori cycle.\textsuperscript{90} This process occurs primarily in the liver and renal cortex.\textsuperscript{90} Finally, lactate can be transaminated with glutamate to alanine and a-ketoglutarate. This reaction is reversible, and therefore pyruvate can be resynthesized and subsequently used in gluconeogenesis.\textsuperscript{90}

Hyperlactatemia associated with sepsis can result from at least four different pathophysiologic pathways.\textsuperscript{90,91,95,96}

- Increased lactate production during anaerobic metabolism. Hypoxia limits the conversion of pyruvate into acetyl coenzyme A.
- Cytopathic hypoxia. A number of different but mutually compatible mechanisms have been proposed as initiators of cytopathic hypoxia. These include diminished delivery of pyruvate into the mitochondrial Krebs cycle, inhibition of key mitochondrial enzymes that are involved either in the Krebs cycle (PDH enzyme complex) or electron transport chain and activation of the enzyme poly (ADP-ribose) polymerase family, member 1 (PARP-1).
- Increased lactate production during aerobic metabolism. Increased aerobic glycolysis increases intracellular pyruvate concentration when there is no need for ATP production, exceeding the oxidative capacity of the mitochondria. Increased activity of the Na\textsuperscript{+}/K\textsuperscript{+} ATP\textsubscript{asa}, activated by catecholamines, in the presence of cellular normoxia has been related to this pathway of aerobic lactate production.
- Decreased lactate clearance, mainly by the liver and kidneys, could be an important cause of hyperlactatemia in hemodynamically stable septic patients. Different processes can be responsible for the decreased clearance, such as: PDH
enzyme dysfunction, decreased blood flow (decreased lactate delivery) to liver and kidney and persistent cellular hypoxia in hemodynamically stable patients.

Hyperlactatemia and lactic acidosis are not synonymous. In human medicine lactic acidosis refers to a metabolic process characterized by increases in blood lactate (> 5 mmol/l) and a decrease in blood pH (< 7.25) whereas hyperlactatemia refers to increases in blood lactate only. However the association between lactate and $\text{H}^+$, especially in sepsis, is far from straightforward. Increase in lactate levels with or without concomitant acidosis reflex a complex metabolic disturbance in which increased aerobic and anaerobic production and decreased clearance are important elements. Furthermore, the importance of these elements differs in different disease states.

Hyperlactatemia is commonly observed in septic patients and increasing severity of sepsis is hallmarked by hyperlactatemia, however its pathophysiology is still debated. Some studies showed that hypoxia is the predominant cause of hyperlactatemia in critically ill patients. However other studies argued against the fact that tissue hypoxia is the origin of hyperlactatemia in sepsis. Although more recent experimental work has shown that hypoxia is present in sepsis. Several other studies have shown that both critically ill patients and septic patients with hemodynamic instability may have global or regional indicators of low flow or tissue hypoxia. In septic patients many factors can compromise oxygen delivery to the tissues. Among these factors are acute lung injury, as a cause of hypoxemia and alterations in myocardial contractility that compromise cardiac output. Sepsis is characterized by dramatic disturbances in microvascular control with profound changes in cardiac output.
distribution.\textsuperscript{91,96} This results in marked perfusion heterogeneity, with some tissues experiencing substantial decrease in oxygen transport while others being relatively over-perfused.\textsuperscript{91} In addition, the erythrocyte deformability is impaired in septic patients, thus further contributing to derangements in microvascular blood flow.\textsuperscript{96}

Lactate remains a very useful and simple measure of tissue perfusion in septic shock patients.\textsuperscript{92} In humans with septic shock, lactate concentration is correlated with mortality, lactate concentrations > 5 mmol/l are associated with poor outcome.\textsuperscript{91-93} On the other hand decrease in lactate concentration is an indicator of successful resuscitation in septic patients.\textsuperscript{90-92} Duration of hyperlactatemia is a predictor of development of MODS in septic patients, since tissue hypoxia contributes to MOF and death.\textsuperscript{92,104} If the hyperlactatemia is severe (greater than 3 – 4 mmol/l), it is consider a marker of tissue hypoperfusion during sepsis.\textsuperscript{92,93} On the other hand mild hyperlactatemia (2 – 3 mmol/l) in hemodynamically stable patients may represent alterations in lactate production or clearance.\textsuperscript{92,94}

In the horse, hyperlactatemia has been correlated with prognosis and horses with colic in which the lactate levels decreased after treatment had a better outcome.\textsuperscript{105,106} Several severity score systems have been developed in horses with colic, all these systems included lactate.\textsuperscript{107-109} A hyperlactatemia greater than 7.5 mmol/l has been associated with high mortality rate in horses with colic.\textsuperscript{107} However lactate alone was not a good predictor of outcome, therefore lactate needs to be taken into account with several other clinical and clinical pathological parameters in order to have a better assessment of outcome.\textsuperscript{107}
For over 30 years blood lactate concentrations have being used as a marker for tissue hypoperfusion or hypoxia during circulatory shock. However, other factor may coexist, complicating the interpretation of hyperlactatemia. A central feature of sepsis is inadequate tissue perfusion, therefore it is commonly assumed that hyperlactatemia is the result of a deficit in oxygen availability; that is pyruvate is shunted to lactate because it cannot be promptly oxidized. Hyperlactatemia has also been used as an indicator of tissue hypoxia and anaerobic glycolysis even when blood pressure, cardiac output and urine output are within acceptable ranges in septic patients. In these septic patients that are hemodynamically stable a mild persistent hyperlactatemia is usually present. Oxygen debt due to defects in oxygen extraction and increased oxygen demand; are thought to be the origin of occult cell hypoxia that causes lactate overproduction during sepsis. There are several lines of evidence that refute that occult tissue hypoxia is the source of hyperlactatemia in hemodynamically stable septic patients. Some of the evidence against tissue hypoxia in hemodynamically stable patients is as follows:

- There is no improvement in morbidity or mortality in hemodynamically stable human patients by increasing oxygen delivery.
- Stable human septic patients are not frequently dependent on oxygen supply.
- Tissue Po$_2$ and energy stores seem to be unaffected by sepsis.

Several clinical surveys have failed to identify any consistent decrease in lactate concentration in septic patients after volume resuscitation or blood transfusion. Septic patients have an increase in the rate of pyruvate oxidation and a proportional increase in the rate of pyruvate production. Therefore this evidence suggests that there
is no limitation in carbohydrate oxidation but an increase in pyruvate production in critically ill patients, thus preferential shunting of pyruvate to lactate due to tissue hypoxia or impairment in pyruvate dehydrogenase are unlikely and need further study. In septic patients the increase in glucose-pyruvate flux, may be an adaptive mechanism for improved sensitivity of the metabolic response and to provide a more efficient generation of energy. Hyperlactatemia in septic patients may result as a byproduct of the overall acceleration of glycolysis. Further contributing to this process is the insulin like effect of endotoxin. Hyperlactatemia may also have some physiologic benefit in septic patients, in which the acidic environment may aid in the dissociation of oxygen from hemoglobin, thus improving oxygen delivery. Furthermore, the hypothesis that hypoxia is the source of hyperlactatemia in sepsis has not been convincingly demonstrated by experimental studies. It has been suggested that cellular energetics are deranged in sepsis not just because oxygen delivery is impaired, but more importantly because the ability of the cell to utilized the available oxygen is compromised.

Circulatory factors such as neural and humoral components, as well as intrinsic metabolic and vascular control systems, play a central role in ensuring adequate delivery of oxygenated blood to the tissues. Ultimately adequacy of oxygenation is determined at the mitochondrial level, thus dysoxia is defined as a condition in which oxygen levels are so low that mitochondrial respiration can no longer be sustained. In human sepsis, resuscitation procedures based on the correction of global variables of oxygen delivery and utilization have been shown to be inadequate, and regional tissue dysoxia is
A deficit in tissue oxygen extraction has been shown to occur in sepsis and it has been interpreted as a possible cause of dysoxia. Whether this oxygen extraction deficit is caused by regional hypoxia or is the result of a defect in metabolic pathways associated with mitochondrial respiration remains controversial. In hemodynamically stable septic patients, hyperlactatemia is thought to be caused by pathologic redistribution of blood flow giving rise to hidden hypoxic microcirculatory units. Several studies have demonstrated that blood flow distribution during sepsis becomes highly heterogeneous between and within organ systems. This pathologic heterogeneity in sepsis is thought to be caused by disturbed vascular regulation attributable to the presence of inflammatory mediators (cytokines and NO) and the presence of microcirculatory emboli, all resulting in defects in microvascular autoregulation and the ability to recruit sufficient capillaries to meet local oxygen needs. Shunted microvascular units would become hypoxic and hypoxia would manifest as a deficit in oxygen extraction. This pathologic redistribution of blood flow, which results in shunting, could explain why resuscitation procedures are sometimes ineffective in achieving adequate tissue oxygenation. In order to further understand this controversial topic, more recent work has focused on the process underlying microcirculatory oxygenation. This work has shown that severe disruption of the microcirculation is present during sepsis.

As stated above, an alternative hypothesis suggested is the fact that despite adequate oxygen delivery to tissues, during sepsis the cells are incapable of utilizing the available oxygen. In the presence of normal tissue oxygenation, mitochondrial dysfunction can contribute to hyperlactatemia. Mediators released during sepsis, such as TNF, NO and
LPS, are capable of directly impairing oxidative metabolism.\textsuperscript{92,96} This acquired intrinsic mechanism of deranged mitochondrial respiration has been termed cytopathic hypoxia.\textsuperscript{96} A number of different mechanisms have been proposed to explain cytopathic hypoxia.\textsuperscript{96}

- Inhibition of pyruvate dehydrogenase.
- NO mediated inhibition of cytochrome a,a\textsubscript{3}
- Induction of PARP-1 nuclear enzyme by sepsis induced inflammatory mediators.

Besides increased production, a decreased clearance can result in elevation of lactate concentration.\textsuperscript{92,93} Lactate is primarily cleared by the liver; however the kidneys, myocardium and skeletal muscle (when blood lactate in greater than 4 mmol/l) also have some ability to clear lactate.\textsuperscript{91-95} Lactate in the liver is cleared by oxidation via the Krebs cycle or by gluconeogenesis via the Cori cycle.\textsuperscript{93} Changes in hepatic oxidative capacity can be the origin of mild hyperlactatemia in septic patients.\textsuperscript{92,93} A study done with hemodynamically stable septic human patients,\textsuperscript{93} demonstrated that the mild hyperlactatemia in this set of patients is due to altered lactate clearance and not due to lactate overproduction. Therefore it was their conclusion that in hemodynamically stable septic patients, hyperlactatemia is not a reliable indicator of anaerobic metabolism, but rather indicates a defect in lactate utilization.\textsuperscript{93} Lactate can be produced by the splanchnic region (gut), by the liver, by the lungs or by the circulating white blood cells.\textsuperscript{94,95} This increase in lactate production during critical illness is thought to involve a stress induced enhancement of glycolysis and lactate synthesis by non parenchymal cells.\textsuperscript{110} During hypermatobolic stress states, glycolytic flux is augmented as a result of cytokine (TNF, IL-1) mediated increased in glucose uptake.\textsuperscript{110} In patients with decreased
lactate clearance this increase in lactate production can contribute to hyperlactatemia.\textsuperscript{94,95} A recent study demonstrated that lactate release by the splanchnic tissues is very uncommon, even when hyperlactatemia is severe.\textsuperscript{94,95}

During severe sepsis and septic shock, pathological changes may affect lactate metabolism as well as its relation to tissue oxygen debt. Increase in lactate synthesis in the absence of tissue hypoxia may occur when the rate of glucose metabolism exceeds the oxidative capacity of the mitochondria (i.e. catecholamines administration, impairment of PDH and respiratory alkalosis).\textsuperscript{92} The hyperlactatemia that occur with these mechanisms tends to be modest (1-2 mmol/l).\textsuperscript{92}

Administration of endotoxin to experimental animals results in lactic acidosis and decrease systemic oxygen consumption ($\text{VO}_2$), the origin of these abnormalities can be explained by the inability of tissues to increase the oxygen extraction ratio ($\text{ERO}_2$).\textsuperscript{78} The mechanisms that can produce these changes are: decrease in systemic oxygen transport, uneven blood flow distribution resulting in tissue hypoxia and direct effect of LPS on cellular energy metabolism.\textsuperscript{78} Tissue hypoxia, as the source of hyperlactatemia after endotoxin administration, was not supported by a study using endotoxemic rabbits.\textsuperscript{78} In that same study the hypothesis was that endotoxin or a mediator induced by endotoxin were responsible for the hyperlactatemia. The mechanism postulated was the inhibition of several enzymatic reactions, such as: PDH, ADP/ATP or Creatine Kinase.\textsuperscript{78}

In skeletal muscle and other tissues (erythrocytes, vascular smooth muscle, neurons and glia) aerobic glycolysis is linked to ATP provision for the $\text{Na}^+\text{K}^+$ ATPase pump, the activity of this pump is stimulated by epinephrine.\textsuperscript{104} The binding of epinephrine to $\beta_2$
adrenergic receptors, increases the intracellular concentration of cAMP, therefore stimulating the activity of the Na⁺/K⁺ ATPase pump and glycogenolysis. Increased Na⁺/K⁺ ATPase pump activity results in accelerated aerobic glycolysis that is sustained mainly by glycogen derived glucose 6 phosphate. Rapid ATP production fueled by glycogen causes hyperlactatemia and muscle glycogen depletion. Based on these pathophysiologic mechanisms, a different theory for hyperlactatemia has been proposed. These authors proposed that hyperlactatemia is a reflection of increased aerobic glycolysis within the skeletal muscle, secondary to the stimulation of the Na⁺/K⁺ ATPase pump by epinephrine. During shock skeletal muscle generates lactate from its glycogen stores rather than from circulating glucose. Since muscle constitutes 40% of the cell body mass, changes in muscle metabolism in response to sepsis allow it to become the main lactate producer. This hypothesis explains why hyperlactatemia often does not correlate with traditional indicators of tissue hypoxia nor diminishes with increased oxygen delivery. Therefore when other variables [cardiac output (CO), blood pressure and urine output] have returned to normal following resuscitation, the use of blood transfusions and inotropic agents to increase oxygen delivery and lactate clearance, may be unnecessary. Thus a proportion of the increase in blood lactate during sepsis; would be unrelated to tissue hypoperfusion and it is unlikely that it will respond to supranormal oxygen delivery. Therefore sepsis induced hyperlactatemia, in hemodynamically stable patients, results from the epinephrine stimulated aerobic glycolysis, rather than from tissue hypoxia. In septic human patients the concentration of circulating epinephrine is consistently high.
Increased blood lactate concentration in septic patients, reflects a complex metabolic process that involves increased aerobic and anaerobic production and decreased clearance.\textsuperscript{90,94} Depending on the disease state these elements have unequal significance.\textsuperscript{90} Therefore, tissue hypoxia should always be excluded first, since persistent tissue hypoxia can lead to MOF and death.\textsuperscript{94} Tissue hypoxia can be global or localized.\textsuperscript{94} Impairment of mitochondrial performance can also induce hyperlactatemia.\textsuperscript{94} Aerobic lactate production, either global or focal (especially lungs), is the result of the activation of the inflammatory cascade.\textsuperscript{94} Hence hyperlactatemia can be a warning indicator of a very severe inflammatory process.\textsuperscript{94} When lactate clearance is involved, it can be due to abnormal liver metabolism, usually insensitive to hemodynamic manipulation, but also to decreased hepatic perfusion, which can be improved with hemodynamic manipulations.\textsuperscript{94} The relationship between hyperlactatemia and acidosis is complex and is influenced by the presence of sepsis and the severity of the disease.\textsuperscript{90} Understanding the complexity of these processes is important for recognizing the usefulness and limitations of monitoring blood lactate concentrations.\textsuperscript{90,94}

**Effects of endotoxemia on coagulation**

Sepsis initiates alteration of the coagulation mainly by activation of the extrinsic pathway.\textsuperscript{111-118} Although the contact system is activated during sepsis it does not play a significant role in coagulation.\textsuperscript{112,117} The origin of tissue factor remains an unresolved issue.\textsuperscript{117} Sepsis does not lead to denudation of the endothelium and subsequent exposure of circulating blood to tissue factor.\textsuperscript{113} Rather, LPS initiates coagulation pathways by
inducing the expression of tissue factor on neutrophils, circulating monocytes and endothelial cells, in response to IL-1, IL-6 and TNF.\textsuperscript{6,111,113-117} Tissue factor in turn activates a series of proteolytic cascades, which results in the conversion of prothrombin to thrombin, which in turn generates fibrin from fibrinogen.\textsuperscript{6,111} This plays a central role in the initiation of intravascular and extravascular fibrin deposition.\textsuperscript{111} Endothelial cells play a prominent role in all three major pathogenic pathways associated with coagulopathy in sepsis: 1) tissue factor induced thrombin generation, 2) dysfunctional anticoagulant pathway, and 3) blocked fibrinolysis (Figure 3).\textsuperscript{116} On the other hand antiinflammatory cytokines (IL-10) regulate coagulation by inhibiting the expression of tissue factor on mononuclear cells.\textsuperscript{6,115}

The endothelium is a factory of procoagulant and anticoagulant factors.\textsuperscript{113} On the procoagulant side, endothelial cells synthesize von Willebrand factor (vWF), thrombin receptor, inhibitor tissue factor and plasminogen activator inhibitor -1 (PAI-1).\textsuperscript{113} On the anticoagulant side, endothelial cells express tissue factor inhibitor, tissue plasminogen activator (t-PA), heparan, thrombomodulin and the protein C receptor.\textsuperscript{113,115,116} It is important that each of these functions is differentially regulated in time and space, meaning that the hemostatic response varies between organs and blood vessel types.\textsuperscript{113}

t-PA transforms plasminogen to plasmin, and the latter lyases fibrin.\textsuperscript{119} The interaction of t-PA and plasminogen is enhanced 400 folds in the presence of fibrin.\textsuperscript{119} The major inhibitor of t-PA in blood is PAI-1.\textsuperscript{119} The balance between t-PA and PAI-1 is finely regulated and it is responsible for the regulation of intravascular fibrinolysis.\textsuperscript{119} TNF is considered to be the major initiator of the fibrinolytic response during sepsis.\textsuperscript{111,112,116} The
fibrinolytic system is initially enhanced in response to sepsis and endotoxemia, but later is almost completely shut down. The initial increase in fibrinolysis is due to the increase in t-PA. One to two hours after the initial increase in plasminogen activator, plasma concentrations of PAI-1 increase, thus resulting in complete suppression of the fibrinolytic system, leading to an imbalance between coagulation and fibrinolysis. Thus sepsis disrupts the balance between t-PA and PAI-1. The net result is enhanced production and reduced removal of fibrin, leading to the deposition of fibrin clots in small vessels, inadequate tissue perfusion and organ failure (Figure 4).

Endothelial cells are normally antithrombotic due to the expression of thrombomodulin and heparin. Thrombomodulin binds and inactivates thrombin. Protein C is activated when thrombin complexes with thrombomodulin and in the presence of protein C receptor. Once activated protein C (aPC) is formed, it dissociates from an endothelial receptor, before binding its natural cofactor protein S. aPC and protein S block the coagulation cascade by inhibition of factor Va and VIIIa. aPC also inhibits PAI-1 generation and may also provide a negative feedback on inflammation by inhibiting cytokine production, preventing neutrophil activation and inhibiting leukocyte adhesion and rolling. During sepsis, TNF-a and IL-1 cause the down regulation of thrombomodulin, resulting in decreased aPC activity. Dysfunction of the aPC pathway appears central to the thrombotic process in disseminated intravascular coagulation (DIC).
Heparin binds the plasma serine protease antithrombin III (synthesized in the liver), and the complex irreversibly inactivates thrombin and factors Xa and IXa.\textsuperscript{111-113,115,116,118} Formation of thrombin – antithrombin complex is accelerated by an endogenous heparin-like molecule, heparan sulfate, a glycosaminoglycan that is found on the endothelial surface.\textsuperscript{118} Through interaction with glycosaminoglycans, antithrombin may stimulate the endothelial production of prostacyclin, thus exerting an antiinflammatory effect.\textsuperscript{118} During sepsis antithrombin III levels are low due to rapid consumption by the ongoing thrombin formation, impaired synthesis and degradation by elastase from activated neutrophils.\textsuperscript{112,115-117} Thrombin generation is limited by antithrombin III, the protein C system and tissue factor inhibitor.\textsuperscript{116} During sepsis and as a result of endothelial dysfunction, all three regulatory systems are defective.\textsuperscript{6,113,116} The end result is that sepsis causes enhancement of procoagulant state, leading to thrombin generation and fibrin deposition.\textsuperscript{112,113}

Tissue factor inhibitor is the only natural inhibitor of tissue factor and it is synthesized by the endothelial cells.\textsuperscript{115} Endothelial injury occurs as the result of activation and degranulation of neutrophils.\textsuperscript{111} Endothelial injury exposes sub-endothelial collagen and tissue factor, thus activating the coagulation cascade and platelet adhesion.\textsuperscript{111} TNF and IL-1 affect the endothelial function by down regulating the expression of thrombomodulin, release of PAF and vWF, expression of leukocyte adhesion proteins and possibly expression of tissue factor.\textsuperscript{111} During sepsis there is a depletion of tissue factor inhibitor.\textsuperscript{115} However in human septic patients the concentrations of tissue factor inhibitor are not diminished from normal.\textsuperscript{116} These
changes transform the endothelium from antithrombotic to prothrombotic and these changes vary between different vascular beds.\textsuperscript{111,113} Thus sepsis induces vascular bed specific changes in the hemostatic balance.\textsuperscript{113} Platelets also become activated during sepsis.\textsuperscript{111,114} Endothelial injury, by exposing collagen, stimulates platelet adhesion and aggregation.\textsuperscript{111,115} Injured endothelial cell also express platelet-endothelial adhesion molecule (PECAM), which promotes platelet adhesion and aggregation.\textsuperscript{111} Endothelial dysfunction characterized by the decrease of PGI\textsubscript{2} and ADP\textsubscript{asa} production also causes platelet activation.\textsuperscript{111} The major mediators of platelet activation during sepsis are thrombin and PAF.\textsuperscript{111} PAF is produced by TNF stimulated endothelial cells and by activated neutrophils, macrophages and platelets.\textsuperscript{111} Platelets of horses are more sensitive to PAF when compared with platelets of dogs, rats, rabbits, sheep and guinea pigs.\textsuperscript{111,120} Platelets form the initial mechanical plug and have procoagulant and proinflammatory properties.\textsuperscript{115} Platelets also are the principal contributors of membrane binding sites for the coagulation complexes.\textsuperscript{115}

Thrombocytopenia is a frequent abnormality diagnosed in septic patients.\textsuperscript{121} Platelets are consumed during DIC in horses and humans.\textsuperscript{117,121,122} A second mechanism responsible for increase platelets destruction is an immune mediated process linked with platelet-associated immunoglobulin G.\textsuperscript{121} Bone marrow suppression has also been ascribed as a cause of sepsis induced thrombocytopenia.\textsuperscript{121} A newly described entity, hemophagocytosis, is characterized by unrestrained proliferation and activation of monocytes and macrophages that actively ingest hematopoietic cells.\textsuperscript{121} This process is carried out principally in the liver and spleen.\textsuperscript{121}
Local and systemic coagulation disorders, including disseminated intravascular coagulation (DIC), are a common problem in sepsis.\textsuperscript{6,111,115-117,119,122} The derangement in the coagulation system includes activation of coagulation, depression of inhibitory mechanisms of coagulation and inhibition of the fibrinolytic system.\textsuperscript{112-114} The major procoagulant events at the sites of inflammation include expression of tissue factor, altered thrombogenicity of endothelial cells, vasoconstriction and activation of platelets.\textsuperscript{111,114,115} These procoagulant pathways are balanced by natural inhibitory systems, including tissue factor pathway inhibitor, antithrombin III, protein C system and fibrinolytic pathway.\textsuperscript{115} Thrombomodulin on the endothelial surface integrates these pathways.\textsuperscript{115} The depletion of platelets and coagulation proteins due to the extensive and ongoing activation of the coagulation system can induce severe bleeding complications.\textsuperscript{112} The end result is the development of concurrent thrombosis and bleeding, a process called DIC, a common complication of sepsis.\textsuperscript{112,115,123} DIC is a syndrome characterized by activation of coagulation pathways and a dysfunction in the natural inhibitory pathways and the fibrinolytic system, leading to widespread microvascular thrombosis and profuse bleeding.\textsuperscript{115-117,122,123} The molecular pathogenesis of DIC in sepsis involves a complex series of interactions triggered by damage to the endothelium.\textsuperscript{115}

DIC in horses is difficult to identify because the coagulopathy commonly occurs in a compensated form, only detected by laboratory abnormalities and attended by few clinical signs.\textsuperscript{122,123} Worsening of the primary disease can cause the subclinical coagulopathy to rapidly evolve to a severe DIC with obvious clinical signs of
microvascular thrombosis and or tendency to hemorrhage. 122 Sepsis and colic are the 
most common causes of DIC in the horse. 122,123 The generation of microvascular thrombi 
in various organs, ultimately can contribute to the pathogenesis of MOF. 112,116,117 In the 
horse microthrombus formation at the afferent renal arteriole produces ischemic cortical 
necrosis and subsequent oliguria and azotemia.122 Acute tubular necrosis follows and 
further impairs renal function. 122 In horses with primary gastrointestinal disease, 
intestinal microthrombus due to DIC further complicates the clinical condition. 122 
Furthermore, microthrombi can cause digital ischemia and secondary laminitis. 122,123 
Thrombosis in the large veins, especially after catheterization, is also a common 
complication of DIC in the horse.122,123 In the horse pulmonary involvement in DIC is 
rarely observed. 122 The equine liver has a large capacity to generate and replenish 
clotting factors, therefore a chronic intermittent to low grade procoagulant stimulus is 
rarely attended by hemorrhage.122,123 Most of the hemorrhagic manifestations of DIC in 
horses are a result of thrombocytopenia and or the anticoagulant effects of fibrinogen 
degradation products.122 The most common signs of DIC in the horse are petechial or 
echymotic hemorrhage on mucus membranes and a tendency to bleed from venipuncture 
sites. 122 However once horses reach the point of having clinical signs of hemorrhage 
there is little that can be done to reverse fulminant coagulopathy. 123 

In the horse as well as in humans, DIC is an elusive syndrome to diagnose, due to the 
lack of test sensitivity and specificity and the dynamic nature of the process. 117,122,123 In 
clinical practice the diagnosis can often be made by a combination of platelet count, 
measurement of global clotting times (aPTT and PT), measurement of one or two clotting
factors and inhibitors (such as antithrombin) and test for fibrin degradation products (FDP’s). It is important to recognize that serial testing is more helpful than single laboratory results in establishing the diagnosis of DIC.

**MODS-MOF-Death**

In human patients there are three different hemodynamic patterns of death in septic shock. Early deaths are due either to distributive shock [low systemic vascular resistance (SVR) and refractory hypotension despite preserve CI] or to a cardiogenic form of septic shock (decreased CI). Late deaths are due to MOF. Multiple organ failure is the most common cause of death in critically ill human patients. The mechanisms for the development of MOF after resuscitation in critically ill patients are not known. Incomplete reversal of tissue hypoxia and continued occult tissue hypoxia could be mechanisms that lead to MOF. Based on the concept of pathologic dependence of oxygen consumption on oxygen delivery in critically ill patients is that occult tissue hypoxia has being suggested as one possible cause of MOF. This concept is supported by some studies and refuted by others.

**Treatment of endotoxemia**

Therapy of septic shock has three main components. The initial priority is to maintain a reasonable mean arterial pressure and cardiac output to keep the patient alive. Secondly the nidus of infection must be identified and eliminated, using
antimicrobial therapy or surgical drainage.\textsuperscript{15,126} Another therapeutic goal is to interrupt the pathogenic sequence leading to sepsis.\textsuperscript{126} Adequate organ system perfusion and function must be maintained, guided by cardiovascular monitoring.\textsuperscript{126} In summary, treatment of sepsis must include early identification and correction of the primary disorder, along with supportive care which is the primary treatment modality.\textsuperscript{15,127,128}

The treatment of septic shock requires an early and vigorous resuscitation.\textsuperscript{126} An integrated approach directed at rapidly restoring systemic oxygen delivery and improving tissue oxygenation has been demonstrated to improve survival in septic human patients.\textsuperscript{129}

Most of the treatments for sepsis are more effective if they are instituted before or coincident with the onset of sepsis, this is because many mediators are released within minutes of the initial insult.\textsuperscript{127} In human medicine the death rates in some subgroups of patients with sepsis induced organ failure have decreased, even though there is no specific therapy for sepsis.\textsuperscript{130} The reduced mortality may be due to changes in the definition of sepsis, better detection and treatment of the underlying infection, or improved supportive care.\textsuperscript{130} Agents tested in large, well designed trials have not reduced overall mortality, though there have been benefits in some subgroups of patients.\textsuperscript{130}

The most common treatments for endotoxemia in horses include: intravenous fluid therapy, nonsteroidal anti-inflammatory drugs, antibiotic therapy, hyperimmune antiendotoxin plasma, dimethyl sulfoxide (DMSO), hypertonic saline solution, heparin
and corticosteroids. Current methods for treatment of endotoxemia in horses focus on one or more of the following interventions:\textsuperscript{8,12,127,131,132}

- Reducing the movement of LPS into the circulation
- Scavenging of LPS to prevent interaction with inflammatory cells
- Inhibition of the synthesis of endogenous pro-inflammatory mediators, released in response to LPS.
- Interference with the effects of inflammatory mediators
- Supportive care.

**Reducing the movement of LPS into the circulation**

This is based on identification and removal of the source of sepsis.\textsuperscript{8} Early surgical removal of a strangulated or ischemic segment of bowel is a key factor for survival of the animal.\textsuperscript{12,131} If the source is a localized infection, then drainage, lavaged and antibiotic therapy should be instituted.\textsuperscript{8,131}

This approach is more difficult or impossible if there is a diffusely inflamed gut wall, such as in colitis.\textsuperscript{131}

**Preventing interaction of LPS with cells**

*Hyperimmune antiendotoxin plasma*

The administration of antibodies directed against the conserved core oligosaccharide and lipid A regions of mutant Gram-negative bacteria (J5 *Escherichia coli* and Re mutant
of Salmonella typhimurium).\textsuperscript{12,15,127,131} This antibodies are intended to neutralize endotoxin from a variety of bacterial species and aid with opsonisation.\textsuperscript{127,131}

There is controversy in the equine and human literature regarding the effectiveness of antiendotoxin antibodies.\textsuperscript{12,15,16,127,128,131,133}

Polymyxin B

Polymyxin B is a cationic amphipathic cyclic polypeptide antibiotic that avidly binds to the anionic lipid A portion of LPS, neutralizing its endotoxin capacity.\textsuperscript{8,12,15,19,84,131,133-135} Because Lipid A is shed by all species of Gram negative bacteria, polymyxin B has a broader endotoxin neutralizing capability compared with serotype specific antiendotoxin antibodies.\textsuperscript{134} In addition Polymyxin B disperses endotoxin aggregates and reduces the ionic density and aqueous solubility of LPS, therefore decreasing its half life in the blood.\textsuperscript{134} Polymyxin B should be administered as early in the disease process as possible.\textsuperscript{8,12,19,134} Experimental studies demonstrated that pretreatment with polymyxin B before endotoxin challenge improved clinical signs and decreased levels of circulating proinflammatory cytokines.\textsuperscript{15,19,134} The effectiveness of polymyxin B is time and dose dependent.\textsuperscript{19} Polymyxin B significantly lowers plasma cytokines (TNF and IL-6) and prostanoids metabolites (TxB\textsubscript{2}), thus decreases proinflammatory mediators of sepsis.\textsuperscript{84,133-135} The recommended dose for polymyxin B in horses is between 1,000 to 5,000 U/kg every 8 to 12 hours.\textsuperscript{12,19,134}
Blocking of the endogenous pro-inflammatory mediators

Nonsteroidal antiinflammatory drugs (NSAIDs)

An important effect of TNF and IL-1 is the induction of phospholipaseA2 (PLA-2) and cyclooxygenase-2 (COX-2) thus the production of eicoanoids, which are responsible for several pathologic changes during inflammation. $^{15}$ NSAIDs inhibit cyclooxygenase, the enzyme responsible for the generation of prostaglandins and thromboxanes. $^{12}$ The acute organ specific vasoconstriction associated with endotoxin administration can be blocked with cyclooxygenase inhibitors. $^{36}$ Cyclooxygenase inhibitors have been shown to delay or prevent the development of systemic hypotension in horses. $^{10,36}$

The most widely used NSAID in equine medicine is flunixin meglumine, a potent inhibitor of the cyclooxygenases (COX-1 and COX-2) which thereby inhibits prostanoid production. $^{8,12,15,128,131,136}$ Flunixin meglumine has been shown to prevent or attenuate early hemodynamic responses to endotoxin administration and to reduce endotoxin induced increases in plasma concentrations of TXA$_2$ and PGI$_2$. $^{8,81,127,128,131,132}$

There is evidence that NSAID’s elicit antiinflammatory effects by alternative COX independent mechanisms. $^{136,137}$ In vitro studies in horses showed that flunixin meglumine inhibits LPS induced i-NOS and nuclear translocation of NFkB. $^{136}$ The inhibition of NFkB activation provides another COX-independent mechanism by which flunixin meglumine can prevent or reduce deleterious effects of LPS. $^{136}$ However it is not clear if these effects would be seen in vivo. $^{136}$ In horses the administration of flunixin meglumine does not reduce the increase in TNF response to administration of LPS. $^{13}$
Interference with the effects of inflammatory mediators

This approach is based on the use of nontoxic endotoxin or lipid A structures. These forms of treatment remain under investigation in equine medicine.

Supportive care

Intravenous fluid therapy

The mainstay of fluid resuscitation in equine medicine has been the use of crystalloid solutions. Intravenous fluid therapy is the most widely used and least controversial treatment for endotoxemia in the horse and in a survey among diplomats of the American College of Veterinary Internal Medicine, Lacted Ringer’s solution was the most commonly used intravenous solution. Intravenous fluid therapy is essential in decreasing mortality and speeding recovery of sick horses and human. In human medicine initial treatment of septic shock includes aggressive volume resuscitation, and the most widely used resuscitation fluids in human septic patients in North America are 0.9% NaCl (normal saline) and Lactated Ringer’s. Design and implementation of an adequate fluid therapy plan require evaluation of the patient’s physical condition, understanding of the principles of body fluid homeostasis and distribution, and selection of the appropriate fluid type (colloid or crystalloid). Shock and sepsis are associated with the failure of the circulatory system to maintain adequate delivery of oxygen and other nutrients, causing cellular and organ dysfunction. In addition to a decrease in arterial vascular tone, venous capacitance increases resulting in a decline in the effective
circulating volume. Moreover, the alteration in the endothelial barrier may result in hypovolemia in the absence of obvious fluid loss. The rationale behind fluid administration in septic patients is as follows: correction of blood and plasma volume (correction of hypovolemia), improvement of cardiac output, normalization of tissue oxygenation, prevention of organ dysfunction and correction of electrolyte and acid base abnormalities. Restoration of blood flow (tissue perfusion) is essential for preventing tissue ischemia and subsequent development of MOF. It is important to recognize that hemodynamic support in septic patients requires consideration of both global and regional perfusion. On the other hand, administration of several liters of fluids, necessary for normalization of hemodynamic variables, can be associated with the adverse effect of extravasation into the interstitial space. In sepsis in particular, this may result in pulmonary edema.

The major question regarding volume support in septic patients is what solution to use: isotonic crystalloids, colloids (and which colloids), or hypertonic saline, and which of these solutions is best in terms of effectiveness and safety. Despite more than twenty years of human and animal studies, the optimal fluid for resuscitation in a given clinical situation remains unclear. Furthermore, the preferred volume expander in patients with sepsis and SIRS is also controversial, and there is no conclusive data demonstrating that the type of fluid has a major impact on outcome. Septic patients can be successfully resuscitated with either fluid type (crystalloids or colloids). Increases in cardiac output and systemic oxygen delivery are proportional to the expansion of the intravascular volume achieved.
crystalloids and colloids are titrated to the same level of filling pressure, they are equally effective in restoring tissue perfusion. However, advocates of colloids argue that hypo-oncotic crystalloids leak from the plasma to excessively expand the interstitial fluid volume, resulting in adverse outcomes as a consequence of pulmonary and peripheral edema, whereas crystalloid supporters argue that leakage of colloid into the interstitial space contributes to edema formation. Furthermore most of the studies have compared only the short term effects of volume infusion, and serial data on the effects of long term volume therapy with different solutions on regulators of the circulation in the critically ill are lacking. What's more, the data from human meta-analyses comparing crystalloids and colloids are conflicting with respect to mortality. These discrepancies are attributed to the poor quality of many of the underlying clinical studies, heterogeneity in the patient population and the fact that none of the clinical studies were designed with mortality as an end point.

The major complications of fluid resuscitation are pulmonary edema and systemic edema. These complications are related to three principal factors: a) increase in hydrostatic pressure, b) decrease in colloid osmotic pressure and, c) increase in microvascular permeability associated with sepsis. Intravenous fluid administration is well tolerated if the microvascular integrity is preserved, but the inflammatory response that occurs in sepsis results in increased vascular permeability. Significant vascular leakage of fluid and albumin causes interstitial edema, which adversely affects organ function. Therefore treating a septic patient, who has increased capillary permeability and low plasma oncotic pressure, with large volumes of crystalloids could
be associated with an increased risk for development of interstitial edema and pulmonary edema, which interferes with tissue oxygen exchange and delay wound healing. On the other hand fluid leakage into the intravascular space produces hypovolemia and reduction of the cardiac output. Decreased cardiac output compromises peripheral and splanchnic perfusion, which in turn causes cardiovascular collapse.

In critically ill patients only 21 to 25 % of the infused volume of crystalloids remains in the intravascular space, while 75 % gains access to the interstitial space. In the clinical setting this can be interpreted as 100 to 200 ml of intravascular volume expansion can be expected after infusion of one liter of isotonic crystalloids. Infusion of large volumes of crystalloids reduces colloidal oncotic pressure, by a dilutional effect, and transiently increases intravascular hydrostatic pressure. Thus these forces will favor the movement of water out of the vascular space.

Intravenous administration of colloids will result in an increase in the plasma colloid oncotic pressure, therefore increasing in plasma volume (Starling’s law). Increased circulating volume increases cardiac preload, arterial blood pressure, cardiac contractility and cardiac output, thereby improving perfusion and oxygen delivery. The currently accepted view in human critical care is that maintenance of colloid osmotic pressure is of secondary importance to maintenance of circulating volume. It is true that the use of well retained colloids may prevent further reductions in colloid oncotic pressure, but very few human intensive care units measure colloid oncotic pressure. Therefore the primary goal in the clinical setting is to restore circulating volume.
In experimental models of sepsis it has been demonstrated that there is no increase in extravascular lung water when hydrostatic pressures are maintained at low levels, indicating that in sepsis the primary determinant of pulmonary extravascular fluid flux appears to be microvascular pressure, rather than colloid osmotic pressure. \textsuperscript{126,154} It can be concluded that when lower filling pressures are maintained there is no difference in the development of pulmonary edema with crystalloids or colloids. \textsuperscript{126} However, if higher filling pressures are required to optimize cardiac performance in patients with ventricular dysfunction, colloids may be more beneficial in preventing pulmonary edema. \textsuperscript{126}

Colloids can be of two types: natural (plasma, whole blood and albumin) or synthetics [hydroxyethyl starch solutions (Hetastarch and Pentastarch), dextran, gelatin and polymerized hemoglobin]. \textsuperscript{79,150,152} It is important to notice that the effects of various colloids differ. \textsuperscript{142,153} There are a variety of colloid solutions that vary in molecular sizes, half lives, colloid oncotic pressure, side effects and costs. \textsuperscript{142} The most commonly used solutions in clinical resuscitation in human medicine are albumin and hydroxyethyl starch (HES). \textsuperscript{56,126}

The remainder of this discussion will deal with HES, a high polymeric glucose compound, manufactured through hydrolysis and subsequent hydroxyethylation from the highly branched starch amylopectin that has volume expansion properties. \textsuperscript{142,151,155-157} HES consists of D-glucose units that are connected within the chain through a-1,4 glycosidic bonds and through a-1,6 glycosidic bonds at the branching points with the hydroxyethyl groups (Figure 5). \textsuperscript{151,155,156,158}
Originally, HES was only characterized by its *in vitro* molecular weight (MW) however this characterization is not sufficient because HES is degraded *in vivo* by α-amylase, independently of the *in vitro* MW. The MW can be regarded as the average molecular weight, which is a simple arithmetic mean, or as weight average molecular weight. The weight average molecular weight is the most commonly used in clinical medicine. Depending on the distribution of weight average molecular weight constituting the particles, colloids can be either monodisperse or polydisperse. Monodisperse colloids consist of molecules of one molecular weight only (e.g. albumin), while polydisperse (e.g. HES) contain a range of different MW.

The degree of substitution of HES (expressed as a number between 0 and 1) indicates the average number of hydroxyethyl groups per glucose unit. The molar substitution ratio is computed by counting the total number of hydroxyethyl groups present and dividing the number by the quantity of glucose molecules. These numbers are not equivalent although they are often incorrectly used interchangeably in the literature. The importance of the degree of substitution is that α-amylase can only degrade unsubstituted glucose units. By changing the degree of substitution, it is possible to influence the degree of enzymatic breakdown and to exert control over the extent and duration of the volume effect. A higher degree of substitution results in a slower breakdown and elimination of the molecule.

The hydroxyethylation can occur at carbon positions C2, C3 or C6 of the glucose molecule, depending on manufacturing. Therefore the final product can vary greatly in the substitution pattern. The substitution type is identified by the C2/C6
hydroxyethylation ratio.\textsuperscript{157} The higher the ratio, that is the higher the number of glucose molecules hydroxyethylated at the C2 atom versus at the C6, the slower the starch is metabolized.\textsuperscript{150,157,158}

In summary the pharmacokinetics of HES are directly related to its particle size (MW), which determines colloidal activity, and to its degree of substitution and hydroxyethylation pattern (C2/C6 ratio) which are the major determinants of metabolism and circulating half life.\textsuperscript{142,150,157}

HES is removed from circulation by two major mechanisms: renal excretion and redistribution.\textsuperscript{156} A third pathway, biliary elimination, is of minimal importance (less than 1 \%).\textsuperscript{79,156} The most important mechanism is renal elimination (removes 70 – 80 \% of the molecules) and it consists of two phases.\textsuperscript{79,151,156} The first occurs almost immediately after administration, because polymers with a MW of less than 50 kD are rapidly eliminated by glomerular filtration.\textsuperscript{142,151,156,157} A second phase of glomerular filtration is more prolonged and occurs as the HES molecule is metabolized.\textsuperscript{142,151} Metabolism results in an average \textit{in vivo} MW that is significantly lower than the average MW of the infused solution.\textsuperscript{150} As stated above the presence of hydroxyethyl groups slows enzymatic degradation, however hydrolysis by a-amylase does occur and serum a-amylase activity increases after HES administration.\textsuperscript{156} Once the product of a-amylase digestion is smaller than 72 kD, it can be renally excreted.\textsuperscript{142,156} Some of these molecules, metabolized by a-amylase, are also excreted in the bile.\textsuperscript{142} The second mechanism of removal from the circulation is redistribution (accounts for 20 to 30 \% of the elimination), which is an uptake and temporary storage of HES in the tissues.\textsuperscript{79,156} The
extravasated molecules are stored in phagocytic cells of liver, lymph nodes and spleen.\textsuperscript{79} Storage of particles is evident on histological examination, especially liver and spleen, where large intracellular vacuoles are seen.\textsuperscript{79} Degradation by lysosomal enzymes occurs over time and organ function is not impaired by vacuole accumulation.\textsuperscript{79} After 24 hours, 23\% of the total dose of HES is extravasated into the interstitial space.\textsuperscript{156} As result of these processes, only 38\% of the initial dose remains in the intravascular space 24 hours post administration, whereas 39\% is excreted in urine and 23\% is sequestrated in tissues.\textsuperscript{156} The duration of volume expansion with HES is approximately 24 hours, although trace amounts can be detected in the circulation for up to 17 – 26 weeks.\textsuperscript{79,126,151,156,157} The elimination rate of HES varies both over time and among species.\textsuperscript{79} Variation in elimination of larger molecules may reflect species differences in serum a-amylase concentrations.\textsuperscript{79,152} Serum a-amylase concentration and activity is less in horses compared with humans.\textsuperscript{79,152} This could imply a potential advantage in horses due to the potentially prolonged duration of effect.\textsuperscript{79} The enzyme a-amylase due to intravascular hydrolysis of large polymers of HES yields a grater number of osmotically active molecules and would serve to sustain the increases in plasma oncotic pressure.\textsuperscript{138}

There are different types of HES solutions, which vary depending on the MW, degree of substitution and C2/C6 ratio.\textsuperscript{157} Data concerning the extent and duration of volume expansion between different HES solutions is difficult to compare.\textsuperscript{157} The most widely used HES solution in human and veterinary medicine in the United States is the HES 450/0.7, meaning that the average MW is 450 kD and the degree of substitution is 0.7 (70\% of its glucose units have a hydroxyethyl group).\textsuperscript{142} HES is composed of a
heterogeneous population of molecules, with 80% of the molecules having MWs between 300 and 2,400 kD.\textsuperscript{79}

The most common cause of septic shock in horses is endotoxemia associated with intestinal disease.\textsuperscript{79} As stated above the primary goal for treatment of shock and sepsis is the restoration of the effective circulating volume, in order to provide adequate tissue perfusion.\textsuperscript{43,79,153} Rapid resuscitation of endotoxemic horses requires rapid administration of large volumes of crystalloids, often in excess of 80 ml/kg.\textsuperscript{79,138} The administration of such large volume of fluid in a septic patient may potentiate the formation of interstitial edema and pulmonary edema, with potential adverse effects on tissue oxygenation and organ function.\textsuperscript{79,140,152} In contrast with crystalloid, resuscitation with colloids preserves the colloid oncotic pressure, thereby limiting transcapillary fluid movement and resulting in a more effective volume expansion.\textsuperscript{152} Resuscitation with colloids requires smaller volumes and shorter infusion times to restore hemodynamic stability and improve tissue oxygen transport.\textsuperscript{138,140,152} The infusion of one liter of hydroxyethyl starch solution expands the plasma volume by 700 ml to 1 L.\textsuperscript{126} It has been shown that colloids improve myocardial contractility and cardiac output.\textsuperscript{140} Despite this apparent advantages a meta-analysis of human studies where crystalloids versus colloids were reviewed, failed to demonstrate any difference in mortality, pulmonary edema or length of hospital stay between crystalloids and colloids.\textsuperscript{140} And as stated above, other meta-analyses also failed to demonstrate differences in outcome.\textsuperscript{126} However, experimental studies with endotoxemic rats resuscitated with a crystalloid (0.9% saline) or a colloid (Hextend, which is 6% Hetastarch in balance electrolyte solution), the
investigators found a longer survival time, less metabolic acidosis and smaller amounts of fluid were needed for the colloidal group.\textsuperscript{141}

In septic human patients treated with long term volume therapy (five days) using HES solution improvements in splanchnic perfusion, oxygenation and improvement in systemic perfusion were noticed.\textsuperscript{67} In HES treated patients the PaO\textsubscript{2}/FiO\textsubscript{2} increased and also oxygen delivery (DO\textsubscript{2}) along with oxygen consumption (VO\textsubscript{2}) increased, these findings were suggestive of an improvement in pulmonary function.\textsuperscript{56} The improvement in oxygenation seen in septic patients treated with HES may have resulted from improvement in the pulmonary microcirculation or from a reduced fluid shift from the intravascular to the interstitial space in the lung, thus less extravascular lung water.\textsuperscript{67} The explanation for these findings was speculative and several potential theories potentially could have played a role, such as protective effects of HES on the microvasculature and occlusive effects of damaged endothelium.\textsuperscript{56} The higher preload (increased CI) in patients treated with HES was thought to be the cause of the decreased in circulating vasopressors (vasopressin, ET-1, adrenaline and noradrenaline) in that study.\textsuperscript{67} On the other hand the beneficial rheological effects of HES may have improved the microcirculation and tissue perfusion, therefore further contributing to the lesser activation of the vasopressor system.\textsuperscript{67}

HES may have the ability to seal selectively the endothelial “pores” or gaps that develop in the microvessels after different forms of injury including sepsis.\textsuperscript{156} By this means HES may prevent the leakage of plasma proteins (especially albumin) from the intravascular space, thus preventing the secondary fluid extravasation.\textsuperscript{156} The medium
MW particles, between 100 to 300 kD, are the ones that may act as plugs at the endothelial “pores” or gaps.\textsuperscript{79,152,159} It has been postulated that there are two different sized pores in the capillaries, small pores through which only water and electrolytes can flow and large pores through which large molecules such as albumin can flow.\textsuperscript{83,151} According to this theory it is the number of the pores rather than the size, that increases in patients with capillary leak syndrome.\textsuperscript{151} The oncotic pressure produced by HES would not affect the flow through the large pores but it would decrease the flow through the small pores that are assumed to account for the major porosity of the capillaries, both in health and in sepsis.\textsuperscript{83,151} There are no clinically applicable techniques for measuring the magnitude of microvascular changes during vascular leak states, the only clinical indicator would be an unexpectedly short duration of action (volume expansion) of the infused colloid.\textsuperscript{159}

HES has the ability to modify the endothelial inflammatory response and by these means attenuate the permeability dysfunction associated with sepsis.\textsuperscript{152} In one of the few long term studies (five days) using human septic patients, patients treated with volume therapy with HES solution had a significant reduction in the plasma concentration of soluble endothelial leukocyte adhesion molecule 1(ELAM-1), soluble ICAM-1 and soluble VCAM-1.\textsuperscript{43} This could also explain the reason why this therapy produces less tissue edema and injury.\textsuperscript{43,151} Decreased circulating adhesion molecules may indicate diminished endothelial damage or represent decreased endothelial activation.\textsuperscript{151} It has been suggested that HES inhibits P-selectin expression.\textsuperscript{43,151} Because leukocyte – endothelial cell interaction are a prerequisite for trans-endothelial emigration and
infiltration of leukocytes into the tissues, attenuation of this pathway would reduce tissue injury. Thus by binding to surface receptors HES molecules could alter the expression of adhesion molecules. Moreover HES could decrease the expression of adhesion molecules by acting as a scavenger for oxygen free radicals and possibly by decreasing the release of cytokines (IL-6). The effect on reduction of IL-6 is important because this cytokine exerts detrimental effects at the microcirculatory level. HES improves macro and microcirculatory flow, resulting in less expression or less release of adhesion molecules in the circulation, thereby reducing leukocyte adhesion.

The release of xanthine oxidase, an oxidant generating enzyme, after ischemia reperfusion injury, may play an important role in MOF. The liver and the gut contain the highest tissue activity of this enzyme. Several studies have documented that HES solutions reduce reperfusion injury in animal models. Also in vitro work has shown that HES scavenges hydroxyl radicals, therefore HES can act as an antioxidant. In a rabbit ischemia reperfusion model, it was shown that HES decreased organ injury after ischemia reperfusion by preventing the decrease the ascorbic acid (plasma antioxidant properties) and decreasing the release of xanthine oxidase.

Adverse effects of HES solutions include alterations of hematological, immunological, renal and reticuloendothelial function. In human medicine there is controversy regarding the adverse effects of HES on transplanted kidneys. Patients with severe sepsis or septic shock are at risk of ischemic renal injury, and they require large amounts of plasma volume expanders. Prevention of renal dysfunction is of paramount importance in septic patients, because there is no curative treatment for
acute tubular necrosis. A significantly greater incidence of acute renal failure was notice in the HES treated group compared with a gelatin treatment group in human septic patients. It is recommended that HES be avoided in patients with renal dysfunction or at risk of developing acute renal failure.

Anaphylactic reactions are mediated by antigen antibody interactions in subjects previously exposed to a drug, on the other hand anaphylactoid reactions are considered to be caused by the direct effects of the drug on mast cells and basophils, with the release of histamine and other mediators. These two types of reactions are clinically indistinguishable. Because the existence of preformed antibodies against HES is extremely rare, it has also been postulated that HES directly activates the complement to mediate anaphylactoid reactions. In human medicine the rate of anaphylactoid reactions to HES is approximately 0.006% of the infusions.

Plasma viscosity is determined by the number and physical properties of macromolecules in plasma. Plasma viscosity is an important factor that contributes to the microcirculatory disturbances that characterize shock. Highly substituted HES is less desirable because it increases plasma viscosity. On the other hand medium MW with low C2/C6 ratio and low MW starches decrease plasma viscosity and have better rheological properties.

A major concern regarding use of HES relates to its effects on hemostasis. However, there is conflicting and controversial information in the literature regarding the effects of HES solutions on coagulation. In addition the true risk for bleeding complications of patients treated with HES solutions is difficult to predict, unless the
patient has a known coagulopathy in which case HES should not be used. ¹⁵⁶,¹⁵⁷,¹⁶²

However, clinical bleeding is greatly influenced by the quantity of HES infused, the preparation selected, whether single or multiple infusions are given, the nature of other fluids infused, and the medical condition of the patient (e.g. underlying coagulation abnormality or thrombocytopenia). ¹⁵⁰,¹⁵⁷,¹⁶² In critically ill patients hemostasis can occur by the activation of the coagulation cascade via the intrinsic or extrinsic systems. ⁵⁶ The released of inflammatory mediators (TNF and IL-1), may have additional effects on coagulation. ⁵⁶ Moreover, patients with septic shock are at risk of developing DIC and thrombocytopenia. ¹⁴³

Hydroxyethyl starch can cause dose dependent decreases in factor VIII and vWF activity and prolongation of PTT. ¹²⁶,¹⁵¹,¹⁵⁷,¹⁶² These effects appeared to be primarily dilutional, with only minor clotting abnormalities and no increased incidence of bleeding has been noted in patients with septic shock. ¹²⁶,¹⁵⁷ These abnormalities may only be relevant in patients with initially low levels of clotting factors and or patients undergoing surgery. ¹⁵¹ Factor VIII is an important factor for maintaining a normal aPTT. ¹⁵⁷,¹⁶² HES 480/0.7 (Hetastarch) produces a decrease in factor VIII that is beyond those effects attributed to hemodilution, and presumably are due to additional mechanisms. ¹⁵⁷,¹⁶² It also has been shown that the bleeding complications of HES are due to an acquired von Willebrand syndrome. ¹⁵⁷,¹⁵⁸

With HES 480/0.7, bleeding complications are reported to have occurred after the use of high doses for several days. ¹⁵⁷,¹⁵⁸,¹⁶² On the other hand HES 480/0.7 produces minor effects on clotting when infused to human or animals in moderate amounts (not
exceeding 20 ml/kg or 1,500 ml total volume over twenty four hours).\textsuperscript{157,162,164,165} Platelet count either remained normal or decreased transiently, always remaining above the levels required for normal hemostasis (>100,000/ml).\textsuperscript{157,165} Plasma clotting times and specific clotting proteins either remained normal or exhibited slight alterations.\textsuperscript{157,165} Even when the clotting parameters were abnormal, specific clotting proteins were present in amounts sufficient to ensure effective hemostasis.\textsuperscript{157,165} Therefore the coagulation effects of moderate doses of HES 480/0.7 were transient and trivial.\textsuperscript{157,165}

It has been reported that after a single administration of HES the concentration of fibrinogen decreases.\textsuperscript{158} This effect is probably due to an acceleration of fibrin polymerization, and its effects on hemostasis are considered irrelevant.\textsuperscript{158} In a study with healthy human volunteers, HES 480/0.7 decreased fibrinogen concentration immediately after infusion. An alteration that was attributed to dilutional effects.\textsuperscript{162}

Thus the major effects of HES on coagulation are dilution of plasma clotting factors, additional decrease in factor VIII, and accelerated fibrin clot formation in the last stages of clotting.\textsuperscript{157}

An equine study was carried out to investigate the effects of HES in normal ponies.\textsuperscript{138} Ponies were divided in three groups those infused with HES at a dose of 10 ml/kg of HES and 20 ml/kg of HES and a control group were 80 ml/kg of saline was infused, all infusions were done within a two hour period.\textsuperscript{138} In the HES groups dose dependent alterations over time on vWF and factor VIII were observed.\textsuperscript{138} The effects on these coagulation proteins were beyond the dilutional effects of HES.\textsuperscript{138} The maximal decrease in factor VIII activity was coincidental with the maximal reduction in vWF activity,
suggesting the relationship of vWf as a carrier protein for factor VIII, direct effects of HES on factor VIII could have been responsible. A difference from other human and animal studies is that the aPTT and partial thrombin time (PTT) values were not prolonged, as would be expected based on the decreased in factor VIII concentration. For APTT to be markedly prolonged the activity of a single coagulation factor must be reduced to less than 30% of normal or the activity of multiple coagulation factors must be reduced. Therefore it can be assumed that the reduction in factor VIII were not great enough. The primary hemostasis was evaluated by measuring cutaneous bleeding time. Bleeding time is considered the most physiologically and clinically relevant test of the platelet response to vascular injury. Minimal effects were seen in this parameter in ponies treated with HES. A reduction in platelet count attributed to dilutional effect was observed in the group with the highest HES dose.

After massive doses of HES 480/0.7 (> 25% of the total blood volume), laboratory abnormalities were detected in all aspects of hemostasis and overt hemorrhage was frequently observed. These experiments were mostly carried out in dogs, in which large amounts of blood was removed and replaced by HES. Hemostasis abnormalities included thrombocytopenia, decreased platelets adhesion, decreased in the concentration of several clotting factors, prolonged clotting time and abnormal formation of the fibrin clot (lack of tight meshwork, friable and decreased tensile strength). These effects were considered to be caused just by hemodilution. Alterations in aPTT, PT and platelet count were not significant in human septic patients resuscitated with HES.
(6 % Hetastarch), using doses of $4,934 \pm 1,354$ ml over 24 hours, when compared with albumin resuscitated patients.\textsuperscript{143}

Pentastarch and HES 200/0.5, available in Europe, exert fewer effects on coagulation when compared with HES 480/0.7 (Hetastarch).\textsuperscript{157} In systematic studies evaluating the influence of a ten day therapy of the most commonly used HES products in Europe, differences among products were found.\textsuperscript{157} Prolonged APTT was found for 10 % HES 200/0.62/10.\textsuperscript{157,158} No changes on the APTT that went beyond the dilutional effect were found for low MW HES 70/0.5/4 and 10 % HES 200/0.5/6.\textsuperscript{157,158} Thus depending on the duration of therapy and the colloid used the aPTT is affected differently.\textsuperscript{158} The prolonged aPTT is indicative of a disorder of the intrinsic clotting system, mainly attributed to abnormalities in factor VIII-vWf complex.\textsuperscript{157,158} vWf supports the adhesion of platelets to the injured blood vessels.\textsuperscript{158} A decreased in factor VIII-vWf complex rarely leads to spontaneous bleeding but may considerably prolong bleeding after even small injuries.\textsuperscript{158} In human patients with von Willebrand syndrome a decreased in factor VIII is observed, probably caused by a decreased in vWf which serves as a carrier for factor VIII.\textsuperscript{158} So far the exact mechanisms by which HES inhibits factor VIII-vWf complex, is not known.\textsuperscript{158} It is possible that there is an acceleration of the \textit{in vivo} elimination of the factor VIII-vWf complex after attachments of starch molecules.\textsuperscript{157,158} The decrease in the VIII-vWf complex is more pronounced with higher doses of HES, larger initial MW, higher C2/C6 ratio, and in particular, higher degree of substitution.\textsuperscript{150,157,158} Therefore by choosing HES with a low \textit{in vivo} MW and a low degree of substitution, the bleeding complications could be minimized.\textsuperscript{157} However, other
work suggested that the use of a rapidly degradable HES (HES 200/0.5/6) in healthy human volunteers at a total dose of one liter, decreased factor VIII and vWf activities.\textsuperscript{150} Also the platelet adhesive function was altered; this was coincidental with the alterations in vWf.\textsuperscript{150} Another effect seen with the higher substituted (HES 200/0.62) starch, was the reduction of factor XI and XII.\textsuperscript{157,158} This finding suggested that alteration of the intrinsic system may not be restricted to factor VIII, however more study is require to test this hypothesis.\textsuperscript{157,158}

There are no studies that have demonstrated a decrease in platelet number, beyond dilutional effect, in association with HES therapy.\textsuperscript{158} A small but significant decrease in platelet volume was observed during a ten day infusion protocol, of medium to low molecular weight HES.\textsuperscript{157,158} The reduction in platelet volume was more pronounced with the higher \textit{in vivo} MW starch.\textsuperscript{157,158} The decrease in platelet number depends on the dose and volume effect of the starches used.\textsuperscript{158} The importance of decreasing platelet volume is not clear.\textsuperscript{150,157,158} The effects of platelet volume on platelet function is of little importance since the platelet aggregation is only slightly inhibited.\textsuperscript{157,158,168} It is suspected that this minor inhibition in platelet aggregation is related to the decrease in factor VIII-vWf complex.\textsuperscript{158,168} Reduction in this complex may result in reduced binding to platelet membrane receptor glycoproteins GP Ib and GP IIb/IIIa which may in turn result in decreased platelet aggregation.\textsuperscript{158,168} Use of rapidly degradable HES (HES 200/0.5/6) in healthy human volunteers suggested that the platelet adhesive function was altered; this was coincidental with the alterations in vWf.\textsuperscript{150}
The use of a low molecular weight HES in septic patients for a period of five days, at a dose that varied between 1,060 ml on day 1 to 5,030 ml on day 5, did not demonstrate significant difference in the APTT and PT values compared with an albumin treated group.\(^{56}\) Another short term study using low molecular weight HES (maximum dose of 2,000 ml) in septic patients did not detect significant changes in APTT, PT and platelet count.\(^{169}\) However a significant decrease in factor VIII was seen in comparison with albumin, although no clinical bleeding was observed.\(^{169}\)

HES solutions that are difficult to metabolize have the advantage of a long lasting volume effect.\(^{157}\) However, studies suggest that the use of HES with high initial MW or HES with high \textit{in vivo} MW, has unfavorable effects on coagulation.\(^{150,157}\) High MW HES affects blood coagulation, even if given over a limited period of time.\(^{150}\) Slowly degradable medium MW HES (with a high degree of substitution and high C2/C6 ratio) also impairs coagulation after repeated administration.\(^{150}\) These side effects can be avoided through the use of medium MW HES (with a low degree of substitution and low C2/C6 ratio) or low MW HES that is easier to degrade.\(^{150,157}\) These solutions have the disadvantage of shorter duration of effect due to their rapid elimination, this can be compensated by the use of repeated administrations.\(^{157}\) Recommendations limit the maximum dose of HES to 10 to 20 mg/kg/day due to concerns of adverse hematological, immunological, renal and reticuloendothelial function.\(^{138,142,157,159}\)

Experience with hypertonic saline solution (HSS) in septic shock is limited.\(^{126,145,146}\) Furthermore, administration of HSS during the early stages of septic shock when the patient is not yet hyperdynamic has not been investigated.\(^{145}\) However HSS resuscitation
is suggested to be a valuable alternative to traditional fluid regimes in the treatment of different types of shock, including endotoxin shock. Several prospective, randomized studies in human trauma patients have shown beneficial effects for resuscitating trauma patients with induced hypovolemia. There is also data supporting its use after severe hemorrhagic shock. However, there is a lack of uniformity in methods evaluating HSS which makes comparisons between studies difficult.

In cardiovascular compromised subjects, across species and forms of shock, the major advantages of HSS are the rapid expansion of blood volume for a small volume administered, improvement in cardiac contractility (increase cardiac output), increased blood pressure, decrease in systemic vascular resistance (precapillary vasodilatation), improvement in microvascular perfusion and decrease in lactate concentration.

The most important mechanism of action of HSS is the instantaneous mobilization of endogenous fluid along the osmotic gradient with increase of intravascular volume. This fluid shift is most important at capillary beds with swollen endothelium, the more swollen the endothelium, the greater the effect of HSS in reducing the hydraulic resistance and improving tissue perfusion. Therefore, capillary blood flow substantially increases. Experimental studies also suggest that HSS in combination with colloid solution improves microcirculation in sepsis. HSS also prevents vascular dysfunction and restores microcirculation by reopening capillaries. The end result is a beneficial redistribution of regional blood flow to the heart, kidneys and splanchnic organs.
In the last five years, a revived interest in HSS has occurred after certain reports demonstrated HSS mediated immune protection.\textsuperscript{37} The microcirculation is the main target of systemic inflammatory injury induced by sepsis.\textsuperscript{37} HSS induces profound effects on neutrophil function.\textsuperscript{37,41,42,177} Moreover, elevated levels of sodium inhibit exaggerated neutrophil responses, resulting in moderated proteolytic enzyme liberation, decreased respiratory burst and reduced expression of adhesion molecules (L-selectin and CD11b) on neutrophil surface.\textsuperscript{37,40-42,144,178,179} This has the effect of attenuating end organ injury.\textsuperscript{42} On the other hand endothelial expression of adhesion molecules (ICAM-1) is also altered by HSS.\textsuperscript{37,178}

Neutrophil sequestration within the lungs is a prerequisite for the development of acute lung injury during sepsis and therapy with HSS reduces the neutrophil accumulation within the lungs.\textsuperscript{42,144} Thus interference with neutrophil adhesion contributes to the antiinflammatory effects of HSS, preventing lung injury.\textsuperscript{41,144,178} Neutrophil degranulation with the release of proteases is the main mechanism of lung injury.\textsuperscript{42} Experimental studies have demonstrated that HSS resuscitation enhances clearance of bacteria from the lung and is associated with decreased bacteremia.\textsuperscript{42}

Data supports the fact that the administration of HSS increases IL-10 and reduces TNF and the oxidative burst, explaining the reason for the lesser degree of injury following HSS administration.\textsuperscript{144,179} Because HSS does not completely abrogate proinflammatory pathways, there is an adequate balance between proinflammatory and anti-inflammatory cytokines, thus maintaining the ability to fight bacteria efficiently.\textsuperscript{144}
The potential effects in blocking the cascade that leads to septic shock can be advantageous in the treatment of septic patients if therapy is instituted early enough in the course of the disease.\textsuperscript{144,145} Hemodynamic resuscitation can reduce the inflammatory response in sepsis and reduce the ischemia-reperfusion phenomena.\textsuperscript{144,171} Therefore the beneficial effects of HSS in septic shock are twofold, hemodynamic and immunomodulatory.\textsuperscript{144} These antiinflammatory effects could reduce the response to sepsis and attenuate later MOF.\textsuperscript{42,145,171,180}

Hemorrhage induces immunosuppression and enhances susceptibility to sepsis and MOF.\textsuperscript{181,182} The use of a “two hit” model (hemorrhage followed by cecal ligation and puncture) in mice showed that HSS attenuated the septic response and improved survival when compared to isotonic solution.\textsuperscript{181} It has been demonstrated that HSS enhances immune function, exerts a positive effect on T cells, increases proinflammatory cytokines, reverses PGE\textsubscript{2} induced immunosuppression and by decreasing plasma IL-4 and PGE\textsubscript{2} restores hemorrhage induced splenocyte suppression.\textsuperscript{45,181-183}

In summary a number of studies have affirmed that HSS prevented bacterial translocation and enhanced containment of infection during hemorrhagic shock.\textsuperscript{42,180,181,184,185} HSS attenuates the receptor mediated activation of proinflammatory cascades, while enhancing the host response to significant bacterial challenge.\textsuperscript{42} The blunting of adhesion molecule expression by HSS reduces aberrant endothelial cell – neutrophil interactions, resulting in less organ and tissue injury.\textsuperscript{37} Inflammatory insults followed by HSS administration consistently demonstrate fewer leukocyte interactions in
different microvascular beds. Thus by preventing vascular damage HSS decreases microvascular permeability.

HSS improves myocardial contractility and systemic microcirculation by an instantaneous mobilization of endogenous fluids and an increase in preload, direct myocardial stimulation, peripheral vasodilatation, redistribution of blood flow (in particular the mesenteric flow), reduction of tissue and endothelium edema and improving blood viscosity through hemodilution and reduction in erythrocyte size. Hypertonicity mobilizes fluid from high capacitance sources (venules) to increase intravascular volume. Thus, HSS leads to an increase in arteriolar flow at the expense of temporary venoconstriction but results in a generalized improvement in microcirculatory patency. Vasomotion, which is the continuous rhythmic contraction and relaxation of a vessel, is increased with HSS and this has been proposed as another mechanism by which HSS improves microhemodynamics. In addition the reduction in endothelium edema may improve tissue perfusion.

The hemodynamic effects of HSS plus colloid are more pronounced when compared with a normal crystalloid, however the effects are short lived (180 minutes).

In anesthetized horses, treatment with HSS after experimental hemorrhage, was associated with an increase in cardiac output, stroke volume, mean arterial pressure, mean pulmonary artery pressure and left ventricular end diastolic pressure. Peripheral
vascular resistance decreased.\textsuperscript{139} The plasma volume and urine output were also increased in horses treated with HSS compared with control.\textsuperscript{139} Infusion of HSS in horses with experimentally induced endotoxemia produced a more rapid decrease in pulmonary arterial pressure, increased cardiac output and decreased peripheral vascular resistance, compared to the administration of isotonic saline solution.\textsuperscript{88} In horses that underwent experimentally induced hemorrhagic shock and subsequently treated with HSS, a significant decrease in arterial and venous pH, venous bicarbonate and venous base excess concentrations were observed.\textsuperscript{186} These changes could be attributed to reperfusion of ischemic areas, an increase in anaerobic metabolism and lactate production or a combination of the two.\textsuperscript{186}

Small volume resuscitation is defined as a rapid infusion of 7.2 – 7.5 \% HSS, at a dose between 3 to 6 ml/kg, with or without addition of colloids.\textsuperscript{37,139,144,170-174,186} The advantages of small volume resuscitation are its ease of transport, speed of administration and rapid correction of hemodynamics.\textsuperscript{37} The short duration of circulatory effects of HSS has been attributed to the rapid equilibration of the hyperosmolar solute between the extracellular and intracellular compartments.\textsuperscript{171} The effects of HSS are short lived, thus in order to sustain the hemodynamic effect for a longer period of time is that colloids have been used in combination with HSS, and this combination seems necessary to significantly affect clinical outcome.\textsuperscript{170,171,173} There is a synergistic effect by combining HSS that increases plasma osmolality and mobilizes intracellular water, and colloids that increase the plasma oncotic pressure, thus conserving the volume effect.\textsuperscript{171} Experimental studies in septic shock have shown that HSS alone or in combination with HES produced
hemodynamic improvements; but these effects have a short period of action. Small volume resuscitation have been shown to effectively support central hemodynamics and improve the microvascular blood flow by reducing shock induced endothelial swelling and by restoring the microvascular surface area available for tissue oxygen exchange. Treatment of septic human patients with 250 ml of 7.5 % HSS induced a transient (less than 120 minutes) increased in cardiac index and pulmonary capillary wedge pressure. The beneficial effects of HSS on cardiac contractility are related to a direct hyperosmolar effect, restoration of transmembrane potential or decreasing myocardial edema. In septic patients HSS improves contractility, an effect that it is unrelated to changes in coronary blood flow or myocardial oxygen consumption. The hyperosmotic-induced decrease in cell water could be responsible for an increased calcium availability, due to calcium released from the sarcoplasmic reticulum, for contractile activation of the myocardium. The increase in intracellular calcium may enhance contractile protein binding. Experimental data in dogs and pigs however, failed to demonstrate a direct myocardial stimulation effect of HSS. Therefore the circulatory effects were attributed to rapid augmentation of ventricular preload (endogenous fluid mobilization) and reduction of afterload. Following HSS resuscitation there is an increase in venous return due to plasma volume expansion; this is an important contributor to the rapid improvement of cardiovascular function. The increase in cardiac output and restoration of peripheral blood flow could also be mediated by vasodilating substances (PGI2) released after HSS infusion. Differences between studies regarding the mechanism of improved cardiac performance can be attributed to
different anesthetic regimens, different species used, different shock models and different volumes infused.  

The combination of HSS and HES in anesthetized healthy humans, revealed an increase in left ventricular preload and a decrease in left ventricular afterload. In this study the improvement in left ventricular function was caused mainly by the decreased afterload rather than by an enhancement of left ventricular contractility. The combination of HSS and colloid solution given to septic patients, induced an increase in cardiac output and oxygen delivery, however no changes were noticed in VO\textsubscript{2} and oxygen extraction ratio. The increase in cardiac filling pressures due to volume expansion and the decrease afterload due to a decrease in systemic vascular resistance, contributed to the increment in cardiac output. HSS draws fluid from intercellular space, microvasculature and red blood cells, thus increasing the intravascular volume. Skeletal muscle, which constitutes a large body mass, is the principal source of fluid mobilization. Plasma volume increases by 3 to 5 ml for each milliliter of HSS infused, these effects are immediate although they dissipate within ten minutes.

The small intestinal mucosa is particularly susceptible to the ischemic and inflammatory insults caused by endotoxemia and sepsis, and that in itself constitutes a source of toxic mediators that may cause MOF and an increase in mortality in septic patients. The use of small volume resuscitation (HSS plus Dextran) during experimental endotoxin shock in pigs, effectively prevented microcirculatory and metabolic deterioration of the intestinal mucosa. The beneficial effects of small
volume resuscitation on intestinal mucosal perfusion markedly increased survival in pigs during the 300 minute experiment. 170

Administration of HSS causes a significant increase in sodium and chloride serum concentrations and plasma osmolality. 186 The primary risk of HSS infusion is the induction of hypertonic states due to sodium load. 126,146

The decrease in total peripheral resistance is the main factor responsible for the hypotension that occurs immediately after HSS infusion. 144,173 Hypotension has also been observed in healthy anesthetized humans treated with a combination of HSS and HES. 176 Hypotension has been attributed to increases in serum osmolality with or without changes in plasma sodium concentration. 176 Hypotension is independent of changes in autonomic activity and may involve endothelium dependent mechanisms. 173 The degree of arterial hypotension depends of the rate of administration of HSS. 176 This problem can be avoided by administering HSS at a rate no greater than 1ml/kg/minute. 173,176

In dehydrated patients the use of HSS may not produce an appropriate fluid shift due to pre-existing depletion of extracellular volume. 173 In addition the potent diuretic effects HSS can cause continued fluid loss, exacerbating the hypovolemia. 173 However experimental data show no adverse effects of HSS resuscitation in dehydrated animals. 173,189,190

However, it is considered overall a safe, simple and effective solution for providing rapid restoration of cardiovascular function without excessive fluid accumulation. 37,146,173,183
Studies have also suggested that HSS could have deleterious effects on coagulation
due to anticoagulant effects on platelets and clotting factros.\textsuperscript{177,191} It has been advocated
that HSS can further worsen coagulopathy in patients with already hemostatic
abnormalities.\textsuperscript{177,191} \textit{In vitro} studies demonstrated that HSS has strong anticoagulant and
anti platelet ability.\textsuperscript{191} By replacing 5\% to 10\% of the normal plasma with HSS,
investigators have found prolongation of PT and impairment in platelet aggregation and
function.\textsuperscript{177,191} A higher percentage of plasma needed to be replaced by HSS in order to
appreciate prolongation of aPTT.\textsuperscript{177,191}
Introduction to the experimental protocol

Endotoxemia is the leading cause of death in the horse and represents the major cause of death in horses with gastrointestinal disease (colic, proximal enteritis or colitis).\textsuperscript{9,11,15,16} Regardless of intensive experimental and clinical research, the optimal fluid for resuscitation in a given clinical situation remains unclear.\textsuperscript{56,140} Moreover, the preferred volume expander in patients with sepsis and SIRS is also controversial, and there is no conclusive data demonstrating that the type of fluid has a major impact on outcome.\textsuperscript{43,56,140,151} Small volume resuscitation has been recommended for resuscitation in critically ill horses.\textsuperscript{192} However there is a lack of controlled clinical trials assessing the effects of the combination of HSS and HES solutions for resuscitation in horses with critical illness and specifically with endotoxemia or sepsis. Several advantages had been attributed to the use of small volume resuscitation such as, ease of transport, speed of administration and rapid correction of hemodynamics.\textsuperscript{37} These properties make the use of small volume resuscitation especially attractive for ambulatory veterinarians when the situation demands quick patient stabilization prior to transport of the horse to a referral institution.

Due to the wide use of small volume resuscitation in some referral institutions and the potential advantage for its use in emergency field situations, we felt that this topic needed to be critically evaluated in a controlled experimental manner in order to determine any advantages or deleterious effects.
Experimental endotoxemia in horses has been induced with different dose protocols and routes of administration. Early studies of experimentally induced endotoxemia in horses utilized total dosages varying between 10 to 400 ug/kg, different routes and times of administration were used in these studies. More recent studies utilized low doses of endotoxin ranging from 1 ug/kg to 0.03 ug/kg, and again the routes of administration and administration protocols varied among studies. The model used in this study of equine endotoxic shock in halothane anesthetized horses could be categorized as a sublethal model; this model was chosen for this study in order to produce measurable hemodynamic and hematological abnormalities with a given time frame.

This model of experimentally induced severe endotoxic shock is probably more severe than most commonly seen cases of clinical endotoxemia in horses. As this was the first experiment assessing low volume resuscitation in horses it was deemed appropriate to initially evaluate this therapy in a severe model. Future research looking at the effects of small volume resuscitation in horses with a less severe experimental endotoxemia model should be performed in order to have a more complete understanding of the used of this therapy in different situations.

We hypothesized that the treatment of severely endotoxemic horses with a combination of HSS followed by HES would be superior to the use of large volume isotonic fluid resuscitation or small volume isotonic fluid administration (control). This hypothesis was tested in vivo in anesthetized horses. The null hypotheses were:

1. The use of HSS-HES has no hemodynamic effects in septic horses when compared with the ISO and control groups.
2. The use of HSS-HES has no effects in coagulation profile of septic horses when compared to the ISO and control groups.

3. The use of HSS-HES has no effects in the venous blood gas parameters when compared with the ISO and control groups.

4. The use of HSS-HES has no effects in the arterial blood gas parameters when compared with the ISO and control groups.
I. Experimental animals (Table 1)

Eighteen adult horses were selected from the research population of the Marion duPont Scott Equine Medical Center. Age ranged between five and twenty three years old. The horses were housed outdoors with *ad lib* access to hay, pasture and water. Twelve hours prior to the experiment the horses were placed in a stall, and feed but not water was withheld. The horses were assessed to be normal based on clinical examination (including a thorough cardiac and respiratory system auscultation), complete blood cell count and serum biochemistry profile performed the day prior to the experiment. The study protocol was approved by the University Animal Care and Use Committee.

II. Experimental groups

Each horse was randomly assigned to one of the three experimental groups (six horses in each group). After completion of endotoxin infusion, the horses were treated intravenously using a volumetric infusion pump $^b$ over a period of thirty minutes with:

- Group I (CONTROL) subjects received a small volume bolus (15 ml/kg) of balanced polyionic crystalloid solution $^a$. The bolus volume was equivalent to the volume given during treatment of horses in Group III.

- Group II (ISOTONIC SOLUTION) (ISO) subjects received a large volume bolus (60 ml/kg) of the same balance polyionic crystalloid solution $^a$. 
Group III (HYPERTONIC SALINE PLUS HETASTARCH) (HSS-HES) subjects received a bolus of 5 ml/kg of hypertonic saline solution (7.2 % sodium chloride) followed by 10 mg/kg of a colloid solution (Hetastarch).

III. Induction of endotoxic shock

Endotoxic shock was induced by the infusion of *E. coli* endotoxin (serotype 055:B5) at a dose of 50 ug/kg. The endotoxin solution was prepared within two hours of being used, by diluting the calculated amount of endotoxin based on body weight in 150 ml of 0.9 % sodium chloride solution.

The endotoxin solution was administered as follows:

- **Bolus rate:** 20 ug/kg of endotoxin were administered as an intravenous bolus infusion (1,800 ml/hr) using a volumetric infusion pump. A total volume of 60 ml of endotoxin solution was administered to each horse over a period of two minutes.

- **30 ug/kg of endotoxin were administered as an infusion rate (180 ml/hr) using a volumetric infusion pump. A total volume of 90 ml of endotoxin solution was administered to each horse over a period of 30 minutes.

IV. Instrumentation

The day of the experiment the skin over the right jugular vein was clipped, aseptically prepared and blocked with 2 % lidocaine solution. With the horse standing, before sedation and induction of general anesthesia, three different catheters were aseptically introduced, into the right jugular vein, as follows:
• In the upper portion of the jugular vein an indwelling 12 gauge x 13 cm intravenous catheter \( g \) was placed. This catheter was used to inject anesthetic solutions, endotoxin infusion and maintenance balanced polyionic crystalloid solution \( a \).

• In the mid portion of the jugular vein a percutaneous sheath introducer (7 – 7.5 Fr) \( h \) was placed. A 110 cm polyethylene tubing PE 240 \( i \) introduced through it was used to measure central venous pressure and injection of the cold dextrose solution for cardiac output measurement.

• In the lower portion of the jugular vein a second percutaneous sheath introducer (7 – 7.5 Fr) \( h \) was placed. A 110 cm 7 Fr balloon tipped thermodilution catheter \( k \) was aseptically advanced to the pulmonary artery; its location was confirmed by recognition of the characteristic blood pressure values and waveform \( j \). This catheter was utilized for measuring pulmonary artery pressure and cardiac output by the thermodilution technique.

V. Anesthetic protocol

All horses were sedated with intravenous xylazine \( m \) (average 0.5, range 0.39 – 0.75 mg/kg IV). After the horse was sedated, assessed by clinical observation and decreased heart rate, general anesthesia was induced with guaifenesin \( n \) (average 41.5 +/- 7.0 mg/kg IV) and ketamine hydrochloride \( o \) (2.2 +/- 0.2 mg/kg IV).

The horses were positioned in left lateral recumbency. An endotracheal tube was placed and anesthesia maintained with halothane \( p \) in oxygen (ET \( \text{hal} \) = 1.2 x MAC
approximately) using an intermittent positive pressure ventilation $^a$ ($ET_{CO_2} = 40 +/- 5$ mmHg). Respiratory gases were analyzed continuously for $O_2$, $CO_2$ and halothane concentration using a gas analyzer $^{aa}$. Throughout the experiment 2.5 ml/kg/h of a balanced polyionic crystalloid solution $^a$ was given intravenously, using a volumetric infusion pump $^b$.

VI. Instrumentation and stabilization

The skin over left cephalic vein was clipped and aseptically prepared. A 14 gauge x 20 cm over the wire catheter $^l$ was aseptically placed, this catheter was utilized for venous blood sampling.

The skin over the right facial artery was clipped and aseptically prepared. A 20 gauge x 2.5 cm catheter $^r$ was aseptically placed in the facial artery. The catheter was used to continuously monitor arterial pressure $^{bb}$ and for arterial blood sample collection.

A 110 cm polyethylene tubing PE 240 $^i$ was aseptically advanced through the mid jugular vein catheter introducer into the right atrium. Its location was verified by observation of the characteristic wave form on an oscilloscope $^j$.

Catheter patency was maintained by flushing the catheters with 10 ml of heparinized saline solution (5 u heparin/ml).

The horses were allowed to stabilize for a period of 60 minutes. The horse was considered to be stable once the predetermined anesthetic plane was achieved and arterial blood pressure and end tidal halothane and $CO_2$ had not changed within the last 10 minutes.

VII. Sample collection
Samples for hematological analysis and hemodynamic recordings were taken at various time points during the experimental period. Thus data collection occurred at following time points (Figure 6).

- End of stabilization period (time – 62)
- End of the two minute endotoxin bolus (time – 60)
- End of the 30 minutes endotoxin infusion (time – 30)
- End of infusion of the test solution (time 0)
- After infusion of test solution 5, 15, 30, 60, 90, 120, 150, 180 minutes.

VIII. Hematologic parameters

Venous blood samples were collected form the cephalic vein catheter by gentle aspiration into syringes.

Blood collected in a 3 ml heparinized syringe was used for venous blood gas analysis using a blood gas analyzer \(^1\). Samples were collected at: -62, 5, 15, 30, 60, 90, 120, 150 and 180 minutes and were analyzed immediately (within two minutes).

Blood collected in a syringe was distributed in three different vacuum tubes \(^u\) for analysis as follows:

- Coagulation profile was determined using 4 ml of blood placed into vacuum tubes \(^u\) containing sodium citrate. Partial thrombin time (APTT) and prothrombin time (PT) were determined immediately using an electronic coagulation analyzer \(^w\). Collection time points were: -62, 60 and 180 minutes.
• For determination of platelet count, 6 ml of blood collected at -62, 60 and 180 minutes were placed into a vacuum tube \( u \) containing ACD. Platelets were measured with the use of an automatic blood cell count machine \( z \).

• At -62, 30, 90, 120 and 180 minutes 3 ml of blood was collected in a vacuum tube \( u \) containing lithium heparin. The samples were centrifuged and the serum was frozen for future studies.

Arterial blood samples were drawn anaerobically from the catheter in the facial artery into a heparinized 3 ml syringe at -62, 5, 60, 120 and 180 minutes. The samples were analyzed immediately using a blood gas analyzer \( v \).

IX. Hemodynamic parameters

Arterial blood pressure was directly measured using the right facial artery catheter. Systolic, diastolic and mean arterial pressures were continuously monitored by the pressure monitor \( bb \). The values were recorded at the following time points -62, -60, -30, 0, 5, 15, 30, 60, 90, 120, 150 and 180 minutes.

Pulmonary arterial pressures were directly measured via the distal port of the thermodilution catheter inserted in the pulmonary artery. Systolic, diastolic and mean pulmonary pressures were recorded at the following time points: -62, -60, -30, 0, 5, 15, 30, 60, 90, 120, 150 and 180 minutes.

The central venous pressure was measured via the PE tubing inserted into the right atrium. Systolic, diastolic and mean venous pressures were recorded \( j \) at the following time points: -62, -60, -30, 0, 5, 15, 30, 60, 90, 120, 150 and 180 minutes.
Electrocardiogram and heart rated were continuously monitored using a modified base apex lead configuration. These values were recorded at the following time points: -62, -60, -30, 0, 5, 15, 30, 60, 90, 120, 150 and 180 minutes.

Cardiac output was measured by using the thermodilution technique as previously described by Muir et al. Briefly, 50 ml of 5% dextrose at 0 °C was manually injected into the right atrium. The thermodilution curve was obtained by the use of an electronic cardiac output meter and computerized integrator. Four consecutive cardiac output measurements were obtained. The outlier was discarded and an average was calculated using the other three measurements and recorded for analysis. Cardiac output measurements were always made during the expiratory pause. The times at which cardiac output measurements were performed are as follows: -62, -30, 5, 30, 60, 90, 120, 150 and 180 minutes.

After the experiment protocol ended halothane anesthesia was deepened and all horses were euthanized with an intravenous bolus injection of potassium chloride.

X. Statistical analysis

Data was analyzed with the aid of statistical software (SAS) (SAS Institute, Cary NC), by ANOVA for repeated measures after confirmation of appropriate statistical assumptions for this test by examining a plot of residuals. The data that did not meet normality of distribution was log transformed. Post hoc pairwise comparison between groups were made using Tukey-Kramer’s test. Results were expressed as mean +/- Std Dev. The level of significance was set at P < 0.05.
Results

Analysis of Arterial Blood Samples

The mean arterial pH was significantly different (P < 0.05) over time for each group (Table 2). Thus the arterial pH decreased significantly over time for all treatment groups (Figure 7). The mean arterial pH values were significantly (P < 0.05) lower in the ISO group compared with control. Thus the treatment of endotoxic horses with large volumes of isotonic fluids exerts a negative effect on arterial pH. The treatment with HSS-HES did not produce a significantly (P < 0.05) different effect on arterial pH compared with the control group.

The mean arterial sodium concentration was significantly different (P<0.05) over time for each group (Table 3). As expected with the use of HSS the concentration of arterial sodium in that group was significantly (P<0.05) greater when compared with the control and ISO group (Figure 8). The peak arterial sodium concentration was seen five minutes after the HSS-HES infusion was finished.

The concentration of arterial ionized calcium (iCa) significantly (P<0.05) decreased five minutes after treatment in all groups and then gradually increased, the iCa concentration at time 180 minutes was not significantly different among groups (Table 4). The decrease in arterial iCa concentration for HSS-HES group was significantly (P<0.05) less at time five minutes compared with the other two groups (Figure 9). The concentration of iCa was significantly (P<0.05) greater in the HSS-HES group compared to the other two groups.
Arterial PaO₂ decreased significantly from base line in all treatment groups (Table 5, Figure 10). Statistically no differences were found between groups. However the arterial PO₂ in the HSS-HES group did not decrease below 100 mmHg for the period of the experiment, while in the ISO and control groups PO₂ decreased below 100 mmHg at time 60 minutes.

Arterial CO₂ increased significantly (P<0.05) from base line in all treatment groups over time (Table 6, Figure 11); however the difference between groups was not statistically significant.

Arterial bicarbonate and arterial base excess were analyzed. Statistical significance among groups was not found for these variables. The arterial bicarbonate and base excess significantly (P < 0.05) decreased from base line in all treatment groups (Table 7 and Figures 12 and 13).

Arterial potassium concentration was analyzed. Statistical significance among groups was not found for this variable (Table 8).

Analysis of Venous Blood Samples

The venous pH decreased significantly (P < 0.05) from base line for all treatment groups (Table 9 and Figure 14). The mean venous pH values were significantly (P < 0.05) lower for the ISO and HSS-HES groups compared with control (Figure 14).

The packed cell volume (PCV) significantly (P < 0.05) increased from base line in all treatment groups between time 15 minutes and 180 minutes. A significant difference (P <
0.05) of the PCV was seen when the control and HSS-HES groups were compared, this difference was appreciated for all time points (Table 10). This difference was more marked at time 30 minutes due to a decline in the PCV of the HSS-HES group. A significantly (P < 0.05) less increase in the PCV for the HSS-HES group was seen when compared with control (Figure 15). The ISO group PCV was not significantly different from either of the other groups.

There was no statistically significant difference in the total protein concentration between treatment groups (Table 11). The total protein concentration significantly (P < 0.05) decreased from the base line value for times 5, 15 and 30 minutes in all groups (Table 11). Although not statistically significant a trend towards a greater decrease in total protein concentration was observed in the ISO group (Figure 16).

As observed with the arterial sodium concentration, with the use of HSS the concentration of venous sodium in that group was significantly (P<0.05) greater when compared with the control and ISO group (Table 12). The peak venous sodium concentration was seen five minutes after the HSS-HES infusion was finished (Figure 17).

Following the same pattern as the sodium concentration, chloride concentration was significantly (P<0.05) greater in the HSS-HES group compared with the other two groups (Table 13, Figure 18). The concentrations of chloride in the ISO and control groups were not significantly different between each other.

The venous ionized calcium (iCa) concentration significantly (P<0.05) decreased from base line in all groups (Table 14). The decrease in the venous iCa was significantly
less for the HSS-HES group when compared with the other two groups (Table 14, Figure 19). These changes followed the same pattern as seen with arterial blood iCa (Table 4).

The creatinine concentration significantly ($P<0.05$) increased from baseline between 15 minutes to 180 minutes for all groups (Table 15). A significant ($P<0.05$) difference was observed when groups ISO and HSS-HES groups where compared over time. The concentration of creatinine was significantly ($P<0.05$) higher for the HSS-HES group compared with the ISO group (Figure 20). The creatinine concentration in the ISO group showed a lower trend when compared with the other two groups, although statistical significance was not achieved between groups ISO and control.

The venous lactate concentration increased significantly ($P<0.05$) from the base line value for all groups. However no significant difference was achieved between groups (Table 16). A trend towards lower lactate concentration, at all time points, was seen for the HSS-HES group when compared with the other two groups (Figure 21).

Significant differences in venous bicarbonate concentration between groups were not found. However a significant difference ($P < 0.05$) from the base line venous bicarbonate concentration was seen at all time points for all there groups (Table 17, Figure 22).

The venous $\text{CO}_2$, venous $\text{O}_2$, venous potassium, venous glucose and venous urea were analyzed. Statistical significance was not found between groups for any of these variables. However differences were seen at several time points. The most interesting
observation was the significant (P<0.05) decreased in venous PO$_2$ for all time points compared with the base line value (Tables 18, 19 and 20).

Analysis of Coagulation Profile

The platelet count decreased significantly (P<0.05) in all groups from the base line value. However statistical difference between groups was not achieved (Table 21, Figure 23). The PT time increased significantly (P<0.05) in all groups from the base line value. There were no significant differences between treatment groups (Table 21, Figure 24). aPTT significantly increased from base line in all groups. Significant differences between treatment groups were not found (Table 21, Figure 25).

Analysis of Hemodynamic Parameters

A significant (P<0.05) change in CO from the base line value was observed in all groups at times 5, 30, 60 and 90 minutes. The differences in CO between groups did not reach statistical significance (Table 22). The CO in all groups followed the same pattern of increased CO that peaked between time 5 and 30 minutes and then progressively decreased for all time points, returning to a value significantly not different from base line at time 120 minutes (Figure 26).

A significant (P<0.05) increase in mean central venous pressure (MCVP) from the base line value was seen in all groups at time 0 minutes. The increase in MCVP was
significantly (P<0.05) greater for the ISO group and sustained for 60 minutes when compared with the control and HSS-HES groups (Table 23, Figure 27). Although not significant, the increment in MCVP for the HSS-HES group tended to be greater when compared with the control group. At time 180 minutes the MCVP for the ISO and HSS-HES groups return to base line values while the MCVP in the control group was below the baseline value at 60 – 120 minutes (Figure 27).

For all groups a significant (P<0.05) difference in mean pulmonary arterial pressure (MPAP) was seen at times -60, -30, 0, 5, 15, 30, 60, 150 and 180 minutes compared with the base line value. However, significant differences for mean pulmonary arterial pressures were not found between groups (Table 24). For all groups the MPAP significantly (P<0.05) increased from base line after endotoxin administration (given between times -60 and -30 minutes) (Figure 28). In the group treated with large volume of isotonic fluids, a more pronounced trend towards increase in MPAP was observed, the MPAP peaked at the end of treatment with large volume of isotonic fluids (time 0 minutes) (Figure 28). A more moderate trend towards increased in MPAP was can be appreciated after treatment with HSS-HES (Figure 28). In the control group the opposite effect was observed, with a trend towards decreased in MPAP (Figure 28).

Mean arterial pressure showed a significant (P<0.05) increase from base line after the end of the endotoxin infusion (time -30 minutes) for all groups (Table 25, Figure 29). After time -30 minutes a trend towards hypotension was observed for all groups (Figure 29). From time 5 minutes to time 180 minutes the decreased in blood pressure was significantly different from base line for all groups (Table 25, Figure 29). The most
precipitously decrease in MAP was observed in the HSS-HES group (Figure 29); however this finding did not reach statistical significance when compared with the other two groups. During the study period only the control group’s arterial blood pressure returned to the base line volume at time 180 minutes, while in the other two groups it remained below base line (Figure 29).

Significant difference for heart rate between groups was not found at any time point (Table 26, Figure 30). The heart rate was not significantly different within groups from base line at any time points.

The end-tidal CO$_2$ and the inspired O$_2$ were not significantly different among groups, throughout the experimental protocol (Table 27).
Discussion

Hemodynamics

Substantial alterations were observed in the hemodynamic status of all subjects following endotoxin administration. In all treatment groups in this study, cardiac output (CO) initially increased from baseline value after administration of endotoxin. The increase was significant compared to the baseline value in all groups and if expressed as a percentage it was equivalent to an average increase of 62.5% at time 5 minutes. These findings are typical during the hyperdynamic phase of endotoxemic shock, and are characterized by high CO and low systemic vascular resistance. It is known that the CO is normal or increased during septic shock and that the ventricular function is abnormal. The left ventricle maintains a normal to elevated CO despite left ventricular depression because there is a decrease in systemic vascular resistance and blood pressure, thus reduced left ventricular afterload. Myocardial depression is seen in patients suffering from septic shock, this is reflected by reduced post resuscitation LVEF, ventricular dilatation and flattening of the Frank Starling relation. Hypotension, in the presence of increase in CO indicating decrease in systemic vascular resistance, was seen in all groups in the present study. However a more pronounced trend towards hypotension was seen in the HSS-HES treated horses. It is important to take into account that the cardiovascular effects of sepsis vary according to the time course and the severity of septic shock, and in the case of experimentally induced sepsis vary with the animal model, route of administration and dosage.
Interestingly the increase in CO was not affected by the treatment administered and after times 5 to 30 minutes the CO declined in all groups to approximately the baseline value. However, between times 30 to 120 minutes a trend towards a higher CO was seen for the HSS-HES treated group. This may be due to the fact that HSS improves myocardial contractility and microcirculation.\textsuperscript{37,146,170,171,173} Colloids (HES) also have been shown to improve cardiac contractility, thus further improving CO.\textsuperscript{67,140} It is also known that colloids (HES) preserve colloid oncotic pressure, thus are more effective in expanding plasma volume, in restoring hemodynamic stability and improving oxygen tissue delivery when compared with crystalloids.\textsuperscript{67,138,140,152} In addition HES has the ability to seal endothelial gaps and to modify endothelial inflammation, thus potentially counteracting the deleterious effects of capillary leak syndrome of sepsis.\textsuperscript{67,79,152,156,159} Consequently the beneficial effects of small volume resuscitation on CO were suggested in this model and could have explained the transient trend towards a higher CO in the HSS-HES group. Although in this experimental model the favorable effects of low volume resuscitation were short lasting, based on the observation that at 150 minutes the CO for the HSS-HES group dropped to the same value as the other two experimental groups.

It is important to understand that the left and right ventricles are under different influences, and this is particularly true during shock states.\textsuperscript{1} The right ventricular afterload is increased during acute phase of sepsis due to pulmonary hypertension, thus the right ventricular CO tends to be decreased.\textsuperscript{1,2} The failure of the right heart to pump blood causes the CVP (right atrial pressure) to rise, and in turn the backward force of the
rising right atrial pressure on the veins of the systemic circulation decreases venous return to the heart. The net effect is that the blood dams up into the peripheral circulation (distensible bag) instead of returning to the heart. This effect is potentiated by the fact that the venous and arterial dilatation occur during distributive shock secondary to sepsis.

In the present study a significantly increase in MCVP (mean peak of 36.8 mmHg) was measured in the ISO group after administration of 60 ml/kg of isotonic crystalloid fluids given as a bolus, even thought this dose of fluid volume was slightly less than the conventional equine resuscitative dose of 80 ml/kg. It is known that there is a limit in the amount of venous return that a normal heart can pump, in humans is up to 2.5 times the normal venous return before the heart becomes a limiting factoring controlling CO. When the right heart function curve reaches its plateau (CVP 6 to 12 mmHg in humans) it indicates that the right heart filling pressure has reached a limit. Thus the use of fluid therapy beyond the critical right filling pressure, in attempt to increase the CO, leads to volume overload.

Volume loading has several deleterious effects, as follows: 1) increasing the right ventricular pressure impedes coronary flow, 2) shifts the ventricular septum to the left which decreases the left ventricular compliance and 3) developing of peripheral and pulmonary edema. In septic human patients, the major complications of fluid resuscitation are pulmonary and systemic edema. Septic patients develop alteration of the endothelial barrier due to SIRS and capillary leak syndrome ensues. As a consequence these patients have the inability to contain fluids and proteins within the
vascular space and tissue edema develops.\textsuperscript{56,74,75} Endothelial damage has been documented in horses.\textsuperscript{10} Volume overload in the presence of vascular leakage and myocardial dysfunction with the secondary accumulation of blood in the peripheral circulation, favors edema formation, in turn edema causes hypovolemia and reduces CO.\textsuperscript{79,126} Furthermore the reduction of CO compromises peripheral perfusion, leading to cardiovascular collapse.\textsuperscript{79} Tissue edema may potentiate tissue injury by reducing oxygen tension secondary to increasing the distance for oxygen diffusion to the cells, thus affecting organ function.\textsuperscript{126,140,142,152}

In this study the significant increase in CVP after ISO treatment was an indication of volume overload. Due to the deleterious effects of volume overload stated above myocardial depression must be kept in mind in a septic patient and the effects of volume overload to a failing heart could further worsen the patient’s clinical condition. On the other hand, edema was subjectively seen in the periorbital area only in the ISO group after the infusion of crystalloids in our study. A trend towards a higher lactate concentration was also observed in the ISO group. These observations suggest that peripheral edema could have played a role in the development of the hyperlactatemia seen in the ISO group. A less severe hyperlactatemia was observed in the HSS-HES group. This observation could lead to the speculation that the less pronounced hyperlactatemia was due to a reduced amount of tissue edema and better tissue perfusion in the HSS-HES treated group.

In the control group a marked decrease in CVP was observed. In the HSS-HES group the CVP did not decrease as much. A significant difference between these two groups
was not found. Interestingly the total volume infused in both groups was the same (15 ml/kg) over the same time period (30 minutes). Thus the volume expansion effect of small volume resuscitation was suggested in our study.

The large increase in the intravascular fluid volume likely responsible for the increased CVP observed in the ISO group could have explained the marked decrease in plasma protein compared with the other two groups. Interestingly the PCV did not decrease with the increase in the extracellular fluid volume. In fact a significant increased from the base line PCV value was seen in all treatment groups between time 15 minutes and 180 minutes. A possible explanation could be that the release of stressor hormones during endotoxemia could have induced splenic contraction resulting in an increase in circulating numbers of red blood cells.

Pulmonary hypertension is a well described abnormality that occurs in horses with experimental induced endotoxemia, TxA$_2$ is the primary mediator of pulmonary hypertension in endotoxemic horses.$^9$, $^{10}$, $^{16}$, $^{80}$, $^{81}$, $^{84}$ As expected in the present study the MPAP in all groups significantly increased from baseline after endotoxin infusion. A pronounced upward trend for MPAP was observed for the ISO group at time 0 minutes (after administration of large volume of isotonic fluids). The increased in MPAP seen in the ISO group was also coincidental with a larger, but not significant, decrease in PaO$_2$ and increase in PaCO$_2$ seen in this group, thus pulmonary edema and a more severe impairment in gas exchange was likely present in the ISO group.

Pulmonary edema is a major complication of fluid resuscitation.$^{126}$, $^{140}$ The increase in MPAP leads to an increase in pulmonary capillary pressure therefore the hydrostatic
pressure rises with the net effect of fluid movement out of the pulmonary capillaries and pulmonary edema develops. Furthermore, pulmonary endothelial damage has been documented in endotoxemic horses and likely could contribute to the development of pulmonary edema.\textsuperscript{44} In an equine study where a similar total endotoxin dose as in our study was utilized, but with a different infusion protocol, pulmonary edema was not found; however this study did not rule out an increase in microvascular permeability.\textsuperscript{29} In this study all groups developed a significant decrease in PaO\textsubscript{2} and increase in PaCO\textsubscript{2} over time. The greater increased in MPAP seen in the ISO group coincided with a larger, but not significant, decrease in PaO\textsubscript{2} and increase in PaCO\textsubscript{2} seen in the ISO group. Thus pulmonary edema and a more severe impairment in gas exchange was likely present in the ISO group. In our study pulmonary edema was not directly measured, thus we hypothesized but could not confirm that pulmonary edema played a greater role in the pulmonary dysfunction observed in the ISO group. Hypoventilation could also have been a contributing factor in the increased PaCO\textsubscript{2}. Hypoxemia can be explained by the increase in the thickness of the diffusion barrier due to pulmonary edema, ventilation perfusion inequalities, hypoventilation and shunting.

The increased MPAP was also coincidental with the increase in CVP in the ISO group. As stated above the increase in CVP was evidence of volume overload. The pulmonary hypertension (increase in MPAP) could also have contributed to the increase in CVP by increasing the resistance that the right ventricle needed to overcome (right ventricular afterload) in order to pump the venous return into the pulmonary artery.\textsuperscript{2} The MCVP was not significantly different between control and HSS-HES groups, thus the
HSS-HES exerted a more moderate increase in CVP than ISO. Furthermore the right ventricular afterload represented by the degree of pulmonary hypertension was less in the HSS-HES group. Thus it could be theorized that, in our model, the combination of HSS-HES had a more beneficial effect on cardiovascular and pulmonary function. It appeared that HSS-HES improved the ability of the myocardium to pump the venous return during sepsis along with a decrease on right ventricular afterload, thus improving the overall right ventricular function. An alternative explanation could be that the volume effect exerted by the combination of HSS-HES is not as pronounced as the volume effect produced by the bolus administration of large volumes of crystalloids. Furthermore the left ventricular function may also be improved by the treatment of septic horses with HSS-HES based on the fact that a trend towards a higher CO was seen for that group in our study. The more pronounced hypotension seen in the HSS-HES group could have accounted for the higher CO in this group. Thus we could not say conclusively that the HSS-HES combination exerted a beneficial cardiac function.

It was not surprising to observe that hypotension in the HSS-HES group was more severe than in the other groups, although significance was not found between groups. It has been documented that HSS causes hypotension secondary to decrease in total vascular resistance.\textsuperscript{144,173} The degree of arterial hypotension depends of the rate of administration of HSS and by administering HSS at a rate of less than 1ml/kg/minute hypotension can be avoided.\textsuperscript{173,176} The infusion rate of HSS in our horses was 0.3 ml/kg/minute, this conservative rate could have accounted for the mild but not significant hypotension in the HSS-HES group.
Based on this evidence it can be concluded that the treatment of septic or endotoxemic horses with large volumes of isotonic fluids (60 ml/kg bolus) is potentially deleterious due to the increases in CVP and MPAP observed here. It is clear that patients with septic shock have a decreased augmentation of ventricular performance in response to fluid resuscitation.¹ In early septic shock the reduction of ventricular contractility is a major cause of myocardial depression.¹

The results of arterial blood gas analysis indicated a more pronounced decrease in PaO₂ and increase in PaCO₂ in the ISO group when compared with the other two groups, although this did not achieve significance. Pulmonary edema secondary to administration of large volume of fluids, relative decrease in CO and development of capillary leak syndrome; were all possible playing a role in the development of a more pronounced, although did not reach statistical significance, decrease in PaO₂ and increase in PaCO₂.

Further supporting the impairment in gas exchange and decreased oxygen delivery to tissues was the fact that the hyperlactatemia observed in the ISO group was more severe that in the HSS-HES group and similar to the untreated group.

After administration of HSS-HES a less pronounced increase in MPAP was observed. Evidence of volume overload (increase in CVP) was not seen in this treatment group, the changes in CVP in this treatment group followed the same pattern as the control group.

These findings along with the trend towards a higher CO between times 30 and 120 minutes suggested a more beneficial effect of the combination of HSS-HES compared with the ISO group regarding cardiovascular function. However a more severe hypotension, although not statistically significant, was seen for the HSS-HES group. The
hypotension in this group could have also played a role in maintaining slightly higher CO in HSS-HES treated horses.

Arterial and venous blood gas

A mixed metabolic and respiratory acidosis was observed in all three groups. The metabolic component of the acidosis was characterized by the decreases in bicarbonate and more negative base excess seen for all groups over time, a significant decreased in this variables was observed for all time points when compared with the base line value. The respiratory component was represented by the increase in PaCO$_2$, which significantly increased from base line in all groups. When arterial pH was measured the pH for the ISO group was significantly different compared with control horses. The greater decrease in the ISO group’s pH was likely due to a trend towards a higher PaCO$_2$ (hypercabia), lower bicarbonate and more negative base excess, seen in that group. Even though not significant, those changes suggested a more severe respiratory and metabolic acidosis in the ISO group. The hypercabia seen in the ISO group is coincidental with the likelihood of more severe pulmonary edema present in that group.

Venous pH analysis revealed a significantly lower pH in the ISO and HSS-HES groups compared with control. A more severe mixed (metabolic and respiratory) acidosis was likely responsible for the pH difference. However assessment of the respiratory component of the acidosis based upon venous blood gas analysis needs to be interpreted with caution. In humans venous pH is considered an acceptable substitute of arterial pH.
However, in human critical care the analysis of venous pH for evaluation of the respiratory function in patients with respiratory disease is an effective screening test for detection of hypercarbia up to a cut-off of 45 mmHg. The PvCO$_2$ was above this cut off for all three groups, thus based on human data it could be argued that PvCO$_2$ was not a good indicator of hypercarbia in our study. Important is the fact that the venous samples were drawn from the cephalic vein, thus truly represent local tissue perfusion of the forelimb. The marked hyperchloremia observed in the HSS-HES group could have also been responsible for the lower pH measured in venous blood. However the hypernatremia may have played a compensatory role, and prevented the development of hyperchloremic metabolic acidosis in the HSS-HES group, by maintaining electroneutrality. It is difficult to explain why the pH in the HSS-HES group is lower in the venous blood sample than in the arterial blood sample when compared with the other two groups. A possibility is the fact that different machines were used for the analysis of venous and arterial samples. On the other hand, all the elements used to interpret pH were not present in both samples (arterial and venous).

**Electrolytes**

Hypocalcemia is a common finding in humans and animals with sepsis. The causes of hypocalcemia in sepsis are multifactorial and not well understood. Inflammatory mediators released during sepsis such as TNF-a, IL-1, IL-6 and IL-8 may play a role in the abnormal calcium metabolism seen during sepsis. It has been shown that in endotoxemic horses and septic humans the parathyroid hormone (PTH)
response to hypocalcemia is variable.\textsuperscript{203-205} Parathyroid gland dysfunction and SIRS are likely responsible for the hypocalcemia seen in septic horses.\textsuperscript{204,205}

In the present study serum ionized calcium (iCa\textsuperscript{++}) decreased in all groups after administration of endotoxin. Interestingly the hypocalcemia was significantly less severe in the HSS-HES group when compared with the other two groups. The more pronounced hypocalcemia in the ISO and control groups could have been potentiated by the use of fluids with very low calcium concentration (5 mEq/L). However the solutions used in the HSS-HES group were Ca\textsuperscript{++} free. Thus we postulated that the combination of HSS-HES exerted a protective effect on the endotoxemia induced hypocalcemia. The mechanisms of this possibly protective effect are not known at this time and further investigations are required.

The clinical significance of hypocalcemia results from deleterious effects on different organ systems such as neuromuscular, cardiovascular and gastrointestinal.\textsuperscript{205} Furthermore, severe hypocalcemia can also result in death.\textsuperscript{205} Therefore it is obvious that calcium supplementation would be beneficial and indicated for the treatment of sepsis induced hypocalcemia. The protective effects on endotoxin induced hypocalcemia seen in the horses treated with HSS-HES are desirable in order to prevent the deleterious effects of hypocalcemia. Treatment of sick horses in field conditions, where Ca\textsuperscript{++} supplementation is not always available, with small volume resuscitation prior to referral could potentially limit serious adverse effects of hypocalcemia.

As expected the administration of HSS caused a significant hyperchloremia and hypernatremia in the HSS-HES group compared with the other two groups. It is known
that HSS administration causes an increase in serum sodium and chloride concentration and that a hypertonic state is the main risk factor associated with the use of HSS solutions.\textsuperscript{126,146,186}

The plasma total protein concentration decreased in all groups after treatment. The greatest decrease in the plasma total protein was seen in the ISO group at time 5 minutes, after administration of large volume crystalloids. Interestingly a decrease in PCV was not seen, to the contrary the PCV increased from the base line value, likely the released of stressor hormones and consequent splenic contraction accounted for this finding. After that time the trend for PCV and total protein concentration was towards higher concentrations, likely reflecting the decrease in plasma volume possible due to the shifting of fluids out of the vascular space.

Creatinine concentration was significantly higher in the HSS-HES group when compared with the ISO group. A trend towards a higher BUN concentration was also seen in the HSS-HES groups compared with the other two groups. The origin of the azotemia can be due to pre renal, renal or post renal factors. The severe shock model created in our study caused hypotension in all treatment groups, hypotension was worse in the HSS-HES treated horses. Therefore decrease renal perfusion, could have played a role in the development of pre-renal azotemia which was more severe in the HSS-HES group. In septic human patients a significantly greater incidence of acute renal failure was noticed in patients treated with HES compared with gelatin treatment.\textsuperscript{163} In humans it has been recommended that HES should be avoided in patients with renal dysfunction or at risk of developing acute renal failure.\textsuperscript{56,163} In our study the shock model was severe and
some degree of renal insult was likely to be present, however the time frame for acute renal failure to develop was considered not to be long enough. Activation of the tubulo-glomerular feedback mechanism due to the high chloride serum concentration could also have contributed to the renal origin of azotemia. Post renal azotemia was unlikely to be a factor in this experiment. At this time we do not know if the combination of HSS-HES can potentiate the deleterious renal effects produced by HES. In our study only the combination was used and the effects of each solution can not be separated, thus more work needs to be done in horses to determine the exact effect of each solution and the combination of them on renal function. Thus, based on our data and human data, at the present time, HES should be avoided or used with caution in patients with risk of developing renal failure until more information comes available.

**Coagulation**

Thrombocytopenia is a common abnormality during sepsis and different mechanisms can account for its development in septic patients. Sepsis induced thrombocytopenia is most often attributed to increased consumption or decreased production of platelets. It has been shown that the replacement of as little of 5% of the plasma with HSS has deleterious effects on platelet aggregation and platelet function. There are no studies that have demonstrated a decrease in platelet number, beyond dilutional effect, in association with HES therapy. The alteration in factor VIII-vWF complex has been suggested as the cause of decrease in platelet aggregation and adhesive function.
It was not surprising that thrombocytopenia was present in all of the treatment groups in our study after induction of endotoxemia. For the study period of our experiment and the dose regimen utilized, the combination of HSS-HES did not cause a more severe decrease in platelet number compared with the control and ISO groups. It is suggested by experimental work, it is likely that a reduction in platelet function is present in patients treated with HSS-HES. Platelet function was not directly assessed in our study.

It can be concluded that severe endotoxemia induces a marked thrombocytopenia in horses. However statistical significance with regard to platelet count between groups was not reached and an equivalent trend towards decrease in platelet count was observed in all groups. Thus in our experimental model of severe endotoxemia and for the time of the experimental protocol, HSS-HES did not have a greater effect in platelet count beyond endotoxemia induced thrombocytopenia. However, the potential adverse effects of HSS-HES on platelet function coupled with the low platelet number, induced by sepsis, could potentiate hemostatic abnormalities seen in septic patients. Moreover abnormalities in primary hemostasis were not observed in any of the experimental groups.

Despite the alterations in platelet numbers suggestive of hemostatic dysfunction, abnormalities in primary hemostasis were not observed in any of the experimental groups. An unexpected finding in our study was the fact that PT and aPTT remained within the normal limits for the HSS-HES group, while for the other two groups a marked elevation was observed. Even though statistical significance between groups was not found. However the trend for the PT and aPTT in the HSS-HES group showed that the tendency of these values was towards higher values. Thus if the study would have been
carried out for a longer period of time it is likely the PT and aPTT would have fallen outside the normal ranges. However in this model of endotoxemia and for the time the study was carried out no abnormalities in secondary hemostasis were detected.

There is concern regarding the adverse effects of HES on coagulation, however the information is conflicting and controversial.\textsuperscript{138,150,156} It is known that the adverse effects of HES on coagulation are influenced by the quantity infused, the preparation selected, the dose and whether single or multiple doses are used, the nature of other fluids infused, and the medical condition of the patient.\textsuperscript{150,157,162} Studies have also suggested that HSS could have deleterious effects on coagulation due to anticoagulant effects on platelets and clotting factors.\textsuperscript{177,191}

This study demonstrated that endotoxemia causes thrombocytopenia in severely endotoxemic horses, and that the degree of thrombocytopenia was independent of the fluid therapy administered. Thus in this experimental model and with the HSS-HES doses used no effects on primary hemostasis were observed. However it could be argued that the platelets function and not the number could have been negatively influenced by HSS-HES. Even though we did not directly investigate platelet function in this study, gross evidence of primary hemostatic disorders was not appreciated. More research needs to be done in order to elucidate the more intricate mechanisms by which HSS-HES could affect hemostasis. In the meantime we can conclude that these solutions should be use with caution in patients with severe impairment of hemostasis.

It has been suggested that HES decreases factor VIII beyond dilutional effects.\textsuperscript{157,162} We know that factor VIII is important in maintaining a normal aPTT and that its activity
should be reduced to less than 30% of normal for aPTT to be prolonged. In this study the aPTT for the HSS-HES group was within normal limits for the duration of the experimental protocol. This suggests that for the study period deleterious effects on factor VIII were not present or if present were not severe enough to cause an increase in aPTT, explaining why the aPTT remained within normal ranges in the HSS-HES group.
Conclusion/Summary

Endotoxic or septic shock plays an important role contributing to the morbidity and mortality in horses with gastrointestinal and other disease processes. Thus it is important to investigate the effects of different intravenous solution used for resuscitation in equine medicine and to elucidate their beneficial and deleterious effects in septic shock patients.

Our study demonstrated some deleterious effects that infusion of large volume of isotonic crystalloid solutions (60 ml/kg bolus) has on the cardio-pulmonary system of halothane anesthetized horses with severe septic shock. Also there were indications of some beneficial effects of small volume resuscitation with HSS-HES on the cardiopulmonary system; however differences did not reach statistical significance preventing definitive conclusions to be drawn from this model.
Footnotes

a. Normosol® - R. Abbott Laboratories, Chicago, IL, USA.
b. Flo-Gard® 6301, Baxter. Deerfiled, IL, USA.
c. Varistaltic® Pump Junior Model, Monostat. New York, NY, USA.
d. Equi-Phar Equine 7 HSS. Vedco, Inc. St Joseph, MO, USA.
e. Hespan®, B Brown Medical Inc. Irvine, CA, USA.
f. Lidocaine HCL 2%. Abbott Laboratories, Chicago, IL, USA.
g. MILACATH®, Mila international, Inc, USA.
h. Arrow® International
i. Intramedic, Becton Dickinson
k. Elecaths®, Electro-catheter corporation, Rahway, NJ
l. Guide Wire Style catheter, Mila international, Inc, USA.
m. Sedazine®. Fort Dodge Animal Health. Fort Dodge, IA, USA.
n. USP, Boehringer Ingelheim Chemicals, Inc., Petersburg, UA.
o. Vetalar®, Fort Dodge Animal Heath. Fort Dodge, IA, USA.
p. Holocarbon Laboratories, River Edge, NJ
q. J. P. Medical Dist. Co. Inc., Phoenix, AZ
r. Quick-Cath®, Baxter. Deerfiled, IL, USA.
s. E coli 055:B5. Sigma – Aldrich CO. St Louis, MO, USA.
t. Stat Profile M, NOVA Biomedical. Waltham, MA, USA.


\textsuperscript{a.} Vacutaner\textsuperscript{\textregistered}, Becton Dickinson VACUTANER systems. Franklin Lakes, NJ, USA.

\textsuperscript{b.} Radiometer ABL 505, Diamond Diagnostics. Holliston, MA, USA.

\textsuperscript{c.} SCA 2000\textsuperscript{TM}, Veterinary Coagulation Analyzer, Synbiotics. San Diego, CA, USA.

\textsuperscript{d.} Cardiomax II Model 85, Columbus Instruments International Corporation. Columbus, OH, USA.

\textsuperscript{e.} CMX/IBM Software, Columbus Instruments International Corporation. Columbus, OH, USA.

\textsuperscript{f.} 9110 Biochem, Allentown, PA, USA.

\textsuperscript{g.} Poet IQ 8500, Criticare Systems, Inc., Waukesha, WI.

\textsuperscript{h.} Datascope 2000, Datascope Corp, Mahwah, NJ.

\textsuperscript{i.} Datascope 2000, Datascope Corp, Mahwah, NJ.
Figures

Figure 1. Chemical structure of LPS from gram negative bacteria.
Figure 2. Frank Starling ventricular performance relation for three different patient groups. Data points plotted represent the mean pre volume and post volume infusion values of end diastolic volume index (EDVI) and left ventricular stroke work index (LVSWI) for each patient group. Control patients demonstrated a normal increase in both EDVI and LVSWI in response to volume infusion. The absolute increases in EDVI and LVSWI in patients with sepsis without shock were less than those of control subjects, but the slope on the curve is similar to that for control patients. Patients with septic shock had a greatly diminished response and demonstrated a marked rightward and downward shift to their Frank Starling relation. Published with permission. 206
Figure 3. Pathogenic mechanisms in disseminated intravascular coagulation. Adapted from\textsuperscript{116}
Figure 4. Sepsis causes a disruption of the normal hemostatic balance between procoagulant and anticoagulant mechanisms. Tissue factor expression is enhanced, ultimately leading to enhanced formation of fibrin clots. PAI-1 levels are increased, thus causing failure of normal fibrinolytic mechanism. Sepsis also causes a decrease in the level of protein C and other natural anticoagulants, thus resulting in further procoagulant effects. The end result is the enhanced formation of fibrin clots in the microvasculature, leading to impaired tissue oxygenation and cell damage. Published with permission 6
Figure 5. Chemical structure of HES molecule. Published with permission\textsuperscript{156}
Figure 6. The Diagram outlines the experimental design and time course of the experimental protocol. The squared with numbers represent the different time points in minutes.
Figure 7. Mean arterial pH +/- standard deviation. Significant decrease (P < 0.05) of arterial pH over time for all treatment groups. Significant difference (P < 0.05) was observed between the ISO and control groups.
Figure 8. Mean arterial sodium (mmol/L) +/- standard deviation. The graph shows the significant (P<0.05) hypernatremia that developed in the HSS-HES group after infusion of the treatment solutions. The hypernatremia for the HSS-HES group persisted throughout the experimental protocol. The sodium concentration in the HSS-HES group was significantly greater than in the ISO and control groups.
Figure 9. Mean arterial ionized calcium (mg/dl) +/- standard deviation. A significant (P<0.05) significant difference in iCa concentration was detected among groups between time 5 minutes and 120 minutes. The iCa concentration at time 180 minutes was not significantly different among groups.
Figure 10. Mean arterial PaO₂ +/- standard deviation. The graph shows a significant (P<0.05) decreased in PaO₂ following from base line in all treatments.
Figure 11. Mean arterial PaCO$_2$ +/- standard deviation. The graph shows the significant increase in arterial PCO$_2$ from base line (P<0.05) in all treatment groups.
Figure 12. Mean arterial HCO$_3^-$ (mEq/L) +/- standard deviation. The graph shows the significant decrease in arterial HCO$_3^-$ from base line (P<0.05) in all treatment groups.
Figure 13. Mean arterial base excess (mEq/L) +/- standard deviation. The graph shows the significant decrease in arterial base excess from base line (P<0.05) in all treatment groups.
Figure 14. Mean venous pH +/- standard deviation. The graph shows the significant decrease in venous pH for all treatment groups over time. A significantly (P < 0.05) greater decrease in pH was observed for the ISO and HSS-HES groups compared to the control group.
Figure 15. Mean venous PCV (%) +/- standard deviation. The graph shows the significant increase in PCV over time for all groups. The PCV was significantly (P < 0.05) higher in the control group compared with the HSS-HES group.
Figure 16. Mean venous total protein (g/dl) +/- standard deviation. The total protein concentration significantly (P < 0.05) decreased from the base line value for times 5, 15 and 30 minutes in all treatment groups. A trend towards a more marked decrease in total protein was seen in the ISO group at all time points except 150 minutes.
Figure 17. Mean venous sodium (mmol/L) +/- standard deviation. The graph represents significant (P<0.05) increased in the venous sodium concentration seen in the HSS-HES group compared with the other two groups.
Figure 18. Mean venous chloride (mmol/L) +/- standard deviation. The graph illustrates the significantly (P<0.05) higher concentration of chloride in the HSS-HES group compared with the control and ISO groups.
Figure 19. Mean venous ionized calcium (mg/dl) +/- standard deviation. The graph represents the significant decrease in iCa concentration from base line in all treatment groups. iCa concentration decreased significantly (P<0.05) less in the HSS-HES group than in the other two groups.
Figure 20. Mean venous creatinine (mg/dl) +/- standard deviation. The graph signifies the azotemia that developed over time in all groups. A significant (P<0.05) difference was observed when the ISO and HSS-HES groups were compared.
Figure 21. Mean venous lactate (mmol/L) +/- standard deviation. The graph depicts the increase in the lactate concentration in venous blood over time for all groups. A trend towards a lower lactate concentration was seen in the HSS-HES group.
Figure 22. Mean venous bicarbonate (mEq/L) +/- standard deviation. The graph depicts the significant (P < 0.05) decrease in venous bicarbonate concentration seen in all groups from the base line value.
Figure 23. Mean platelets (cells/μl) +/- standard deviation. The graph depicts the decrease in platelet concentration for all treatment groups over time. A significant (P<0.05) decrease in platelet concentration was seen for all groups compared with the base line value.
Figure 24. Mean prothrombin time (seconds) +/- standard deviation. The graph shows the elevation of PT for all groups over time. The PT value for the HSS-HES group remained within normal ranges for the study period.
<table>
<thead>
<tr>
<th>Time (minutes)</th>
<th>Control</th>
<th>ISO</th>
<th>HSS-HES</th>
</tr>
</thead>
<tbody>
<tr>
<td>60</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>180</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Figure 25. Mean partial thrombin time (seconds) +/- standard deviation. The graph shows the elevation of aPTT for all groups over time. The aPTT value for the HSS-HES group remained within normal ranges for the study period.
Figure 26. Mean cardiac output (ml/kg/min) average +/- standard deviation. The graph shows the elevation in CO, with a peak reached between times 5 and 30 minutes. A significant (P<0.05) change in CO from the base line value was observed in all groups at times 5, 30, 60 and 90 minutes. The CO value returned to a value not significantly different from base line at 120 minutes.
Figure 27. Mean central venous pressure (mmHg) +/- standard deviation. Graph showing the changes in MCVP for all treatment groups over time. A significant (P<0.05) increase in MCVP was observed for the ISO group.
Figure 28. Means of mean pulmonary arterial pressures (mmHg) +/- standard deviation.

This graph depicts the changes in MPAP over time for all three experimental groups. In all groups significant differences form the base line value of MPAP was seen at times -60, -30, 0, 5, 15, 30, 60, 150 and 180 minutes.
Figure 29. Means of mean arterial pressure (mmHg) +/- standard deviation. This graph demonstrates the changes in arterial blood pressure over time for all three treatment groups.
Figure 30. Means of mean arterial pressure (mmHg) +/- standard deviation. The graph depicts the changes in heart rate over time for all three treatment groups.
Tables

Table 1. Shows the weight, age and breed and group allocation of the horses used in the experiment. Statistical significance was not found among groups regarding age or weight.

<table>
<thead>
<tr>
<th>HORSE</th>
<th>GROUP</th>
<th>WEIGHT (kg)</th>
<th>AGE (yr)</th>
<th>BREED</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>CONTROL</td>
<td>688</td>
<td>9</td>
<td>TB</td>
</tr>
<tr>
<td>2</td>
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</tr>
<tr>
<td>3</td>
<td>CONTROL</td>
<td>460</td>
<td>23</td>
<td>TB</td>
</tr>
<tr>
<td>4</td>
<td>CONTROL</td>
<td>640</td>
<td>20</td>
<td>ARBX</td>
</tr>
<tr>
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<td>CONTROL</td>
<td>500</td>
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<td>TB</td>
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<td>X</td>
</tr>
<tr>
<td>AVERAGE</td>
<td></td>
<td>561</td>
<td>12.5</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>ISOTONIC</td>
<td>560</td>
<td>14</td>
<td>TB</td>
</tr>
<tr>
<td>8</td>
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<td>TB</td>
</tr>
<tr>
<td>9</td>
<td>ISOTONIC</td>
<td>400</td>
<td>20</td>
<td>TB</td>
</tr>
<tr>
<td>10</td>
<td>ISOTONIC</td>
<td>460</td>
<td>7</td>
<td>SADDL</td>
</tr>
<tr>
<td>11</td>
<td>ISOTONIC</td>
<td>500</td>
<td>12</td>
<td>QH</td>
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<tr>
<td>12</td>
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</tr>
<tr>
<td>AVERAGE</td>
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<td>490</td>
<td>12.8</td>
<td></td>
</tr>
<tr>
<td>13</td>
<td>HSS-HES</td>
<td>440</td>
<td>5</td>
<td>QH</td>
</tr>
<tr>
<td>14</td>
<td>HSS-HES</td>
<td>500</td>
<td>9</td>
<td>TB</td>
</tr>
<tr>
<td>15</td>
<td>HSS-HES</td>
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<td>OLD</td>
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<tr>
<td>16</td>
<td>HSS-HES</td>
<td>460</td>
<td>20</td>
<td>QH</td>
</tr>
<tr>
<td>17</td>
<td>HSS-HES</td>
<td>480</td>
<td>6</td>
<td>PAINT</td>
</tr>
<tr>
<td>18</td>
<td>HSS-HES</td>
<td>560</td>
<td>17</td>
<td>TB</td>
</tr>
<tr>
<td>AVERAGE</td>
<td></td>
<td>493</td>
<td>11.8</td>
<td></td>
</tr>
</tbody>
</table>
Table 2. Mean arterial pH +/- standard deviation. Three treatment groups (Control, ISO and HSS-HES) were evaluated at five different times (-62, 5, 60, 120 and 180 minutes).

<table>
<thead>
<tr>
<th>TIME (min)</th>
<th>CONTROL a</th>
<th>ISO b</th>
<th>HSS-HES b</th>
</tr>
</thead>
<tbody>
<tr>
<td>-62</td>
<td>7.445 +/- 0.043</td>
<td>7.409 +/- 0.035</td>
<td>7.420 +/- 0.011</td>
</tr>
<tr>
<td>5</td>
<td>7.308 +/- 0.042</td>
<td>7.224 +/- 0.029</td>
<td>7.246 +/- 0.050</td>
</tr>
<tr>
<td>60</td>
<td>7.265 +/- 0.040</td>
<td>7.198 +/- 0.026</td>
<td>7.205 +/- 0.059</td>
</tr>
<tr>
<td>120</td>
<td>7.219 +/- 0.040</td>
<td>7.156 +/- 0.034</td>
<td>7.173 +/- 0.066</td>
</tr>
<tr>
<td>180</td>
<td>7.191 +/- 0.045</td>
<td>7.122 +/- 0.039</td>
<td>7.158 +/- 0.056</td>
</tr>
</tbody>
</table>

*Variables with the same letter were significantly different at P<0.05*

Table 3. Mean arterial sodium (mmol/L) +/- standard deviation. Three treatment groups (Control, ISO and HSS-HES) were evaluated at five different times (-62, 5, 60, 120 and 180 minutes).

<table>
<thead>
<tr>
<th>TIME (min)</th>
<th>CONTROL a</th>
<th>ISO b</th>
<th>HSS-HES a, b</th>
</tr>
</thead>
<tbody>
<tr>
<td>-62</td>
<td>136.3 +/- 2.2</td>
<td>136.8 +/- 2.8</td>
<td>136.3 +/- 4.1</td>
</tr>
<tr>
<td>5</td>
<td>135.6 +/- 3.2</td>
<td>135.2 +/- 2.1</td>
<td>146.3 +/- 2.6</td>
</tr>
<tr>
<td>60</td>
<td>136.3 +/- 3.1</td>
<td>135.0 +/- 1.9</td>
<td>145.5 +/- 2.6</td>
</tr>
<tr>
<td>120</td>
<td>135.8 +/- 2.8</td>
<td>134.5 +/- 1.5</td>
<td>144.8 +/- 3.2</td>
</tr>
<tr>
<td>180</td>
<td>135.5 +/- 1.9</td>
<td>134.5 +/- 2.2</td>
<td>145.3 +/- 3.4</td>
</tr>
</tbody>
</table>

*Variables with the same letter were significantly different at P<0.05*
Table 4. Mean arterial ionized calcium (mg/dl) +/- standard deviation. Three treatment groups (Control, ISO and HSS-HES) were evaluated at five different times (-62, 5, 60, 120 and 180 minutes).

<table>
<thead>
<tr>
<th>TIME (min)</th>
<th>CONTROL *</th>
<th>ISO *</th>
<th>HSS-HES a,b</th>
</tr>
</thead>
<tbody>
<tr>
<td>-62</td>
<td>4.99 +/- 0.46</td>
<td>5.23 +/- 0.31</td>
<td>5.17 +/- 0.64</td>
</tr>
<tr>
<td>5</td>
<td>4.33 +/- 0.40</td>
<td>3.83 +/- 0.30</td>
<td>5.09 +/- 0.27</td>
</tr>
<tr>
<td>60</td>
<td>4.48 +/- 0.59</td>
<td>4.15 +/- 0.60</td>
<td>5.41 +/- 0.20</td>
</tr>
<tr>
<td>120</td>
<td>4.52 +/- 0.44</td>
<td>4.43 +/- 0.37</td>
<td>5.14 +/- 0.70</td>
</tr>
<tr>
<td>180</td>
<td>4.77 +/- 0.49</td>
<td>4.37 +/- 0.32</td>
<td>5.32 +/- 0.27</td>
</tr>
</tbody>
</table>

Variables with the same letter were significantly different at P<0.05

Table 5. Mean arterial PaO₂ +/- standard deviation. Three treatment groups (Control, ISO and HSS-HES) were evaluated at five different times (-62, 5, 60, 120 and 180 minutes).

<table>
<thead>
<tr>
<th>TIME (min)</th>
<th>CONTROL</th>
<th>ISO</th>
<th>HSS-HES</th>
</tr>
</thead>
<tbody>
<tr>
<td>-62</td>
<td>453.2 +/- 63.3</td>
<td>475.7 +/- 55.4</td>
<td>444.2 +/- 145.3</td>
</tr>
<tr>
<td>5</td>
<td>187.0 +/- 131.2</td>
<td>145.5 +/- 81.4</td>
<td>253.0 +/- 168.8</td>
</tr>
<tr>
<td>60</td>
<td>89.3 +/- 30.3</td>
<td>98.3 +/- 40.7</td>
<td>166.3 +/- 102.2</td>
</tr>
<tr>
<td>120</td>
<td>79.4 +/- 10.5</td>
<td>89.9 +/- 34.2</td>
<td>121.6 +/- 41.0</td>
</tr>
<tr>
<td>180</td>
<td>83.7 +/- 6.4</td>
<td>62.7 +/- 7.9</td>
<td>112.4 +/- 44.3</td>
</tr>
</tbody>
</table>
Table 6. Mean arterial PaCO\(_2\) +/- standard deviation. Three treatment groups (Control, ISO and HSS-HES) were evaluated at five different times (-62, 5, 60, 120 and 180 minutes).

<table>
<thead>
<tr>
<th>TIME (min)</th>
<th>CONTROL</th>
<th>ISO</th>
<th>HSS-HES</th>
</tr>
</thead>
<tbody>
<tr>
<td>-62</td>
<td>41.8 +/- 5.3</td>
<td>43.4 +/- 4.6</td>
<td>40.6 +/- 6.9</td>
</tr>
<tr>
<td>5</td>
<td>44.3 +/- 7.6</td>
<td>50.1 +/- 4.9</td>
<td>47.0 +/- 5.9</td>
</tr>
<tr>
<td>60</td>
<td>46.0 +/- 3.5</td>
<td>50.8 +/- 5.5</td>
<td>48.5 +/- 4.7</td>
</tr>
<tr>
<td>120</td>
<td>50.7 +/- 3.0</td>
<td>54.0 +/- 7.2</td>
<td>50.8 +/- 5.4</td>
</tr>
<tr>
<td>180</td>
<td>55.8 +/- 4.0</td>
<td>57.1 +/- 9.4</td>
<td>53.2 +/- 6.1</td>
</tr>
</tbody>
</table>

Table 7. Arterial bicarbonate concentration (mEq/L) and arterial base excess (mEq/L) +/- standard deviation. Three treatment groups (Control, ISO and HSS-HES) were evaluated at five different times (-62, 5, 60, 120 and 180 minutes).

<table>
<thead>
<tr>
<th>TIME (min)</th>
<th>Arterial bicarbonate (mEq/L) +/- std. dev.</th>
<th>Arterial base excess (mEq/L) +/- std. dev.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>ISO</td>
</tr>
<tr>
<td>-62</td>
<td>28.3 +/- 2.9</td>
<td>26.9 +/- 2.3</td>
</tr>
<tr>
<td>5</td>
<td>21.5 +/- 2.4</td>
<td>19.9 +/- 1.4</td>
</tr>
<tr>
<td>60</td>
<td>20.3 +/- 2.4</td>
<td>19.0 +/- 1.8</td>
</tr>
<tr>
<td>120</td>
<td>20.1 +/- 2.3</td>
<td>18.2 +/- 1.7</td>
</tr>
<tr>
<td>180</td>
<td>20.7 +/- 2.8</td>
<td>17.8 +/- 2.1</td>
</tr>
</tbody>
</table>
Table 8. Arterial potassium concentration (mEq/L) +/- standard deviation. Three treatment groups (Control, ISO and HSS-HES) were evaluated at five different times (-62, 5, 60, 120 and 180 minutes).

<table>
<thead>
<tr>
<th>TIME (min)</th>
<th>Arterial K (mEq/L) +/- std. dev.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
</tr>
<tr>
<td>-62</td>
<td>3.6 +/- 0.3</td>
</tr>
<tr>
<td>5</td>
<td>2.8 +/- 0.3</td>
</tr>
<tr>
<td>60</td>
<td>2.8 +/- 0.2</td>
</tr>
<tr>
<td>120</td>
<td>3.0 +/- 0.2</td>
</tr>
<tr>
<td>180</td>
<td>3.5 +/- 0.5</td>
</tr>
</tbody>
</table>

Table 9. Mean venous pH +/- standard deviation. Three treatment groups (Control, ISO and HSS-HES) were evaluated at nine different times (-62, 5, 15, 30, 60, 90, 120, 150 and 180 minutes).

<table>
<thead>
<tr>
<th>TIME (min)</th>
<th>Mean venous pH +/- std. dev.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CONTROL a, b</td>
</tr>
<tr>
<td>-62</td>
<td>7.422 +/- 0.045</td>
</tr>
<tr>
<td>5</td>
<td>7.322 +/- 0.031</td>
</tr>
<tr>
<td>15</td>
<td>7.310 +/- 0.025</td>
</tr>
<tr>
<td>30</td>
<td>7.300 +/- 0.025</td>
</tr>
<tr>
<td>60</td>
<td>7.283 +/- 0.030</td>
</tr>
<tr>
<td>90</td>
<td>7.264 +/- 0.028</td>
</tr>
<tr>
<td>120</td>
<td>7.243 +/- 0.029</td>
</tr>
<tr>
<td>150</td>
<td>7.226 +/- 0.034</td>
</tr>
<tr>
<td>180</td>
<td>7.215 +/- 0.035</td>
</tr>
</tbody>
</table>

Variables with the same letter were significantly different at P<0.05
Table 10. Mean venous PCV (%) +/- standard deviation. Three treatment groups (Control, ISO and HSS-HES) were evaluated at nine different times (-62, 5, 15, 30, 60, 90, 120, 150 and 180 minutes).

<table>
<thead>
<tr>
<th>TIME (min)</th>
<th>Control a</th>
<th>ISO b</th>
<th>HSS-HES a</th>
</tr>
</thead>
<tbody>
<tr>
<td>-62</td>
<td>32 +/- 6</td>
<td>30 +/- 2</td>
<td>25 +/- 7</td>
</tr>
<tr>
<td>5</td>
<td>38 +/- 5</td>
<td>33 +/- 6</td>
<td>31 +/- 5</td>
</tr>
<tr>
<td>15</td>
<td>41 +/- 4</td>
<td>35 +/- 5</td>
<td>36 +/- 5</td>
</tr>
<tr>
<td>30</td>
<td>44 +/- 4</td>
<td>37 +/- 6</td>
<td>32 +/- 8</td>
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<tr>
<td>60</td>
<td>45 +/- 5</td>
<td>41 +/- 3</td>
<td>38 +/- 9</td>
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<tr>
<td>90</td>
<td>47 +/- 5</td>
<td>44 +/- 3</td>
<td>42 +/- 13</td>
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<td>47 +/- 9</td>
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<td>42 +/- 12</td>
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<td>150</td>
<td>48 +/- 6</td>
<td>47 +/- 4</td>
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</tr>
<tr>
<td>180</td>
<td>51 +/- 4</td>
<td>46 +/- 2</td>
<td>40 +/- 8</td>
</tr>
</tbody>
</table>

Variables with the same letter were significantly different at P<0.05

Table 11. Mean venous total protein (g/dl) +/- standard deviation. Three treatment groups (Control, ISO and HSS-HES) were evaluated at nine different times (-62, 5, 15, 30, 60, 90, 120, 150 and 180 minutes).

<table>
<thead>
<tr>
<th>TIME (min)</th>
<th>Control</th>
<th>ISO</th>
<th>HSS-HES</th>
</tr>
</thead>
<tbody>
<tr>
<td>-62</td>
<td>5.5 +/- 0.5</td>
<td>6.1 +/- 0.4</td>
<td>5.6 +/- 0.5</td>
</tr>
<tr>
<td>5</td>
<td>4.7 +/- 0.5</td>
<td>3.5 +/- 0.6</td>
<td>4.3 +/- 1.3</td>
</tr>
<tr>
<td>15</td>
<td>4.9 +/- 0.5</td>
<td>3.7 +/- 0.4</td>
<td>4.9 +/- 0.5</td>
</tr>
<tr>
<td>30</td>
<td>5.2 +/- 0.7</td>
<td>4.2 +/- 0.5</td>
<td>5.1 +/- 0.6</td>
</tr>
<tr>
<td>60</td>
<td>5.5 +/- 0.9</td>
<td>4.5 +/- 0.6</td>
<td>5.4 +/- 0.5</td>
</tr>
<tr>
<td>90</td>
<td>5.7 +/- 0.7</td>
<td>4.9 +/- 0.6</td>
<td>5.8 +/- 0.4</td>
</tr>
<tr>
<td>120</td>
<td>6.0 +/- 0.6</td>
<td>5.4 +/- 0.4</td>
<td>5.8 +/- 0.5</td>
</tr>
<tr>
<td>150</td>
<td>5.8 +/- 1.3</td>
<td>5.6 +/- 0.4</td>
<td>5.4 +/- 1.2</td>
</tr>
<tr>
<td>180</td>
<td>6.2 +/- 0.5</td>
<td>5.6 +/- 0.5</td>
<td>5.9 +/- 0.6</td>
</tr>
</tbody>
</table>
Table 12. Mean venous sodium (mmol/L) +/- standard deviation. Three treatment groups (Control, ISO and HSS-HES) were evaluated at nine different times (-62, 5, 15, 30, 60, 90, 120, 150 and 180 minutes).

<table>
<thead>
<tr>
<th>TIME (min)</th>
<th>CONTROL a</th>
<th>ISO b</th>
<th>HSS-HES a, b</th>
</tr>
</thead>
<tbody>
<tr>
<td>-62</td>
<td>139.0 +/- 1.1</td>
<td>139.8 +/- 3.1</td>
<td>137.0 +/- 5.8</td>
</tr>
<tr>
<td>5</td>
<td>138.7 +/- 1.9</td>
<td>137.5 +/- 2.8</td>
<td>150.0 +/- 4.0</td>
</tr>
<tr>
<td>15</td>
<td>138.2 +/- 1.7</td>
<td>137.8 +/- 2.9</td>
<td>150.0 +/- 2.8</td>
</tr>
<tr>
<td>30</td>
<td>138.5 +/- 2.1</td>
<td>137.8 +/- 3.2</td>
<td>149.8 +/- 2.3</td>
</tr>
<tr>
<td>60</td>
<td>140.2 +/- 2.6</td>
<td>138.2 +/- 2.3</td>
<td>149.0 +/- 3.2</td>
</tr>
<tr>
<td>90</td>
<td>140.2 +/- 2.3</td>
<td>138.2 +/- 2.5</td>
<td>148.7 +/- 3.3</td>
</tr>
<tr>
<td>120</td>
<td>140.3 +/- 2.7</td>
<td>138.3 +/- 2.7</td>
<td>149.0 +/- 4.3</td>
</tr>
<tr>
<td>150</td>
<td>141.0 +/- 2.2</td>
<td>139.0 +/- 2.1</td>
<td>148.5 +/- 3.0</td>
</tr>
<tr>
<td>180</td>
<td>140.5 +/- 1.9</td>
<td>139.3 +/- 2.3</td>
<td>148.7 +/- 3.6</td>
</tr>
</tbody>
</table>

Variables with the same letter were significantly different at P<0.05

Table 13. Mean venous chloride (mmol/L) +/- standard deviation. Three treatment groups (Control, ISO and HSS-HES) were evaluated at nine different times (-62, 5, 15, 30, 60, 90, 120, 150 and 180 minutes).

<table>
<thead>
<tr>
<th>TIME (min)</th>
<th>Control a</th>
<th>ISO b</th>
<th>HSS-HES a, b</th>
</tr>
</thead>
<tbody>
<tr>
<td>-62</td>
<td>99.3 +/- 2.1</td>
<td>100.8 +/- 3.2</td>
<td>102.3 +/- 10.0</td>
</tr>
<tr>
<td>5</td>
<td>101.2 +/- 4.0</td>
<td>99.7 +/- 2.6</td>
<td>116.5 +/- 6.3</td>
</tr>
<tr>
<td>15</td>
<td>99.3 +/- 2.0</td>
<td>99.8 +/- 3.1</td>
<td>115.3 +/- 3.8</td>
</tr>
<tr>
<td>30</td>
<td>100.3 +/- 2.3</td>
<td>99.8 +/- 3.5</td>
<td>114.8 +/- 2.9</td>
</tr>
<tr>
<td>60</td>
<td>102.3 +/- 2.4</td>
<td>100.8 +/- 2.9</td>
<td>114.7 +/- 4.3</td>
</tr>
<tr>
<td>90</td>
<td>102.3 +/- 1.4</td>
<td>101.0 +/- 3.4</td>
<td>114.3 +/- 4.1</td>
</tr>
<tr>
<td>120</td>
<td>102.7 +/- 4.7</td>
<td>99.8 +/- 3.3</td>
<td>114.0 +/- 5.2</td>
</tr>
<tr>
<td>150</td>
<td>102.3 +/- 3.2</td>
<td>99.2 +/- 1.3</td>
<td>114.3 +/- 5.6</td>
</tr>
<tr>
<td>180</td>
<td>101.3 +/- 1.9</td>
<td>100.5 +/- 3.2</td>
<td>113.3 +/- 3.6</td>
</tr>
</tbody>
</table>

Variables with the same letter were significantly different at P<0.05
Table 14. Mean venous ionized calcium (mg/dl) +/- standard deviation. Three treatment groups (Control, ISO and HSS-HES) were evaluated at nine different times (-62, 5, 15, 30, 60, 90, 120, 150 and 180 minutes).

<table>
<thead>
<tr>
<th>TIME (min)</th>
<th>CONTROL a</th>
<th>ISO b</th>
<th>HSS-HES a, b</th>
</tr>
</thead>
<tbody>
<tr>
<td>-62</td>
<td>5.80 +/- 0.29</td>
<td>6.13 +/- 0.46</td>
<td>5.92 +/- 0.60</td>
</tr>
<tr>
<td>5</td>
<td>4.70 +/- 0.23</td>
<td>4.41 +/- 0.47</td>
<td>5.09 +/- 0.98</td>
</tr>
<tr>
<td>15</td>
<td>4.92 +/- 0.40</td>
<td>4.52 +/- 0.37</td>
<td>5.56 +/- 0.37</td>
</tr>
<tr>
<td>30</td>
<td>4.76 +/- 0.42</td>
<td>4.37 +/- 0.47</td>
<td>5.41 +/- 0.40</td>
</tr>
<tr>
<td>60</td>
<td>4.33 +/- 0.66</td>
<td>4.50 +/- 0.26</td>
<td>5.51 +/- 0.36</td>
</tr>
<tr>
<td>90</td>
<td>4.62 +/- 0.45</td>
<td>4.45 +/- 0.26</td>
<td>5.65 +/- 0.45</td>
</tr>
<tr>
<td>120</td>
<td>4.42 +/- 0.61</td>
<td>4.63 +/- 0.36</td>
<td>5.43 +/- 0.36</td>
</tr>
<tr>
<td>150</td>
<td>4.29 +/- 0.58</td>
<td>4.74 +/- 0.19</td>
<td>5.24 +/- 0.83</td>
</tr>
<tr>
<td>180</td>
<td>4.43 +/- 0.27</td>
<td>4.57 +/- 0.30</td>
<td>5.49 +/- 0.77</td>
</tr>
</tbody>
</table>

Variables with the same letter were significantly different at P<0.05

Table 15. Mean venous creatinine (mg/dl) +/- standard deviation. Three treatment groups (Control, ISO and HSS-HES) were evaluated at nine different times (-62, 5, 15, 30, 60, 90, 120, 150 and 180 minutes).

<table>
<thead>
<tr>
<th>TIME (min)</th>
<th>Control a</th>
<th>ISO b</th>
<th>HSS-HES b, c</th>
</tr>
</thead>
<tbody>
<tr>
<td>-62</td>
<td>1.28 +/- 0.36</td>
<td>1.08 +/- 0.31</td>
<td>1.53 +/- 0.23</td>
</tr>
<tr>
<td>5</td>
<td>1.62 +/- 0.40</td>
<td>1.12 +/- 0.32</td>
<td>1.55 +/- 0.48</td>
</tr>
<tr>
<td>15</td>
<td>1.65 +/- 0.37</td>
<td>1.23 +/- 0.29</td>
<td>1.75 +/- 0.21</td>
</tr>
<tr>
<td>30</td>
<td>1.75 +/- 0.36</td>
<td>1.30 +/- 0.34</td>
<td>1.82 +/- 0.23</td>
</tr>
<tr>
<td>60</td>
<td>1.78 +/- 0.31</td>
<td>1.45 +/- 0.31</td>
<td>2.15 +/- 0.25</td>
</tr>
<tr>
<td>90</td>
<td>1.93 +/- 0.30</td>
<td>1.57 +/- 0.28</td>
<td>2.35 +/- 0.45</td>
</tr>
<tr>
<td>120</td>
<td>2.08 +/- 0.56</td>
<td>1.70 +/- 0.25</td>
<td>2.45 +/- 0.33</td>
</tr>
<tr>
<td>150</td>
<td>2.17 +/- 0.33</td>
<td>1.92 +/- 0.23</td>
<td>2.40 +/- 0.61</td>
</tr>
<tr>
<td>180</td>
<td>2.38 +/- 0.48</td>
<td>1.92 +/- 0.29</td>
<td>2.77 +/- 0.59</td>
</tr>
</tbody>
</table>

Variables with the same letter were significantly different at P<0.05
Table 16. Mean venous lactate (mmol/L) +/- standard deviation. Three treatment groups (Control, ISO and HSS-HES) were evaluated at nine different times (-62, 5, 15, 30, 60, 90, 120, 150 and 180 minutes).

<table>
<thead>
<tr>
<th>TIME (min)</th>
<th>Control</th>
<th>ISO</th>
<th>HSS-HES</th>
</tr>
</thead>
<tbody>
<tr>
<td>-62</td>
<td>0.95 +/- 0.31</td>
<td>1.10 +/- 0.27</td>
<td>0.72 +/- 0.25</td>
</tr>
<tr>
<td>5</td>
<td>3.27 +/- 0.75</td>
<td>3.15 +/- 1.00</td>
<td>2.68 +/- 0.23</td>
</tr>
<tr>
<td>15</td>
<td>3.88 +/- 0.85</td>
<td>3.47 +/- 1.02</td>
<td>2.82 +/- 0.29</td>
</tr>
<tr>
<td>30</td>
<td>4.08 +/- 0.88</td>
<td>3.97 +/- 1.24</td>
<td>3.16 +/- 0.30</td>
</tr>
<tr>
<td>60</td>
<td>4.32 +/- 0.80</td>
<td>4.42 +/- 1.34</td>
<td>3.56 +/- 0.38</td>
</tr>
<tr>
<td>90</td>
<td>4.63 +/- 0.93</td>
<td>4.78 +/- 1.37</td>
<td>3.76 +/- 0.27</td>
</tr>
<tr>
<td>120</td>
<td>4.60 +/- 0.73</td>
<td>4.88 +/- 1.33</td>
<td>3.84 +/- 0.52</td>
</tr>
<tr>
<td>150</td>
<td>4.77 +/- 1.26</td>
<td>5.15 +/- 1.41</td>
<td>3.74 +/- 0.69</td>
</tr>
<tr>
<td>180</td>
<td>5.05 +/- 1.07</td>
<td>5.25 +/- 1.59</td>
<td>4.04 +/- 0.36</td>
</tr>
</tbody>
</table>

Table 17. Venous bicarbonate concentration (mEq/L) +/- standard deviation. Three treatment groups (Control, ISO and HSS-HES) were evaluated at nine different times (-62, 5, 15, 30, 60, 90, 120, 150 and 180 minutes).

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Control</th>
<th>ISO</th>
<th>HSS-HES</th>
</tr>
</thead>
<tbody>
<tr>
<td>-62</td>
<td>31.5 +/- 1.2</td>
<td>30.7 +/- 2.6</td>
<td>30.9 +/- 3.7</td>
</tr>
<tr>
<td>5</td>
<td>26.2 +/- 3.6</td>
<td>25.0 +/- 2.7</td>
<td>22.7 +/- 5.9</td>
</tr>
<tr>
<td>15</td>
<td>26.4 +/- 2.3</td>
<td>25.2 +/- 2.5</td>
<td>24.0 +/- 2.9</td>
</tr>
<tr>
<td>30</td>
<td>25.4 +/- 2.3</td>
<td>24.7 +/- 2.6</td>
<td>23.0 +/- 2.2</td>
</tr>
<tr>
<td>60</td>
<td>23.7 +/- 2.0</td>
<td>23.6 +/- 2.2</td>
<td>22.2 +/- 2.1</td>
</tr>
<tr>
<td>90</td>
<td>23.1 +/- 2.0</td>
<td>22.4 +/- 1.8</td>
<td>21.8 +/- 2.2</td>
</tr>
<tr>
<td>120</td>
<td>22.4 +/- 3.7</td>
<td>22.1 +/- 2.0</td>
<td>21.2 +/- 2.5</td>
</tr>
<tr>
<td>150</td>
<td>22.9 +/- 1.3</td>
<td>22.3 +/- 2.3</td>
<td>20.7 +/- 3.0</td>
</tr>
<tr>
<td>180</td>
<td>23.8 +/- 2.0</td>
<td>21.6 +/- 2.1</td>
<td>21.7 +/- 2.3</td>
</tr>
</tbody>
</table>
Table 18. Partial venous CO$_2$ (mmHg) and partial venous O$_2$ (mmHg) +/- standard deviations. Three treatment groups (Control, ISO and HSS-HES) were evaluated at nine different times (-62, 5, 15, 30, 60, 90, 120, 150 and 180 minutes).

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>PvCO2 (mmHg) +/- std. dev.</th>
<th>PvO2 (mmHg) +/- std. dev.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>ISO</td>
</tr>
<tr>
<td>-62</td>
<td>48.1 +/- 4.2</td>
<td>49.9 +/- 5.5</td>
</tr>
<tr>
<td>5</td>
<td>50.4 +/- 7.6</td>
<td>56.8 +/- 7.2</td>
</tr>
<tr>
<td>15</td>
<td>52.0 +/- 4.4</td>
<td>57.7 +/- 6.0</td>
</tr>
<tr>
<td>30</td>
<td>51.2 +/- 3.8</td>
<td>56.3 +/- 5.1</td>
</tr>
<tr>
<td>60</td>
<td>49.7 +/- 2.9</td>
<td>55.6 +/- 4.3</td>
</tr>
<tr>
<td>90</td>
<td>50.3 +/- 2.1</td>
<td>55.8 +/- 5.1</td>
</tr>
<tr>
<td>120</td>
<td>51.4 +/- 3.7</td>
<td>59.6 +/- 6.5</td>
</tr>
<tr>
<td>150</td>
<td>54.7 +/- 2.6</td>
<td>61.2 +/- 6.8</td>
</tr>
<tr>
<td>180</td>
<td>58.2 +/- 5.1</td>
<td>60.9 +/- 8.5</td>
</tr>
</tbody>
</table>

Table 19. Venous potassium concentration (mEq/L) +/- standard deviation. Three treatment groups (Control, ISO and HSS-HES) were evaluated at nine different times (-62, 5, 15, 30, 60, 90, 120, 150 and 180 minutes).

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Venous K (mEq/L) +/- std. dev.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
</tr>
<tr>
<td>-62</td>
<td>3.5 +/- 0.2</td>
</tr>
<tr>
<td>5</td>
<td>3.2 +/- 0.3</td>
</tr>
<tr>
<td>15</td>
<td>3.1 +/- 0.2</td>
</tr>
<tr>
<td>30</td>
<td>3.1 +/- 0.3</td>
</tr>
<tr>
<td>60</td>
<td>2.9 +/- 0.2</td>
</tr>
<tr>
<td>90</td>
<td>3.1 +/- 0.3</td>
</tr>
<tr>
<td>120</td>
<td>3.2 +/- 0.5</td>
</tr>
<tr>
<td>150</td>
<td>3.3 +/- 0.3</td>
</tr>
<tr>
<td>180</td>
<td>3.5 +/- 0.2</td>
</tr>
</tbody>
</table>
Table 20. Venous glucose concentration (mg/dl) and venous urea concentration (mg/dl) +/- standard deviation. Three treatment groups (Control, ISO and HSS-HES) were evaluated at nine different times (-62, 5, 15, 30, 60, 90, 120, 150 and 180 minutes).

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Venous glucose (mg/dl) +/- std. dev.</th>
<th>Venous BUN (mg/dl) +/- std. dev.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>ISO</td>
</tr>
<tr>
<td>-62</td>
<td>120 +/- 42</td>
<td>137 +/- 19</td>
</tr>
<tr>
<td>5</td>
<td>173 +/- 64</td>
<td>187 +/- 18</td>
</tr>
<tr>
<td>15</td>
<td>189 +/- 59</td>
<td>199 +/- 15</td>
</tr>
<tr>
<td>30</td>
<td>181 +/- 62</td>
<td>202 +/- 38</td>
</tr>
<tr>
<td>60</td>
<td>173 +/- 76</td>
<td>203 +/- 31</td>
</tr>
<tr>
<td>90</td>
<td>164 +/- 74</td>
<td>194 +/- 34</td>
</tr>
<tr>
<td>120</td>
<td>147 +/- 84</td>
<td>182 +/- 33</td>
</tr>
<tr>
<td>150</td>
<td>125 +/- 73</td>
<td>169 +/- 39</td>
</tr>
<tr>
<td>180</td>
<td>115 +/- 68</td>
<td>150 +/- 35</td>
</tr>
</tbody>
</table>

Table 21. Mean platelets +/- standard deviation (cells/ul), mean prothrombin time (seconds) +/- standard deviation and mean activated partial thrombin time (seconds) +/- standard deviation. Three treatment groups [Control (cont), ISO and HSS-HES] were evaluated at three different times (-62, 60 and 180 minutes).

<table>
<thead>
<tr>
<th>TIME (Min)</th>
<th>Mean +/- std. dev. PLT (cells/ul)</th>
<th>Mean +/- std. dev. PT (seconds)</th>
<th>Mean +/- std. dev. aPTT (seconds)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Normal &gt; 100/ul</td>
<td>Normal = 14 – 22 sec</td>
<td>Normal = 131 – 199 sec</td>
</tr>
<tr>
<td></td>
<td>CONT</td>
<td>ISO</td>
<td>HSS-HES</td>
</tr>
<tr>
<td>-62</td>
<td>116 +/- 38</td>
<td>120 +/- 22</td>
<td>108 +/- 25</td>
</tr>
<tr>
<td>60</td>
<td>58 +/- 5</td>
<td>55 +/- 14</td>
<td>58 +/- 17</td>
</tr>
<tr>
<td>180</td>
<td>58 +/- 15</td>
<td>58 +/- 17</td>
<td>62 +/- 14</td>
</tr>
</tbody>
</table>
Table 22. Mean cardiac output (ml/kg/min) average +/- standard deviation. Three treatment groups (Control, ISO and HSS-HES) were evaluated at nine different times (-62, -30, 5, 30, 60, 90, 120, 150 and 180 minutes).

<table>
<thead>
<tr>
<th>TIME (min)</th>
<th>CONTROL</th>
<th>ISO</th>
<th>HSS-HES</th>
</tr>
</thead>
<tbody>
<tr>
<td>-62</td>
<td>25.2 +/- 4.5</td>
<td>24.9 +/- 8.2</td>
<td>24.3 +/- 5.9</td>
</tr>
<tr>
<td>-30</td>
<td>31.7 +/- 13.9</td>
<td>27.5 +/- 11.6</td>
<td>27.0 +/- 4.0</td>
</tr>
<tr>
<td>5</td>
<td>41.3 +/- 13.7</td>
<td>40.0 +/- 22.6</td>
<td>40.6 +/- 14.2</td>
</tr>
<tr>
<td>30</td>
<td>42.2 +/- 11.7</td>
<td>38.4 +/- 18.5</td>
<td>46.3 +/- 9.3</td>
</tr>
<tr>
<td>60</td>
<td>37.1 +/- 10.7</td>
<td>36.7 +/- 15.1</td>
<td>41.6 +/- 8.1</td>
</tr>
<tr>
<td>90</td>
<td>33.8 +/- 9.1</td>
<td>34.5 +/- 13.6</td>
<td>37.9 +/- 9.6</td>
</tr>
<tr>
<td>120</td>
<td>31.4 +/- 9.7</td>
<td>31.7 +/- 11.0</td>
<td>34.5 +/- 9.2</td>
</tr>
<tr>
<td>150</td>
<td>30.0 +/- 8.6</td>
<td>30.5 +/- 10.6</td>
<td>29.5 +/- 7.8</td>
</tr>
<tr>
<td>180</td>
<td>26.0 +/- 6.8</td>
<td>25.9 +/- 8.3</td>
<td>25.8 +/- 4.9</td>
</tr>
</tbody>
</table>

Variables with the same letter were significantly different at P<0.05

Table 23. Mean central venous pressure (mmHg) +/- standard deviation. Three treatment groups (Control, ISO and HSS-HES) were evaluated at twelve different times (-62, -60, -30, 0, 5, 15, 30, 60, 90, 120, 150 and 180 minutes).

<table>
<thead>
<tr>
<th>TIME (min)</th>
<th>CONTROL b</th>
<th>ISO a,b</th>
<th>HSS-HES a</th>
</tr>
</thead>
<tbody>
<tr>
<td>-62</td>
<td>10.0 +/- 2.5</td>
<td>12.2 +/- 4.2</td>
<td>9.7 +/- 4.7</td>
</tr>
<tr>
<td>-60</td>
<td>14.0 +/- 5.3</td>
<td>17.3 +/- 3.4</td>
<td>15.7 +/- 7.2</td>
</tr>
<tr>
<td>-30</td>
<td>11.2 +/- 3.5</td>
<td>14.3 +/- 4.4</td>
<td>10.5 +/- 6.7</td>
</tr>
<tr>
<td>0</td>
<td>16.2 +/- 3.9</td>
<td>36.8 +/- 5.0</td>
<td>17.7 +/- 5.1</td>
</tr>
<tr>
<td>5</td>
<td>13.5 +/- 4.8</td>
<td>30.2 +/- 4.4</td>
<td>14.7 +/- 6.4</td>
</tr>
<tr>
<td>15</td>
<td>9.2 +/- 4.1</td>
<td>26.3 +/- 4.4</td>
<td>14.5 +/- 5.9</td>
</tr>
<tr>
<td>30</td>
<td>7.0 +/- 3.6</td>
<td>24.2 +/- 3.9</td>
<td>11.2 +/- 5.0</td>
</tr>
<tr>
<td>60</td>
<td>5.5 +/- 2.8</td>
<td>19.0 +/- 2.9</td>
<td>9.5 +/- 4.8</td>
</tr>
<tr>
<td>90</td>
<td>3.8 +/- 2.0</td>
<td>14.8 +/- 3.7</td>
<td>8.0 +/- 5.3</td>
</tr>
<tr>
<td>120</td>
<td>3.8 +/- 1.9</td>
<td>11.7 +/- 4.2</td>
<td>7.5 +/- 6.2</td>
</tr>
<tr>
<td>150</td>
<td>6.0 +/- 1.7</td>
<td>12.2 +/- 4.4</td>
<td>9.8 +/- 6.2</td>
</tr>
<tr>
<td>180</td>
<td>8.2 +/- 4.0</td>
<td>14.0 +/- 5.7</td>
<td>12.7 +/- 4.2</td>
</tr>
</tbody>
</table>

Variables with the same letter were significantly different at P<0.05
Table 24. Mean pulmonary arterial pressure (mmHg) +/- standard deviation. Three treatment groups (Control, ISO and HSS-HES) were evaluated at twelve different times (-62, -60, -30, 0, 5, 15, 30, 60, 90, 120, 150 and 180 minutes).

<table>
<thead>
<tr>
<th>TIME (min)</th>
<th>Control</th>
<th>ISO</th>
<th>HSS-HES</th>
</tr>
</thead>
<tbody>
<tr>
<td>-62</td>
<td>23.3 +/- 3.4</td>
<td>20.8 +/- 3.3</td>
<td>20.2 +/- 7.7</td>
</tr>
<tr>
<td>-60</td>
<td>30.7 +/- 7.1</td>
<td>31.0 +/- 5.5</td>
<td>26.2 +/- 10.8</td>
</tr>
<tr>
<td>-30</td>
<td>32.8 +/- 9.3</td>
<td>29.8 +/- 4.8</td>
<td>25.2 +/- 6.6</td>
</tr>
<tr>
<td>0</td>
<td>26.3 +/- 6.0</td>
<td>41.5 +/- 9.3</td>
<td>31.5 +/- 4.0</td>
</tr>
<tr>
<td>5</td>
<td>27.8 +/- 4.6</td>
<td>40.3 +/- 6.2</td>
<td>30.2 +/- 4.6</td>
</tr>
<tr>
<td>15</td>
<td>24.3 +/- 6.1</td>
<td>36.5 +/- 5.4</td>
<td>30.3 +/- 4.9</td>
</tr>
<tr>
<td>30</td>
<td>23.2 +/- 7.4</td>
<td>33.5 +/- 4.3</td>
<td>28.2 +/- 5.7</td>
</tr>
<tr>
<td>60</td>
<td>20.2 +/- 10.4</td>
<td>29.7 +/- 5.0</td>
<td>25.7 +/- 5.8</td>
</tr>
<tr>
<td>90</td>
<td>18.0 +/- 7.4</td>
<td>26.3 +/- 5.0</td>
<td>24.3 +/- 5.8</td>
</tr>
<tr>
<td>120</td>
<td>19.5 +/- 7.8</td>
<td>25.3 +/- 1.0</td>
<td>25.0 +/- 5.8</td>
</tr>
<tr>
<td>150</td>
<td>22.5 +/- 9.4</td>
<td>24.3 +/- 3.0</td>
<td>27.2 +/- 7.4</td>
</tr>
<tr>
<td>180</td>
<td>26.8 +/- 9.3</td>
<td>25.2 +/- 4.3</td>
<td>29.2 +/- 7.9</td>
</tr>
</tbody>
</table>

Table 25. Mean arterial pressure (mmHg) +/- standard deviation. Three treatment groups (Control, ISO and HSS-HES) were evaluated at twelve different times (-62, -60, -30, 0, 5, 15, 30, 60, 90, 120, 150 and 180 minutes).

<table>
<thead>
<tr>
<th>TIME (min)</th>
<th>Control</th>
<th>ISO</th>
<th>HSS-HES</th>
</tr>
</thead>
<tbody>
<tr>
<td>-62</td>
<td>57.3 +/- 7.6</td>
<td>64.0 +/- 10.3</td>
<td>61.7 +/- 15.2</td>
</tr>
<tr>
<td>-60</td>
<td>60.5 +/- 10.3</td>
<td>58.0 +/- 6.0</td>
<td>60.7 +/- 10.9</td>
</tr>
<tr>
<td>-30</td>
<td>66.0 +/- 10.8</td>
<td>67.0 +/- 11.6</td>
<td>63.3 +/- 12.5</td>
</tr>
<tr>
<td>0</td>
<td>54.2 +/- 9.5</td>
<td>62.7 +/- 8.3</td>
<td>55.0 +/- 10.4</td>
</tr>
<tr>
<td>5</td>
<td>55.0 +/- 7.7</td>
<td>58.8 +/- 6.5</td>
<td>49.8 +/- 9.1</td>
</tr>
<tr>
<td>15</td>
<td>52.3 +/- 6.7</td>
<td>53.0 +/- 5.5</td>
<td>46.3 +/- 7.7</td>
</tr>
<tr>
<td>30</td>
<td>49.5 +/- 6.7</td>
<td>50.2 +/- 5.5</td>
<td>45.8 +/- 4.4</td>
</tr>
<tr>
<td>60</td>
<td>46.2 +/- 6.6</td>
<td>46.7 +/- 3.9</td>
<td>45.5 +/- 2.8</td>
</tr>
<tr>
<td>90</td>
<td>48.0 +/- 6.8</td>
<td>46.7 +/- 2.4</td>
<td>46.2 +/- 4.7</td>
</tr>
<tr>
<td>120</td>
<td>51.0 +/- 8.2</td>
<td>45.3 +/- 3.1</td>
<td>47.2 +/- 4.4</td>
</tr>
<tr>
<td>150</td>
<td>54.2 +/- 6.7</td>
<td>47.2 +/- 7.7</td>
<td>51.0 +/- 5.2</td>
</tr>
<tr>
<td>180</td>
<td>58.0 +/- 9.1</td>
<td>47.7 +/- 9.1</td>
<td>53.0 +/- 4.4</td>
</tr>
</tbody>
</table>
Table 26. Mean heart rate (beats per minute) +/- standard deviation. Three treatment groups (Control, ISO and HSS-HES) were evaluated at twelve different times (-62, -60, -30, 0, 5, 15, 30, 60, 90, 120, 150 and 180 minutes).

<table>
<thead>
<tr>
<th>TIME (min)</th>
<th>Heart Rate (beats per minute) +/- std. dev.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
</tr>
<tr>
<td>-62</td>
<td>34 +/- 2</td>
</tr>
<tr>
<td>-60</td>
<td>36 +/- 6</td>
</tr>
<tr>
<td>-30</td>
<td>40 +/- 6</td>
</tr>
<tr>
<td>0</td>
<td>36 +/- 3</td>
</tr>
<tr>
<td>5</td>
<td>37 +/- 3</td>
</tr>
<tr>
<td>15</td>
<td>37 +/- 4</td>
</tr>
<tr>
<td>30</td>
<td>39 +/- 7</td>
</tr>
<tr>
<td>60</td>
<td>37 +/- 8</td>
</tr>
<tr>
<td>90</td>
<td>38 +/- 8</td>
</tr>
<tr>
<td>120</td>
<td>37 +/- 7</td>
</tr>
<tr>
<td>150</td>
<td>37 +/- 6</td>
</tr>
<tr>
<td>180</td>
<td>36 +/- 6</td>
</tr>
</tbody>
</table>

Table 27. End tidal CO\textsubscript{2} (mmHg) and inspired O\textsubscript{2} (mmHg) +/- standard deviation. Three treatment groups (Control, ISO and HSS-HES) were evaluated at five different times (-62, 5, 60, 120 and 180 minutes).

<table>
<thead>
<tr>
<th>TIME (min)</th>
<th>ETCO\textsubscript{2} (mmHg) +/- std. dev.</th>
<th>FIO\textsubscript{2} (mmHg) +/- std. dev.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>ISO</td>
</tr>
<tr>
<td>-62</td>
<td>35.33 +/- 3.83</td>
<td>35.00 +/- 4.20</td>
</tr>
<tr>
<td>5</td>
<td>38.40 +/- 6.66</td>
<td>34.00 +/- 4.00</td>
</tr>
<tr>
<td>60</td>
<td>32.00 +/- 7.29</td>
<td>33.33 +/- 4.32</td>
</tr>
<tr>
<td>120</td>
<td>34.00 +/- 4.86</td>
<td>29.00 +/- 2.28</td>
</tr>
<tr>
<td>180</td>
<td>36.00 +/- 2.97</td>
<td>28.50 +/- 3.39</td>
</tr>
</tbody>
</table>
References


190. Sondeen JL, Gunther RA, Dubick MA. Comparison of 7.5% NaCl/6% dextran-70 resuscitation of hemorrhage between euhydrated and dehydrated sheep. Shock 1995;3:63-68.


Vita

Lucas Guillermo Pantaleon was born October 6th, 1973 in La Plata, Buenos Aires, Argentina to Marta Maria and Guillermo Pantaleon. He was raised in a small town west of Buenos Aires, Chacabuco.

Lucas attended National High School in Chacabuco and after having spent a year as an exchanged student in Minnesota, United States of America graduated from Fairmont High School in 1991.

After taking some time off, Lucas commenced a veterinary degree at the University of Buenos Aires, Buenos Aires, Argentina. He received his Veterinarian degree in 1999.

After graduation he assisted with vaccine field research for the National Agricultural Service in Buenos Aires and worked as a veterinarian at the Aleman and Mediterraneo Jumping Club in Buenos Aires and at the Santa Maria Stud in Buenos Aires.

In February 2001, Lucas started a fellowship at Hagyard Davidson McGee in Lexington, Kentucky. Then in August 2002, he began a residency in equine internal medicine at the Marion du Pont Scott Equine Medical Center in Leesburg, Virginia and a Masters in Veterinary Science at Virginia Polytechnic Institute.

Lucas’ future plans are to pursue his American veterinary license and find a job in a private practice.