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Published in:
Scandinavian Journal of Clinical & Laboratory Investigation

DOI:
10.3109/00365513.2012.762114

2013

Albumin-induced coagulopathy is less severe and more effectively reversed with fibrinogen concentrate than is synthetic colloid-induced coagulopathy

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Running head: Fibrinogen and dilutive coagulopathy

Abstract

Background: Volume resuscitation is essential to restore normovolemia during hemorrhagic shock, burns and sepsis. However, synthetic colloids cause dilutional coagulopathy. Aims were to determine whether the natural colloid albumin induces a lesser degree of coagulopathy compared to synthetic colloids, and the comparative effectiveness of fibrinogen concentrate to reverse coagulopathy following dilution with these solutions.

Methods: Rotational thromboelastometry-based tests were used to examine coagulation parameters in samples from 10 healthy volunteers, in undiluted blood and samples diluted 1:1 with saline, Ringer's acetate, hydroxyethyl starch (HES) 130/0.4, buffered HES 130/0.4, 3% dextran 60, 6% dextran 70 or 5% albumin. Samples were analyzed before and after addition of 2 mg/mL fibrinogen concentrate.

Results: EXTEM maximum clot firmness (MCF), clot formation time (CFT) and alpha angle (AA) decreased significantly upon dilution with all colloid solutions ($p<0.001$), although a significantly greater coagulopathic effect was seen for samples diluted with synthetic colloid solutions versus albumin ($p\leq0.001$). A significant reduction in the platelet component of clot strength (EXTEM MCF − FIBTEM MCF) was seen for samples diluted with synthetic colloids ($p<0.001$) but not albumin ($p=0.10$). Following addition of fibrinogen, FIBTEM MCF, EXTEM MCF and EXTEM AA were significantly higher, and EXTEM CFT was significantly shorter in samples diluted with albumin versus those treated with HES or dextran ($p\leq0.001$).

Conclusion: Hemodilution using albumin induced a lesser degree of coagulopathy compared with the synthetic colloids HES and dextran. In addition, albumin-induced coagulopathy was more effectively reversed following addition of fibrinogen concentrate compared with coagulopathy induced by synthetic colloids.

Key words

Albumins; dextrans; fibrinogen; hetastarch; Point-of-Care systems; Ringer's acetate; thrombelastography

Introduction

Volume resuscitation is essential to rapidly restore normovolemia in patients with hemorrhagic shock, burns and sepsis. Colloids, such as albumin, hydroxyethyl starches (HES) and dextran, and crystalloids including saline or Ringer's acetate are commonly used to replace fluid volume. However, both colloids and crystalloids can cause coagulopathy by dilution of coagulation factors such as fibrinogen and platelets, resulting in a reduction in clot strength, while colloids may also impair fibrin polymerization and platelet function [1-4].

The coagulopathic effects of resuscitation fluids have been studied by a number of groups using rotational thromboelastometry (ROTEM®) [5-9]. ROTEM-based tests provide information on the dynamics of clot formation and the mechanical strength of the clot. Dilution *in vitro* using either colloids [5, 6, 8, 9] or crystalloids [5, 7, 8] can reduce fibrin-based clot strength measured using the ROTEM-based FIBTEM assay, although synthetic colloids affect coagulation earlier and more extensively than crystalloids [10-13]. Godier *et al.* [5] showed that HES reduced fibrin-based clot strength more than saline or Ringer's lactate. Additionally, Niemi *et al.* [9] showed that the natural colloid albumin may induce fewer coagulation abnormalities than the synthetic colloids HES and gelatin.

In vivo, albumin also causes a lesser degree of coagulopathy compared with HES [11]. In a study of the effects of dilutional coagulopathy in cardiac surgery patients, no coagulopathic effects were seen with 4% albumin, whereas both 6% HES 200/0.5 and 6% HES 130/0.4 induced impairment of coagulation as diagnosed by ROTEM [14]. The use of colloids such as albumin during sepsis or hemorrhage may confer advantages over crystalloids, including enhanced duration of hemodynamic effects and less capillary leakage, meaning that a smaller dose may be required [15, 16].

In addition to volume replacement therapy, resuscitation protocols recommend replacement of coagulation factors such as fibrinogen, which are depleted during massive bleeding or sepsis.

Fresh frozen plasma (FFP) is commonly administered to correct coagulopathy; however, allogeneic blood products such as FFP have prolonged times to administration due to the thawing procedure, do not deliver consistent doses of coagulation factors, and are associated with side-effects including transfusion-associated circulatory overload and transfusion-related lung injury. In addition, the benefit of FFP administration is not yet proven in many clinical settings [17, 18], and a lack of definition exists as to the optimal ratio of FFP:red blood cells:platelets [19].

Treatment with fibrinogen concentrate has emerged as a promising strategy to correct coagulopathy during massive transfusion [10, 20]. In one retrospective report, trauma patients administered with FIBTEM-guided fibrinogen concentrate had a favorable survival rate [21]. FIBTEM-guided fibrinogen concentrate therapy decreased requirements for allogeneic blood products in cardiac surgery patients [22-24].

The potential for fibrinogen concentrate to reverse dilutional coagulopathy has been examined *in vitro* using ROTEM. Fenger-Eriksen *et al*. [10] showed that coagulopathy induced by hemodilution with HES 200/0.5, HES 130/0.4, or dextran 70 may be improved by fibrinogen supplementation. In addition, fibrinogen concentrate may be more efficient than FFP in correcting ROTEM parameters in samples diluted using Ringer's solution [25], although in a similar study examining hemodilution dilution with gelatin, FFP was more effective than fibrinogen in restoring EXTEM MCF; with FIBTEM analysis, MCF improved more by fibrinogen than by FFP [26].

The aims of this study were to use ROTEM-based tests to determine the extent to which albumin-induced dilutional coagulopathy can be reversed using fibrinogen concentrate, and to directly compare *in vitro* the effect of a high dose of fibrinogen concentrate on reversal of 50% dilution using a panel of resuscitation fluids. This is the first direct comparison of the use of fibrinogen concentrate for correction of dilutional coagulopathy induced using a panel of

crystalloids and colloids including 3% dextran 60, 6% dextran 70, buffered and un-buffered HES 130/0.4, 5% albumin, saline, and Ringer's acetate.

Methods and materials

Sampling

A 30-mL blood sample was drawn from 10 healthy volunteers (one woman and nine men, aged 30–40 years) who had given written consent, and who had not taken medication for 7 days preceding blood sampling. The study was approved by the Ethical Board, Lund, Sweden (Registration Number: DNR 484/2008). All procedures were performed in accordance with the Helsinki Declaration of 1975, as revised in 1983.

Venipuncture was performed with a 22-gauge needle (BD Vacutainer PrecisionGlide™ Multi-Sample Needle, Plymouth, UK). Blood samples were collected into plastic vacuum tubes (2.7 mL PET BD Vacutainer® Coagulation Tubes, Stadler, Germany) containing 0.109 M buffered sodium citrate. For each sample, the first tube was discarded.

Hemodilution

Citrated whole blood was diluted 1:1 with either saline, Ringer's acetate (Baxter Medical AB, Sweden), HES 130/0.4 saline solution, pH 4.0–4.5 (Voluven, Fresenius-Kabi, Germany), buffered HES 130/0.4 saline solution, pH 5.7–6.5 (Volulyte, Fresenius-Kabi, Germany), 3% buffered dextran 60, pH 6 (Plasmodex®, MEDA Group, Sweden), 6% dextran 70, pH 4–7 (Macrodex®, MEDA Group, Sweden), or 5% albumin (CSL Behring GmbH, Germany).

Human fibrinogen concentrate (RiaSTAP®, CSL Behring, Germany) was dissolved in sterile water according to the manufacturer's instructions, to a final concentration of 20 mg/mL, and 0.1 mL was added to 1-mL samples of undiluted blood or blood diluted with resuscitation fluids, as described in Table I. This corresponds to a dose of 8 g fibrinogen concentrate

administered to a 70-kg adult (plasma volume calculated from a standard hematocrite of 40%, blood volume (males 70 ml/kg – female 60 ml/kg).

Thromboelastometry

Thromboelastometry analyses were performed within 4 h following blood sampling. All samples were incubated in plugged tubes in a heating block for 30 min directly before thromboelastometry analyses were performed. Thromboelastometry was carried out using a ROTEM® analyzer (TEM International GmbH, Germany) according to the manufacturer's instructions, and ROTEM-based assays were run for 60 min.

The extrinsic coagulation assay (EXTEM) was initiated with tissue factor and the following coagulation parameters were measured: maximum clot firmness (MCF), clotting time (CT), clot formation time (CFT) and alpha-angle (AA). MCF is the maximum firmness of the clot, and fibrin, platelets and FXIII all influence clot strength. CT measures time from the start of measurement until the initiation of clotting (when MCF of 2 mm has been reached). CFT is the time from initiation of clotting until a clot firmness of 20 mm is detected, and AA is the angle of tangent of clot firmness over time at 2 mm amplitude; both CFT and AA represent the speed at which the clot forms, and are primarily influenced by platelets and fibrinogen.

The FIBTEM assay was performed as for the EXTEM assay but with the addition of the platelet inhibitor cytochalasin D, in order to measure the fibrin-based component of clot strength. A surrogate measure of platelet activity was determined by calculating the difference between EXTEM MCF and FIBTEM MCF.

Statistical analysis

All data were analyzed with analysis of variance (ANOVA). A two-way ANOVA was used to study the influence of the different resuscitation fluids on coagulation, and where statistically significant differences were detected, a Dunnett's adjustment was performed to allow for

multiple comparisons between methods. The level of statistical significance was set at $p<0.05$. Mean values and (+/- SEM(standard error of mean) are given in text and figures. Statistical analyses were performed using SPSS software.

Results

Blood diluted using each resuscitation fluid showed a significant decrease in MCF in the EXTEM assay, as compared to undiluted blood ($p<0.001$) [Figure 1a]. Dilution with dextran and HES solutions caused the most pronounced decreases in EXTEM MCF. Both had significantly greater effects on EXTEM MCF than did albumin ($p\leq0.001$). There were no significant differences in EXTEM MCF between samples diluted with crystalloids or albumin.

Upon addition of fibrinogen concentrate, EXTEM MCF increased in all samples (Figure 1a). For samples diluted with saline or Ringer's acetate, EXTEM MCF increased to approximately 100% of MCF values observed in the undiluted sample before addition of fibrinogen concentrate. In samples diluted with albumin, EXTEM MCF recovered to significantly higher levels than in samples diluted with HES or dextran ($p<0.001$). Upon addition of fibrinogen, samples diluted with HES or dextran recovered to 72–83% of EXTEM MCF for the untreated, undiluted blood sample, whereas in the sample diluted with albumin, EXTEM MCF recovered to 95% of that in the untreated, undiluted blood sample.

In the FIBTEM assay, dilution with albumin and the synthetic colloid resuscitation fluids caused a significant decrease in MCF ($p\leq0.02$) [Figure 1b]. Following addition of fibrinogen, FIBTEM MCF increased in all samples, and was significantly higher in samples diluted with albumin than in samples diluted with HES or dextran ($p<0.001$).

Dilution with each resuscitation fluid generally resulted in an increase in EXTEM CT and EXTEM CFT, and a decrease in EXTEM AA (Figure 2), i.e., increased time to initiation of coagulation and formation of the clot. For all parameters measured, dilution with saline and Ringer's acetate had a similar effect on coagulation. Dilution with buffered/unbuffered HES, or 3% dextran 60/6% dextran 70 induced a more extensive coagulopathy than dilution with saline or Ringer's acetate. Dilution with all synthetic colloids also resulted in a longer CT as compared to dilution with albumin; however, this difference was only significant for 6% dextran ($p<0.001$). Dilution with HES and dextran solutions caused a significantly greater change in CFT and AA parameters as compared to dilution with albumin ($p\leq0.001$).

Addition of fibrinogen to the diluted blood samples partially corrected coagulopathy; CT and CFT were reduced, and AA increased upon fibrinogen treatment. Following addition of fibrinogen, samples diluted with albumin exhibited a significantly shorter CFT and greater AA (i.e. improved correction of coagulopathy) as compared to samples diluted with HES or dextran ($p\leq0.001$) [Figure 2]. However, following addition of fibrinogen, CT was not significantly different in samples diluted with albumin, as compared to samples diluted with other solutions, or undiluted samples.

Dilution with resuscitation fluids had no effect on maximum lysis time (data not shown).

The contribution of platelets to clot strength was calculated by subtracting FIBTEM MCF from EXTEM MCF (Figure 3). Compared to undiluted samples, a significant reduction in the platelet component of clot strength was observed for samples diluted with all solutions ($p\leq0.01$), except albumin ($p=0.10$). The 3% and 6% dextran solutions caused the greatest decrease in the platelet component of clot strength; these decreases were significantly greater than that caused by dilution with albumin ($p\leq0.001$). Following addition of fibrinogen concentrate, the platelet component of clot strength was not significantly different for samples

treated with albumin, as compared to those treated with any of the other resuscitation fluids (Figure 3).

Discussion

In this study, we show that albumin induces a less extensive coagulopathy as compared to synthetic colloids, demonstrated by a smaller decrease in EXTEM MCF and AA, and a smaller increase in EXTEM CT and CFT following dilution with albumin. We also show that addition of fibrinogen concentrate effectively reversed albumin-induced coagulopathy, with EXTEM MCF, CFT, CT and AA parameters returning approximately to levels seen before dilution. Furthermore, we show that albumin-induced coagulopathy is more effectively reversed using fibrinogen concentrate (corresponding to an 8-g dose in a 70-kg adult) compared with coagulopathy induced by synthetic colloids such as HES or dextran.

Our results confirm previous findings that coagulopathy induced by dilution with crystalloids is less severe than coagulopathy induced with synthetic colloids [5]. Our results also agree with the ROTEM-based study of Niemi *et al.* [9], which showed that dilutional coagulopathy induced using 4% albumin was less severe than coagulopathy induced with HES 130/0.4, and with two studies which used thrombelastography to show that albumin-induced coagulopathy is less severe than HES-induced coagulopathy [3, 27].

Here, we show that fibrinogen concentrate reversed coagulopathy in samples diluted with albumin more successfully than in samples diluted with synthetic colloids. Following addition of fibrinogen concentrate, EXTEM MCF was fully corrected in samples treated with saline and Ringer's acetate, confirming previous results of Nielsen *et al.* [3] and Fenger-Eriksen *et al.* [10]. In line with the results from these studies, we also report that coagulopathy was not

fully corrected in samples treated with HES or dextran, even though we used a relatively high dose of fibrinogen concentrate. Such high dosages of fibrinogen concentrate may be necessary to correct clot structure defects as being measured by ROTEM-FIBTEM in trauma patients [21] and being in contrast to dosages of 2-4 gram, recommended in many current formula based guidelines and transfusion protocols.

Hemodilution with HES is well-documented as causing coagulopathy through an inhibitory effect on clotting factors [28-30], platelet function [1, 2] and fibrin polymerization [3, 4]. Dextrans also have a negative effect on platelet aggregation [31]. In our study, we observed a significant decrease in the platelet component of clot strength following hemodilution with either HES or dextran, but not with albumin. HES is also known to impair fibrin crosslinking, probably by inhibiting the interaction between activated FXIII and fibrin monomers, resulting in impaired clot formation and a weaker clot that is more susceptible to fibrinolysis [1, 32]. In addition, some groups have reported that HES causes fibrinolysis by diminishing alpha 2-antiplasmin-plasmin interactions [29, 33], although other groups found no effect [34, 35]. Taken together, these observations may explain why, in our study, dilutional coagulopathy caused by albumin was corrected more effectively with fibrinogen concentrate than dilutional coagulopathy caused by synthetic colloids.

Although albumin does not impair coagulation to the same degree as synthetic colloids, it binds calcium ions [36], which are essential for a number of processes in the coagulation cascade, including activation of protein C, correct functioning of the tenase and prothrombin complex, and binding of coagulation factors to phospholipids. This could account for the difference in ROTEM-recorded parameters following dilution with albumin, compared with dilution using saline and Ringer’s acetate. Also, reports suggest that crystalloids may induce a hypercoagulable state [1, 37], with reduced levels of anticoagulants such as antithrombin and

magnesium ions suggested as the cause (although this is generally seen with lower levels of dilution).

A recent clinical study compared mortality of patients with severe sepsis treated with either HES 130/0.42 or Ringer's acetate; those treated with HES had an increased risk of death and were more likely to require renal replacement therapy than those receiving Ringer's acetate [38]. However, in the SAFE study of nearly 7,000 intensive care unit patients, mortality rates were similar between patients randomized to receive either albumin or saline [39]. Clinical studies show that the natural colloid albumin induces a lesser degree of coagulopathy *in vivo*, as compared to HES. Schramko *et al*. [14] showed that coagulopathy was not evident in patients administered with 4% albumin, whereas HES 200/0.5 and HES 130/0.4 both induced impairment of ROTEM-recorded coagulation parameters. In a meta-analysis of 970 patients, significantly more bleeding in cardiac surgery was observed for patients treated with HES, compared to those treated with albumin [40]. In another meta-analysis of 653 patients undergoing cardiopulmonary bypass, the percentage of patients with excessive cardiopulmonary bleeding was higher for patients administered with HES compared to those administered with albumin [41]. Further studies are needed to determine whether the *in vitro* benefits of a reduced degree of coagulopathy and easier reversal with fibrinogen concentrate (or FFP) translates into a clinical benefit *in vivo*.

In this study, we used fibrinogen concentrate to correct coagulopathy following hemodilution. A study by Fenger-Eriksen *et al*. [10] investigated correction of *in vitro* coagulopathy with platelets, and plasma depleted of FII, FVII and FX; both of which had a negligible effect on dilutional coagulopathy. Schramko *et al.* [26] suggested that FFP was more effective in reversing dilutional coagulopathy following dilution with gelatin, because FFP contains physiological concentrations of most coagulation factors. However, the concentration of fibrinogen (an essential precursor for formation of the fibrin-based clot) is present at relatively

low concentrations in FFP, and a similar study by Haas *et al.* reported that fibrinogen concentrate was more effective than FFP in correcting coagulopathy following dilution with Ringer's solution [25]. In patients, use of fibrinogen concentrate may be preferable to FFP as fibrinogen concentrate is not associated with many of the safety concerns associated with allogeneic blood products [18, 42], and fibrinogen concentrate is also quicker to administer.

There are several limitations to performing *in vitro* hemodilution studies using citrated blood, as is the case in this study. The situation *in vitro* may not accurately represent the situation *in vivo*; for example, *in vitro* models do not account for shear stress, release of tissue factor by the endothelium, and activation of procoagulation or fibrinolytic pathways in response to tissue trauma. Our study was performed in blood from healthy volunteers, whereas trauma patients may exhibit a wide range of coagulopathies, and may have varying body temperatures [43]. ROTEM-recorded parameters are also affected by hematocrit; FIBTEM readings may appear higher for samples with lower red blood cell counts [44].

Conclusion

In our study, hemodilution using albumin induced a lesser degree of coagulopathy compared with the synthetic colloids HES and dextran. In addition, albumin-induced coagulopathy was more effectively reversed following *in vitro* addition of fibrinogen concentrate compared with coagulopathy following hemodilution with synthetic colloids. These results support the unique positioning of albumin as a volume expander which gives the benefits of other colloid resuscitation fluids, without such detrimental effects upon the coagulation system. Further studies are needed to determine if these advantages of albumin translate to the treatment of hypovolemia *in vivo,* and to establish whether fibrinogen can be used successfully *in vivo* to treat albumin-induced coagulopathy.

Acknowledgements

Assistance with preparation of this manuscript was provided by medical writers from Meridian HealthComms Ltd. Financial support for this was provided by CSL Behring.

Conflicts of Interest and Source of Funding

Ulf Schött has received honoraria from CSL Behring. Fibrinogen concentrate (RiaSTAP®) was provided by CSL Behring for *in vitro* use in this study. Dag Winstedt and Jennifer Hanna have no conflicts of interest to declare.

Tables

Table I. Diluted and undiluted blood samples with and without fibrinogen concentrate.

Sample number	Undiluted blood	Colloid	Fibrinogen (20 mg/mL)
1.	1 mL	-	-
2.	0.5 mL	0.5 mL saline	-
3.	0.5 mL	0.5 mL Ringer's acetate	-
4.	0.5 mL	0.5 mL HES 130/0.4 saline solution	-
5.	0.5 mL	0.5 mL buffered HES 130/0.4 saline solution	-
6.	0.5 mL	0.5 mL 3% dextran 60	-
7.	0.5 mL	0.5 mL 6% dextran 70	-
8.	0.5 mL	0.5 mL albumin 5 %	-
9.	1 mL	-	0.1 mL
10.	0.5 mL	0.5 mL saline	0.1 mL
11.	0.5 mL	0.5 mL Ringer's acetate	0.1 mL
12.	0.5 mL	0.5 mL HES 130/0.4 saline solution	0.1 mL
13.	0.5 mL	0.5 mL buffered HES 130/0.4 saline solution	0.1 mL
14.	0.5 mL	0.5 mL 3% dextran 60	0.1 mL
15.	0.5 mL	0.5 mL 6% dextran 70	0.1 mL
16.	0.5 mL	0.5 mL albumin 5 %	0.1 mL

Figures

Figure 1. Maximum clot firmness in a) the EXTEM assay and b) the FIBTEM assay, with or without dilution with different resuscitation fluids and *in vitro* addition of fibrinogen concentrate.

■Significant difference as compared to undiluted samples, without fibrinogen concentrate

*Significant difference as compared to samples diluted with albumin, without fibrinogen concentrate

●Significant difference as compared to samples diluted with albumin, with fibrinogen concentrate

Figure 2. a) Clotting time, b) clot formation time, and c) alpha angle in the EXTEM assay, with or without dilution with different resuscitation fluids and *in vitro* addition of fibrinogen concentrate.

■Significant difference as compared to undiluted samples, without fibrinogen concentrate

*Significant difference as compared to samples diluted with albumin, without fibrinogen concentrate

●Significant difference as compared to samples diluted with albumin, with fibrinogen concentrate

Figure 3. Contribution of platelets to clot strength, calculated by EXTEM MCF – FIBTEM MCF, with or without dilution with different resuscitation fluids.

■Significant difference as compared to undiluted samples, without fibrinogen concentrate

*Significant difference as compared to samples diluted with albumin, without fibrinogen concentrate

●Significant difference as compared to samples diluted with albumin, with fibrinogen concentrate

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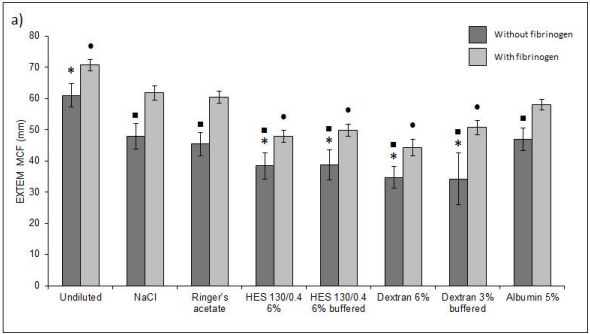
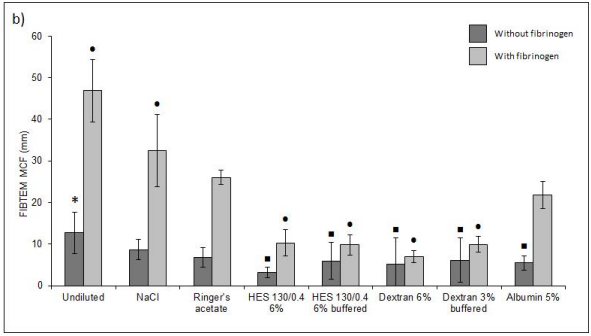
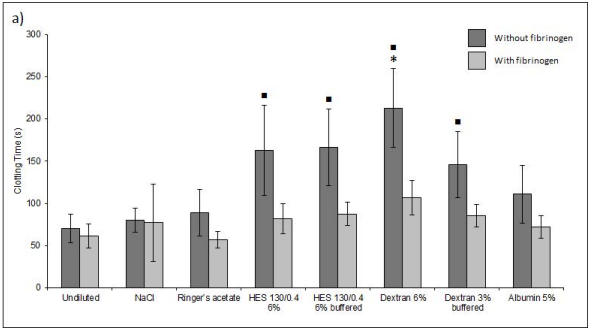
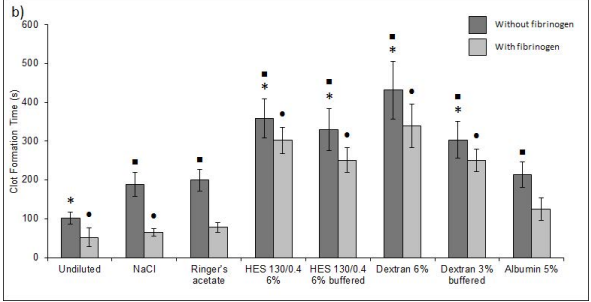
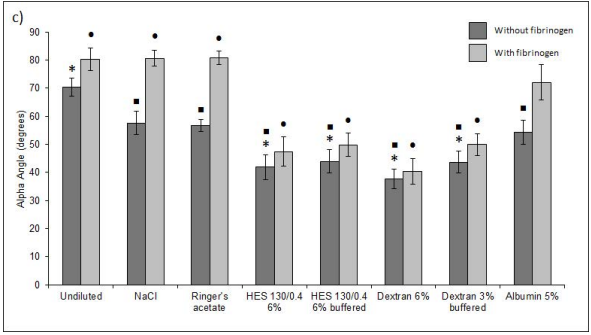
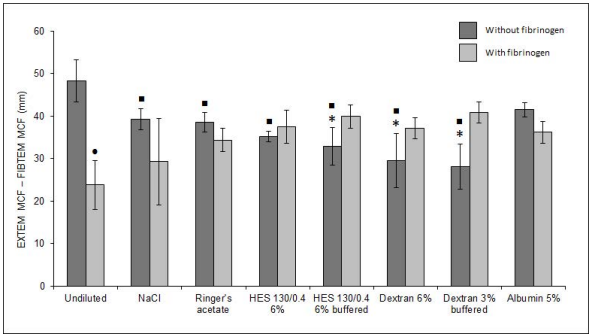

a)
Without fibrinogen
With fibrinogen
EXTEM MCF (mm)
0
10
20
30
40
50
60
70
80
Undiluted
NaCl
Ringer's acetate
HES 130/0.4 6%
HES 130/0.4 6% buffered
Dextran 6%
Dextran 3% buffered
Albumin 5%


b)
Without fibrinogen
With fibrinogen
FIBTEM MCF (mm)
60
50
40
30
20
10
0
Undiluted
NaCl
Ringer's acetate
HES 130/0.4 6%
HES 130/0.4 6% buffered
Dextran 6%
Dextran 3% buffered
Albumin 5%


a)
Clotting Time (s)
300
250
200
150
100
50
0
Without fibrinogen
With fibrinogen
■
*
Undiluted
NaCl
Ringer's acetate
HES 130/0.4 6%
HES 130/0.4 6% buffered
Dextran 6%
Dextran 3% buffered
Albumin 5%


b)
Clot Formation Time (s)
600
500
400
300
200
100
0
Without fibrinogen
With fibrinogen
Undiluted
NaCl
Ringer's acetate
HES 130/0.4 6%
HES 130/0.4 6% buffered
Dextran 6%
Dextran 3% buffered
Albumin 5%


c)
Without fibrinogen
With fibrinogen
Alpha Angle (degrees)
0
10
20
30
40
50
60
70
80
90
Undiluted
NaCl
Ringer's acetate
HES 130/0.4 6%
HES 130/0.4 6% buffered
Dextran 6%
Dextran 3% buffered
Albumin 5%


Without fibrinogen
With fibrinogen
EXTEM MCF – FIBTEM MCF (mm)
60
50
40
30
20
10
0
Undiluted
NaCl
Ringer's acetate
HES 130/0.4 6%
HES 130/0.4 6% buffered
Dextran 6%
Dextran 3% buffered
Albumin 5%