

A Thromboelastometric Evaluation of the Effects of Hypothermia on the Coagulation System

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BACKGROUND: Hypothermia may be accidental or therapeutic. Therapeutic hypothermia is increasingly used as treatment for various conditions, e.g., neuroprotection after cardiac arrest. Hypothermia leads to an impairment of the coagulation system, but the degree of impairment has been difficult to determine. Most studies have been performed on plasma instead of whole blood. We therefore evaluated whole blood investigating the effects of hypothermia on the coagulation system over a wide range of temperatures (25–40°C).

METHODS: Blood was drawn from six healthy volunteers into citrated test tubes. Samples were then placed in water baths with temperatures ranging from 25 to 40°C for 30 min before the coagulation system was studied using rotational thromboelastometry. A contact activator (Ellagic acid) was used for initiation of coagulation. Clotting time, clot formation time, α angle, and maximum clot strength were measured. All tests were run for 60 min and they were performed at the same temperature as the temperature in the water bath.

RESULTS: Coagulation was increasingly impaired with decreasing temperatures in the temperature range studied. All variables measured were significantly impaired in a stepwise pattern ($P < 0.0001$).

CONCLUSIONS: Evaluation using a whole blood analysis shows that hypothermia progressively impairs the coagulation system.

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Hypothermia may be accidentally or therapeutically induced. Accidental hypothermia is often seen in trauma patients during resuscitation and emergency surgery. Therapeutic hypothermia has been used for decades in conjunction with cardiac surgery and extra-corporeal circulation. More recently, mild induced hypothermia is used for neuroprotection in patients successfully resuscitated after cardiac arrest. In these patients, a body temperature of $33 \pm 1^\circ\text{C}$ is induced after the cardiac arrest and maintained for 12–24 h.^{1,2} Mild hypothermia is currently undergoing studies in several other indications, e.g., after neonatal asphyxia, myocardial infarction, and traumatic brain injury.

Previous studies report that hypothermia is accompanied by an impairment of the coagulation system and state that factor activity is severely impaired below 30°C, e.g., at 25°C clotting activity ranges from

0% (FVIII and FIX) to 5% (FII and FVII).³ There are also studies showing that standard clotting tests, e.g., activated partial thromboplastin time, are impaired at low temperatures.^{4–6} However, some of these studies evaluated plasma samples instead of whole blood, thereby potentially omitting the effects of cellular components of the blood, and others were performed on only elements of blood, e.g. platelets in a cell-based study.⁵

One study using thromboelastography (TEG[®]) studied the coagulation at 32 and 36°C and found an impairment of the coagulation system at 32°C.⁷ TEG uses whole blood for the analysis and gives a dynamic picture of the coagulation visualizing both the initiation of clotting and later stages involving both humoral and cellular elements. Rotational thromboelastometry (ROTEM) is a method using similar technology as TEG. ROTEM uses a rotating pin instead of a rotating cup, but studies have shown very close correlation between the two methods with r^2 values of 0.99, justifying straight comparisons between the two methods.⁸

There are no studies using whole blood analysis that have evaluated the effects of hypothermia in a wider range of temperatures $<32^\circ\text{C}$. In this study, we systematically studied the effects of temperature on coagulation using ROTEM over a wider range of temperatures (25–40°C). Using ROTEM we intended to be able to study both humoral and platelet-mediated parts of coagulation.

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MATERIALS

The study was approved by the Swedish central ethics committee. Informed consent was sought from six healthy volunteers who had not taken any drugs for at least 1 wk. An indwelling 1.1 mm catheter was placed in a forearm vein. A series of whole blood samples was collected in 4.5 mL citrate tubes, containing 0.129 M citrate (BD vacutainer systems, Plymouth, UK). Before each sample was collected 5 mL blood was drawn and discarded.

The samples were kept in a water bath for 30–45 min at predetermined temperatures. These temperatures were within 0.5°C of 25, 28, 31, 34, 37, and 40°C respectively. The temperature was controlled using a temperature sensor urinary catheter (Rüsch, Willy Rüsch GmbH, Kernen, Germany). Analyses at the different temperatures were performed in random order for each healthy volunteer.

Samples were analyzed using a ROTEM analyzer (Pentapharm GmbH, Munich, Germany). The analyses were performed after temperature stabilization of the ROTEM analysis instrument at the predetermined temperatures. Coagulation was initiated by adding 20 μ L 0.2 M CaCl₂ and 20 μ L Ellagic acid to 320 μ L of blood according to the standard INTEM procedure described by the manufacturer. INTEM analysis was chosen because it is the most commonly used system in published studies having used ROTEM. All specimens were run in the ROTEM analyzer for 60 min.

Several different analyses are available when using the ROTEM system and we studied clotting time (CT), clot formation time (CFT), α angle (AA) and maximum clot firmness (MCF). The different variables provide information regarding different parts of the coagulation system. CT primarily measures effects on the humoral coagulation steps before fibrin formation whereas CFT reflects primarily fibrinogen to fibrin conversion and to a minor extent the platelet activity. AA reflects similar steps as the CFT whereas MCF primarily provides information regarding platelet activity but is also affected by fibrin production.

Statistical analysis was performed using the program SPSS15.0 for Windows. We used the nonparametric Friedmans test to check for overall differences among the different temperature groups and used Wilcoxon's paired test for *post hoc* analysis where Friedmans test indicated a significant difference. Values are given as median and range. A *P* value <0.05 was regarded as statistically significant.

RESULTS

Three men and three women volunteers between 37 and 51 yr of age were included in the study. No participant withdrew from the study and blood samples were collected uneventfully. Target temperatures in the water bath were kept at target \pm 0.5°C. At

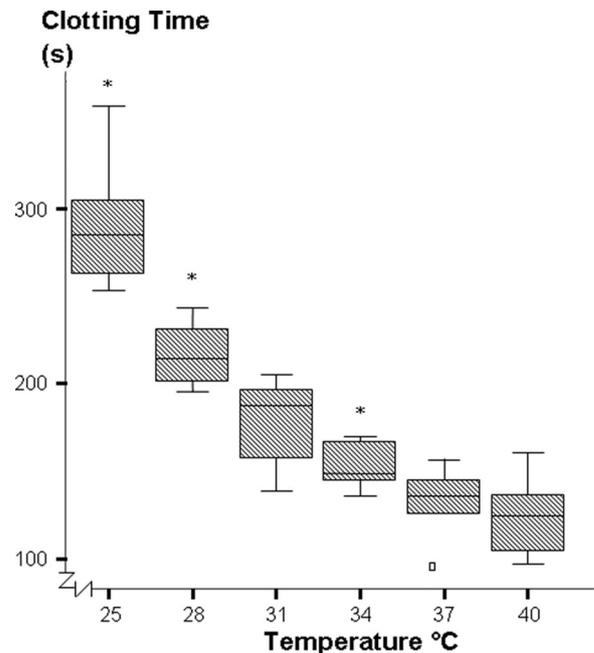


Figure 1. Boxplot describing the relationship between clotting time and temperature. Clotting time was significantly impaired at all temperatures below 37°C ($P < 0.0001$). * indicates a significant difference when compared to 37°C.

37°C the median CT was 136(93–156) s and an impairment was evident when the temperature was decreased ($P < 0.0001$). The CT, when compared to CT at 37°C, was significantly prolonged at 34°C and at cooler temperatures. Maximum prolongation was 121% at 25°C (Fig. 1). Also CFT was significantly prolonged when the temperature was decreased ($P < 0.0001$). The prolongation was significant at all temperatures below 37°C. CFT prolongation was more pronounced than CT prolongation with a CFT of 84 (68–104) s at 37°C and a maximum prolongation of 220% at 25°C (Fig. 2). AA is closely related to CFT and showed an equally significant impairment at all temperatures below 37°C. AA at 37°C was 74° (72–77°) with a maximal difference between 37°C and 25°C of 18% (Fig. 3).

MCF, primarily assessing the platelet function, was the variable least significantly impaired ($P < 0.01$) and the maximum impairment was 8% (Fig. 4) with a MCF of 56.5 (50–62) mm at 37°C. The impairment, when compared to 37°C, was significant at 28 and 25°C.

DISCUSSION

In this study we attempted to isolate the effects of hypothermia on coagulation. To be able to systematically study the temperature correlations over a wider range of temperatures, the study was performed as an *in vitro* study using blood from healthy volunteers. We found a significant correlation between temperature and blood coagulation, with lower temperatures leading to an impairment of the coagulation variables. The impairment is more pronounced for the variables reflecting the humoral part of the coagulation than for

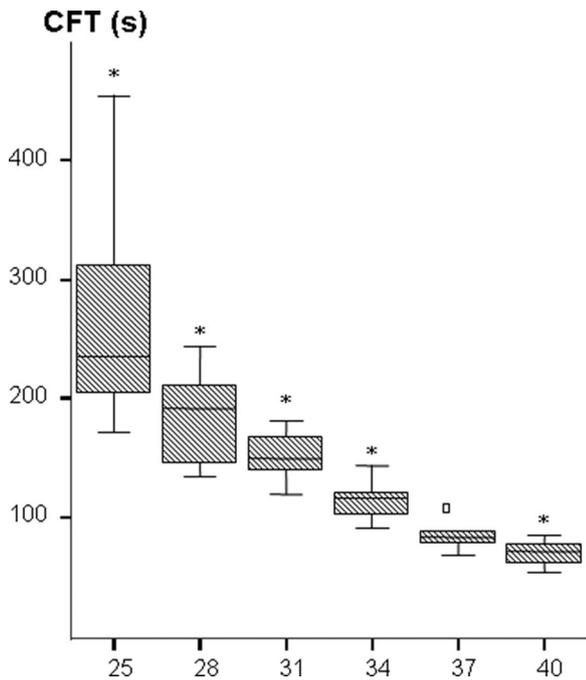


Figure 2. Boxplot describing the relationship between clot formation time and temperature. Clot formation time was significantly impaired at all temperatures below 37°C ($P < 0.0001$). * indicates a significant difference when compared to 37°C.

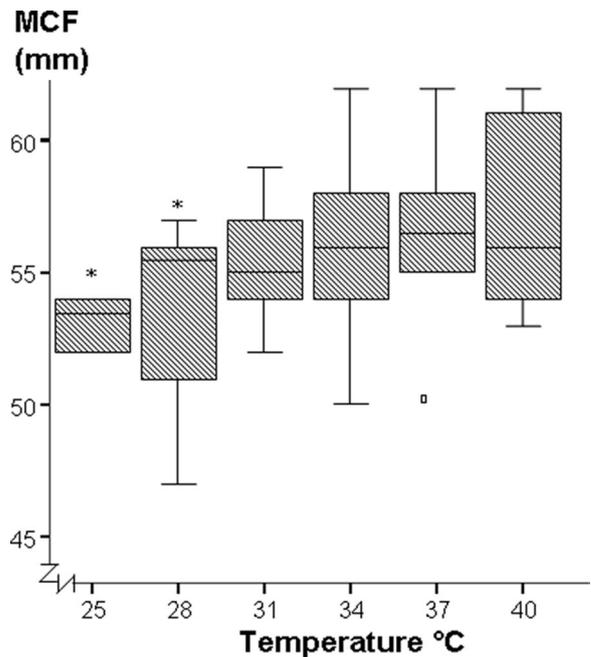


Figure 4. Boxplot describing the relationship between maximum clot firmness and temperature. Maximum clot firmness was significantly impaired at temperatures below 37°C ($P < 0.01$). * indicates a significant difference when compared to 37°C.

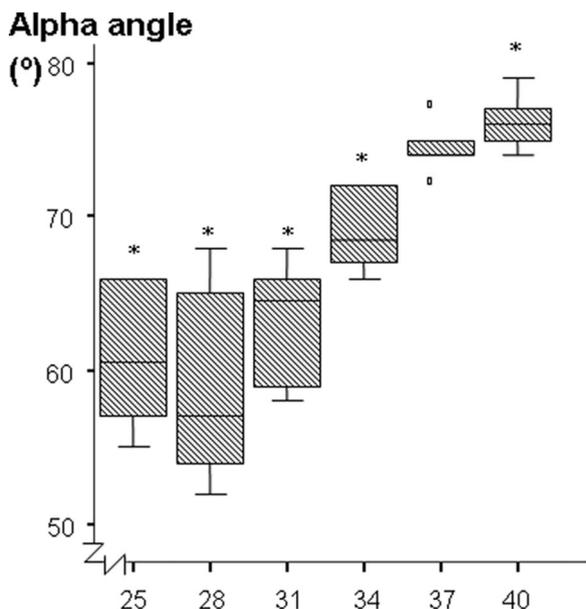


Figure 3. Boxplot describing the relationship between Alpha Angle and temperature. Alpha Angle was significantly impaired at all temperatures below 37°C ($P < 0.0001$). * indicates a significant difference when compared to 37°C.

MCF, the variable that primarily reflects platelet activity. The reasons for this finding suggest that a general impairment of procoagulant enzyme systems occurs that affect hemostatic and platelet activity. However, platelet activity based on our finding appears to be less impaired than the coagulation proteins. However, we did not directly evaluate platelet function. The effect of hypothermia in this study can

be compared to a previous study by Kettner et al. investigating the effects of hypothermia in patients undergoing intracranial surgery.⁹ They studied coagulation in the temperature range from 32 to 36°C using TEG and found an impairment of coagulation at lower temperatures.⁷ At 32°C, the variable equivalent to CT was prolonged by 14% and the equivalent of CFT was prolonged by 50%. In contrast, they found no change in the variable equivalent to MCF.

Another study by Wolberg et al. noted that thrombin is not affected when temperature is decreased from 37 to 33°C.⁵ They also performed activated partial thromboplastin time between 23 and 39°C and found only minor effects when temperature was decreased from 39 to 33°C, but a much a larger impact when temperature was decreased further. Interestingly, they found an impairment of platelet aggregation at 33°C that was further impaired down to 23°C. This finding is in contrast to relatively minor effects on platelet activity in our study. It may be that the isolated impairment of platelet aggregation is of less importance in whole blood analyses, but it may also be that ROTEM is less sensitive to changes in platelet aggregation.

In a rabbit study using TEG and Sonoclot, Shimokawa et al. found that coagulation was impaired at 30°C compared to normal temperature.¹⁰ However, the differences disappeared when coagulation tests were performed at 37°C, even though the blood was drawn at a body temperature of 30°C. They also found that the platelet count decreased after cooling, and speculated whether this could be a contributing cause of the coagulation impairment. This hypothesis is not

supported by our data, as we cooled blood after it was drawn; the platelet count was thus unchanged, and we found a similar impairment as did Shimokawa et al. in rabbits cooled to 30°C.

Impairments of coagulation factor enzymatic activities at low temperatures are well described, but these studies do not report the degree of impairment *in vivo*.^{4,11} In our study, the impairment of coagulation induced by hypothermia is less pronounced than expected from coagulation factor activity studies. However, our study has limitations, including the fact that samples obtained from only six different volunteers. We also only performed ROTEM analyses and did not compare the ROTEM results to standard coagulation tests, including platelet function tests. However, the effects of hypothermia on single factor activity are known already.^{3,5}

The impact of induced hypothermia on coagulation within the range used after cardiac arrest produced minimal effects in our study, but clinical studies have shown a significant increase in blood loss when temperature was decreased from 36.4 to 35.0°C.¹² However, the effects of relatively mild hypothermia (<35.5°C) in trauma patients may have profound effects, and increase mortality in this patient population.¹³ Other studies have also found increased mortality and bleeding in hypothermic patients and Jeremitsky et al. found hypothermia to independently predict worse outcome after traumatic brain injury.^{14,15}

In summary, we performed an *in vitro* dose-response study of the effects of temperature on coagulation using a whole blood analysis and found that decreasing temperatures in the range from 37 to 25°C increasingly impair the coagulation system. Further clinical studies are important to validate our findings, and to understand the role that hypothermia plays in hemostatic alterations in patients.

REFERENCES

1. Nolan JP, Morley PT, Hoek TL, Hickey RW. Advancement life support task force of the international liaison committee on R. Therapeutic hypothermia after cardiac arrest. An advisory statement by the advancement life support task force of the international liaison committee on resuscitation. *Resuscitation* 2003;57:231-5
2. Holzer M, Bernard SA, Hachimi-Idrissi S, Roine RO, Sterz F, Mullner M. On behalf of the collaborative group on induced hypothermia for neuroprotection after cardiac A. Hypothermia for neuroprotection after cardiac arrest: systematic review and individual patient data meta-analysis. *Crit Care Med* 2005;33:414-8
3. Johnston TD, Chen Y, Reed RL II. Functional equivalence of hypothermia to specific clotting factor deficiencies. *J Trauma* 1994;37:413-7
4. Reed RL II, Bracey AW Jr, Hudson JD, Miller TA, Fischer RP. Hypothermia and blood coagulation: dissociation between enzyme activity and clotting factor levels. *Circ Shock* 1990;32:141-52
5. Wolberg AS, Meng ZH, Monroe DM III, Hoffman M. A systematic evaluation of the effect of temperature on coagulation enzyme activity and platelet function. *J Trauma* 2004;56:1221-8
6. Rohrer MJ, Natale AM. Effect of hypothermia on the coagulation cascade. *Crit Care Med* 1992;20:1402-5
7. Kettner SC, Zimpfer M, Kozek SA, Holzer A, Spiss CK, Illievich UM. The effect of graded hypothermia (36 degrees C-32 degrees C) on hemostasis in anesthetized patients without surgical trauma. *Anesth Analg* 2003;96:1772-6
8. Calatzis A, Fritzsche P, Kling M, Hipp R, Stemberger A. An analysis of the correlation of the roTEG coagulation analyser and the Hellige Thromboelastograph D. *Ann Hematol* 1996;72:P87
9. Kettner SC, Sitzwohl C, Zimpfer M, Kozek SA, Holzer A, Spiss CK, Illievich UM. The effect of graded hypothermia (36 degrees C-32 degrees C) on hemostasis in anesthetized patients without surgical trauma. *Anesth Analg* 2003;96:1772-6, table of contents
10. Shimokawa M, Kitaguchi K, Kawaguchi M, Sakamoto T, Kakimoto M, Furuya H. The influence of induced hypothermia for hemostatic function on temperature-adjusted measurements in rabbits. *Anesth Analg* 2003;96:1209-13
11. Meng ZH, Wolberg AS, Monroe DM III, Hoffman M. The effect of temperature and pH on the activity of factor VIIa: implications for the efficacy of high-dose factor VIIa in hypothermic and acidotic patients. *J Trauma* 2003;55:886-91
12. Schmied H, Kurz A, Sessler DI, Kozek S, Reiter A. Mild hypothermia increases blood loss and transfusion requirements during total hip arthroplasty. *Lancet* 1996;347:289-92
13. Krishna G, Sleigh JW, Rahman H. Physiological predictors of death in exsanguinating trauma patients undergoing conventional trauma surgery. *Aust N Z J Surg* 1998;68:826-9
14. Jeremitsky E, Omert L, Dunham CM, Protetch J, Rodriguez A. Harbingers of poor outcome the day after severe brain injury: hypothermia, hypoxia, and hypoperfusion. *J Trauma* 2003;54:312-9
15. Ferrara A, MacArthur JD, Wright HK, Modlin IM, McMillen MA. Hypothermia and acidosis worsen coagulopathy in the patient requiring massive transfusion. *Am J Surg* 1990;160:515-8