

## ORIGINAL ARTICLE

# In vitro evidence of gender-related heparin resistance

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**SUMMARY.** Coagulability varies among men, women, and pregnant women, along a spectrum where the blood of men is the least and that of pregnant women the most coagulable. The effects of differences in coagulation status on the action of heparin cannot be measured by specific laboratory tests such as aPTT or anti-Factor Xa assay. Thromboelastography<sup>®</sup> which measures whole blood coagulation can assess the effect of heparin against differing backgrounds of coagulation. The aim of this in vitro study was to explore differences in heparin effect between men, women and pregnant women. Fifteen male and female staff volunteers, and 15 pregnant women approaching term, donated venous blood, which was added to four cups in two TEG<sup>®</sup> 5000 analysers. In the cups of the analysers was 0.03 mL of saline control, or heparin 0.4, 0.6 or 1 unit/mL. TEG<sup>®</sup> variables *r* and *k*, angle and *MA* were compared across the groups using two way ANOVA. All subject groups demonstrated a significant heparin effect, which was least in the control group and greatest with 1 unit/mL ( $P < 0.0001$ ). Across the subject groups, from men to pregnant women, increasing coagulability was seen, with shortening of *r* and *k* ( $P < 0.04$ ), and increasing angle and *MA* ( $P < 0.0001$ ). A relationship between gender and heparin was significant for *r* and *k* ( $P < 0.02$ ) but not for angle and *MA*. This result assists the case against a one-size-fits-all approach to policies on heparinisation.

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**Keywords:** Blood coagulation; Measurement techniques; Thromboelastography; Drugs: heparin

## INTRODUCTION

Anticoagulation is a relative contraindication to regional blockade. The factors most likely to influence practice are dose and time after dose, and guidelines reflect this.<sup>1</sup> The overall state of coagulation is not taken into account unless it is in failure. The physiological changes of pregnancy, which increase procoagulant factors and decrease natural anticoagulant, are effective by the third trimester.<sup>2</sup> The standard specific laboratory tests for assessing the effects of heparin, the aPTT for unfractionated, and antifactor Xa assay for low molecular weight heparins, will be unaffected by the global hypercoagulation of pregnancy, but general changes occurring in the circulation will reduce bioavailability, necessitating a higher dose.<sup>3</sup>

Thromboelastography<sup>®</sup> (TEG<sup>®</sup>) is a test of whole blood coagulation that is capable of measuring both hypercoagulation and the effects of heparin. The aim of this study was to use TEG<sup>®</sup> to determine if, in vitro, it was possible to measure differences in response to heparin between pregnant and non-pregnant subjects that might be attributable to physiological changes in coagulation.

## METHODS

Following hospital Clinical Research (Ethics) Committee approval and informed consent, men, women and pregnant women were invited to participate in the study, which took place at St. James' University Hospital between January and May 2002.

The men and non-pregnant women were healthy staff volunteers aged between 18 and 55 years. The pregnant women were scheduled for elective caesarean section, with a healthy singleton fetus at 36 weeks' gestation or more. All were questioned about their health, and were excluded if there was a positive history of thrombophilia or malignancy, or any treatment with drugs known to interfere with coagulation or platelet function.

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Four channels of two Thromboelastograms 5000<sup>®</sup> (Hemoscope, Illinois, USA) were used for each subject, with disposable cups and pins. In each cup was placed 0.03 mL of either 0.9% w/v saline or heparin 0.4 units/mL, 0.6 units/mL or 1.0 units/mL, using a Biohit Pro-line<sup>®</sup> (Alpha Laboratories, Hampshire, UK) automatic pipette. The addition of saline alone to one of the four cups acted as a control. In order to avoid syringe or channel bias, the sequence of channels rotated following each sample. Subjects were studied as and when they became available.

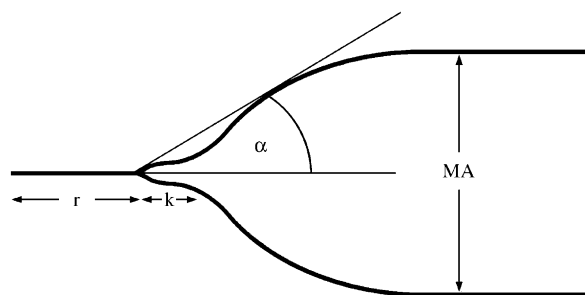
Venous blood was taken using a 22-gauge intravenous cannula inserted into a forearm vein. To avoid clot activation, venepuncture was performed with as little trauma as possible, and the first 2 mL of blood was discarded. Four fresh samples were then taken in sequence with 1-mL syringes, and from each, 0.33 mL of blood was added to each channel of the TEG<sup>®</sup>, to make the final amount in each cup to 0.36 mL. Thromboelastogram analysis was started 4 minutes after venepuncture. All samples were managed according to the recommendations in the Hemoscope<sup>®</sup> manual for native whole blood samples.<sup>4</sup>

The four solutions (sodium chloride and the three heparin dilutions) were prepared fresh, twice weekly, in sterile, refrigerated containers. The heparin solutions were prepared from unfractionated sodium heparin 10 units/mL i.v. flush solution (Leo Pharmaceutical Products, Princes Risborough, UK) diluted to the appropriate concentration using 0.9% sodium chloride.

TEG<sup>®</sup> detects increased stickiness as blood clots in the cuvette. As coagulation begins, fibrin strands form between a thermostatically controlled, warmed cuvette that oscillates through an angle of 4°45', and a pin suspended in the blood that is attached to a torsion wire. With increasing clot strength, more movement of the cuvette is transferred to the torsion wire, transduced, and displayed electronically in the form shown in Fig. 1. The variables recorded by the TEG<sup>®</sup> analyser include  $r$  (mm),  $k$  (mm), angle (degrees) and maximum amplitude,  $MA$ , (mm).  $r$  and  $MA$  are measured, the other variables are derived (Fig. 1).  $r$  and  $k$  values reflect the time to clot initiation, angle and  $k$  reflect rate and strength of clot development, and  $MA$ , the final strength of the clot.  $MA$  is largely determined by fibrinogen and platelets, whilst  $r$  and  $k$  are factor dependent. The effects of heparin are mainly visible in  $r$  and  $k$ .<sup>4</sup>

Personal data including age, sex, height, weight and gestation were recorded for each subject. The  $r$ ,  $k$ , angle and  $MA$  values were recorded from the TEG<sup>®</sup> analyser.

Two way ANOVA was used to analyse the data, facilitated by GraphPad Prism 4 (GraphPad Software, San Diego, USA).  $P < 0.05$  was considered significant. Based on a standard deviation of 15.9<sup>5</sup> to see a difference in the  $r$  value of 10 mm for a  $P$  value  $< 0.05$ , 15



**Fig. 1** Schematic TEG<sup>®</sup> trace showing the variables measured.  $r$  is the distance from the start of the run to where the trace splits with an amplitude of 2 mm;  $k$  is the distance from 2 mm until the amplitude of the trace is 20 mm;  $\alpha$  is the angle of a line drawn tangentially between the start of coagulation and the shoulder of the trace;  $MA$  is the maximum amplitude achieved.  $r$  and  $k$  are factor-dependent.  $MA$  reflects platelet and fibrinogen function. The angle gives a measure of the speed of clot production.

subjects per group were required to achieve a power of 0.85.

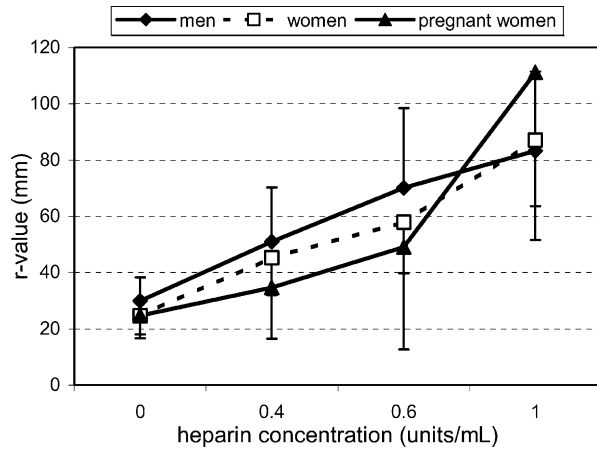
## RESULTS

Fifteen subjects were recruited to each group. The groups were comparable in age, while differences in height and weight were as expected (Table 1).  $r$  and  $k$  values were analysed together. The addition of heparin increased  $r$  and  $k$  values, and decreased angle and  $MA$ . This was the dominant source of variation for  $r$  and  $k$  values ( $F = 71.77$ ), and angle ( $F = 57.13$ ), but was also detectable for  $MA$  ( $F = 29.78$ ). It was unlikely that these changes had arisen by chance ( $P < 0.0001$ ).

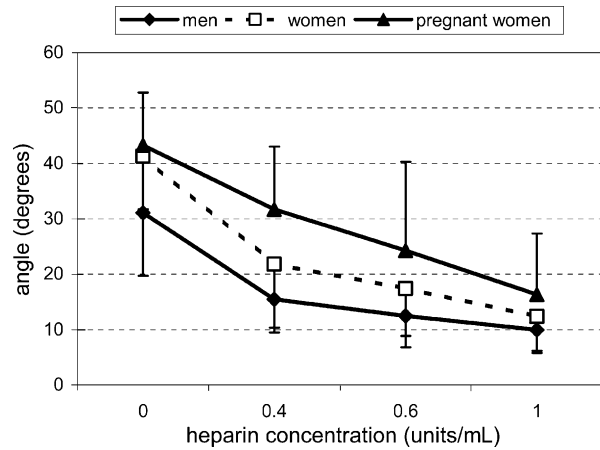
Gender, when considered from men through to pregnant women, was associated with increasing coagulability, shortening of  $r$  and  $k$  values, and increasing angle and  $MA$ . This was the dominant source of variation ( $F = 186.7$ ) for  $MA$  ( $P < 0.0001$ ), but not for angle ( $F = 20.91$ ), and was weak ( $F = 3.52$ ) for  $r$  and  $k$  values. It was unlikely that these changes had arisen by chance ( $P < 0.04$ ). A small interaction was detected between doses of heparin and gender ( $F = 2.24$ ) for  $r$  and  $k$  values ( $P < 0.02$ ), (Figs. 2 and 3). This relationship was not found for angle and  $MA$  (Figs. 4 and 5).

**Table 1.** Personal characteristics for men, women and pregnant women shown as mean (SD)

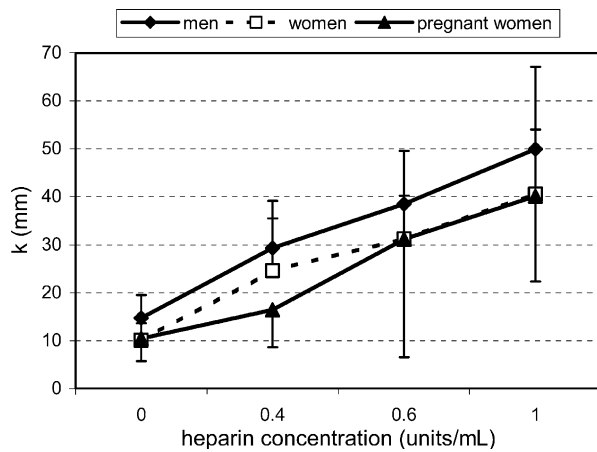
	$n$	Age (years)	Height (cm)	Weight (kg)
Men	15	32.4 (9.4)	179 (3.4)	77.9 (8.0)
Women	15	31.6 (11.6)	168.3 (4.6)	61.6 (7.9)
Pregnant women	15	31.7 (2.1)	164.8 (3.3)	70.7 (5.0)



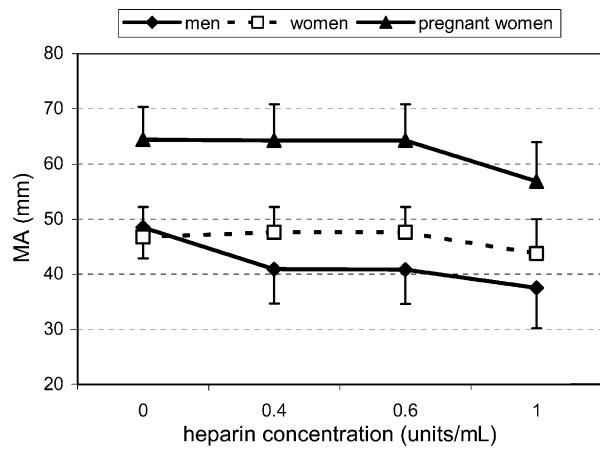
**Fig. 2** Mean TEG<sup>®</sup> *r* values for saline and three concentrations of heparin in men, women and pregnant women. Vertical bars represent standard deviations. *r* values were significantly related to the dose of heparin ( $P < 0.0001$ ).



**Fig. 4** Mean TEG<sup>®</sup> angle for saline and three concentrations of heparin in men, women and pregnant women. Vertical bars represent standard deviations. Angle decreased significantly with dose of heparin ( $P < 0.0001$ ). Variation due to gender alone was also significant ( $P < 0.0001$ ). The interaction between heparin and gender was not significant.



**Fig. 3** Mean TEG<sup>®</sup> *k* values for saline and three concentrations of heparin in men, women and pregnant women. Vertical bars represent standard deviations. *k* values were significantly related to the dose of heparin ( $P < 0.0001$ ). When taken together with *r* value, variation due to gender alone was also significant ( $P < 0.04$ ). The interaction between heparin and gender was significant ( $P < 0.02$ ).



**Fig. 5** Mean TEG<sup>®</sup> MA for saline and three concentrations of heparin in men, women and pregnant women. Vertical bars represent standard deviations. MA changed in proportion to the dose of heparin ( $P < 0.02$ ). Variation due to gender alone was significant ( $P < 0.0001$ ). The interaction between heparin and gender was not significant.

**DISCUSSION**

In choosing the concentrations of heparin for this in vitro experiment it was important that they were small enough to show a graduated response in *r* value, starting with the control, but large enough to trigger a response that could be measured. It is not possible to attribute, with any degree of certainty, an aPTT value to the concentrations used. Heparin 0.4 units/mL is probably sub-therapeutic, while 1 unit/mL produces an effect in the range two to three times control. The highly significant relationship found between *r* value and heparin dose suggests that this has been achieved. Our reference

range for normal men and women for *r* value is 8–40 mm. All control values were inside this range, but all subsequent mean values except that for pregnant women at 0.4 units/mL were outside it. The range of values seen is comparable to those that might be measured in the first hours after a large prophylactic rather than a therapeutic dose of heparin.<sup>6</sup> In vitro testing is independent of the reduced bioavailability due to an expanded plasma volume, but is influenced by any physiological change within blood itself, that is transferable to the TEG<sup>®</sup> cuvette.

The effect of increasing concentration of heparin is best seen for  $r$  and  $k$  values, but also achieves significance for angle and  $MA$ . The data also demonstrate a significant relationship between the groups. Critical appraisal of figures 2–5 reveals that there is a cascade of coagulation through the groups, where men remain the least coagulable, and pregnant women the most coagulable, despite increasing concentration of heparin. This relationship has previously been demonstrated using TEG<sup>®</sup> with native samples, and is stronger with angle and  $MA$ , compared to  $r$  and  $k$ .<sup>7</sup> It is acknowledged that it might be considered incorrect to evaluate changes in coagulation between men, women and pregnant women, in terms of a trend, but this is a convenient and understandable way of viewing the differences. Susceptibility to heparin is modulated by gender, with pregnant women lying at the more coagulable end of the spectrum.

This relationship is not wholly maintained for  $r$  value in the samples treated with heparin 1 unit/mL (Fig. 2), and it is not clear why. Examination of figures 2–5 shows that heparin effect is maintained. For the remaining TEG<sup>®</sup> variables in the samples treated with 1 unit/mL (Figs. 3–5), the gender effect is maintained, indicating that the unexpected finding is limited to the  $r$  value alone, and is associated with unanticipated variability. There are two potential sources of variation. Unfractionated heparin binds in a charge-dependent manner to proteins released from platelets and other cells such as macrophages. This is a non-specific process that influences the bioavailability of the drug in any given individual, giving rise to inter-individual variability.<sup>8</sup> The second source relates to the TEG<sup>®</sup> variables themselves. Of the four variables commonly measured,  $r$  and  $k$  values are consistently less reliable than  $MA$ ,<sup>9</sup> and this is another potential source of variability. A larger group size might have assisted consistency, but interestingly, the reverse in this group has not prevented a positive finding. Low molecular weight heparins bind to plasma proteins to a lesser degree than unfractionated heparin, and had one been used in preference, it is possible that less variability would have been seen.

A saline control was used to represent a zero dose of heparin. This was chosen in preference to a pure native sample because it did not upset the ratio of native blood to diluent in the cup, and the source of the heparin was a solution in saline. However, saline used in this way is responsible for a small degree of accelerated coagula-

tion, though the magnitude of the effect on  $r$  value is clinically insignificant.<sup>10</sup>

The clinical relevance of this study lies in the application of guidelines relating to the performance of regional blockade. Recommendations on the number of hours between interventions are not based on outcome data, for obvious reasons, and reflect normal coagulation in a non-pregnant population.<sup>11</sup> Our TEG<sup>®</sup> data show that the hypercoagulable state of pregnancy modifies the in vitro response to heparin, and suggests that for modest doses of heparin, values for  $r$ ,  $k$  and angle in mildly heparinised pregnant women may resemble those for unheparinised men. Although there are no direct comparisons of normal men and pregnant women in vivo, one case series proposes that for LMWH 8 h, rather than the 10–12 h recommended,<sup>1</sup> is a sufficient lapse between a prophylactic dose of heparin and regional blockade in pregnant women.<sup>11</sup> The current study may be insufficient to justify a change in practice, but adds to the case against a one-size-fits-all approach to heparinisation and regional blockade.

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