THE CHARACTERISATION OF GLOBAL HAEMOSTATIC FUNCTION DURING PREGNANCY AND THE PUERPERIUM USING THROMBOELASTOGRAPHY.

Thesis submitted for the degree of

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by

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In memory of my inspiring Grandmother,

Helena Patricia Maybury

1909-1983.

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CONTENTS

PAGE No.

CHAPTER 1.

INTRODUCTION

Coagulation

In recent years there have been many advances in our understanding of the coagulation system. This offers great opportunities to understand the clinical implications of abnormalities within the system and possible treatment options that may be employed.

The development of a clot is one of the most important concepts in pathology. The 3 factors required for formation of a solid thrombus were described by Rudolf Virchow in 1856: damage to the vessel wall, disruption to laminar blood flow and abnormalities in the composition of blood itself. When one or more of these factors occur, this stimulates the process whereby soluble proteins in the blood interact with one another resulting in the formation of an insoluble fibrin clot. This is coupled with a process which results in the dissolution of the fibrin clot, fibrinolysis. The balance between these systems maintains normal haemostasis. Abnormalities within these pathways may manifest themselves by either excessive bleeding or clot formation which result in many pathological processes such as thromboembolism and myocardial infarction.

The Coagulation Cascade.

The classical coagulation cascade was described in the 1960s by two groups of biochemists who proposed a model of a series of steps in which activation of each clotting factor led to the activation of another resulting in a burst of thrombin generation[1, 2]. The thrombin converts soluble fibrinogen to fibrin, which by the laying down and cross linkage of fibrin strands, forms a blood clot. In the original model, each clotting factor existed as a proenzyme that could be converted to an active enzyme. It was later observed that some pro-coagulants were co-factors and did not posses enzymatic activity. The familiar clotting cascade contains two series of protein chain reactions; the intrinsic and extrinsic pathways each provide a route for the generation of factor X where the pathways converge into a final common pathway which results in thrombin formation (Fig 1.1). The intrinsic pathway contains factors which were considered to be intravascular whereas the extrinsic pathway includes tissue factor (TF), a protein found within the cell membrane and considered to be extrinsic to the circulating blood. The components of the extrinsic and common pathways are reflected clinically in the prothrombin time (PT). The components of the intrinsic and the common pathways are reflected in the activated partial thromboplastin time (APTT).

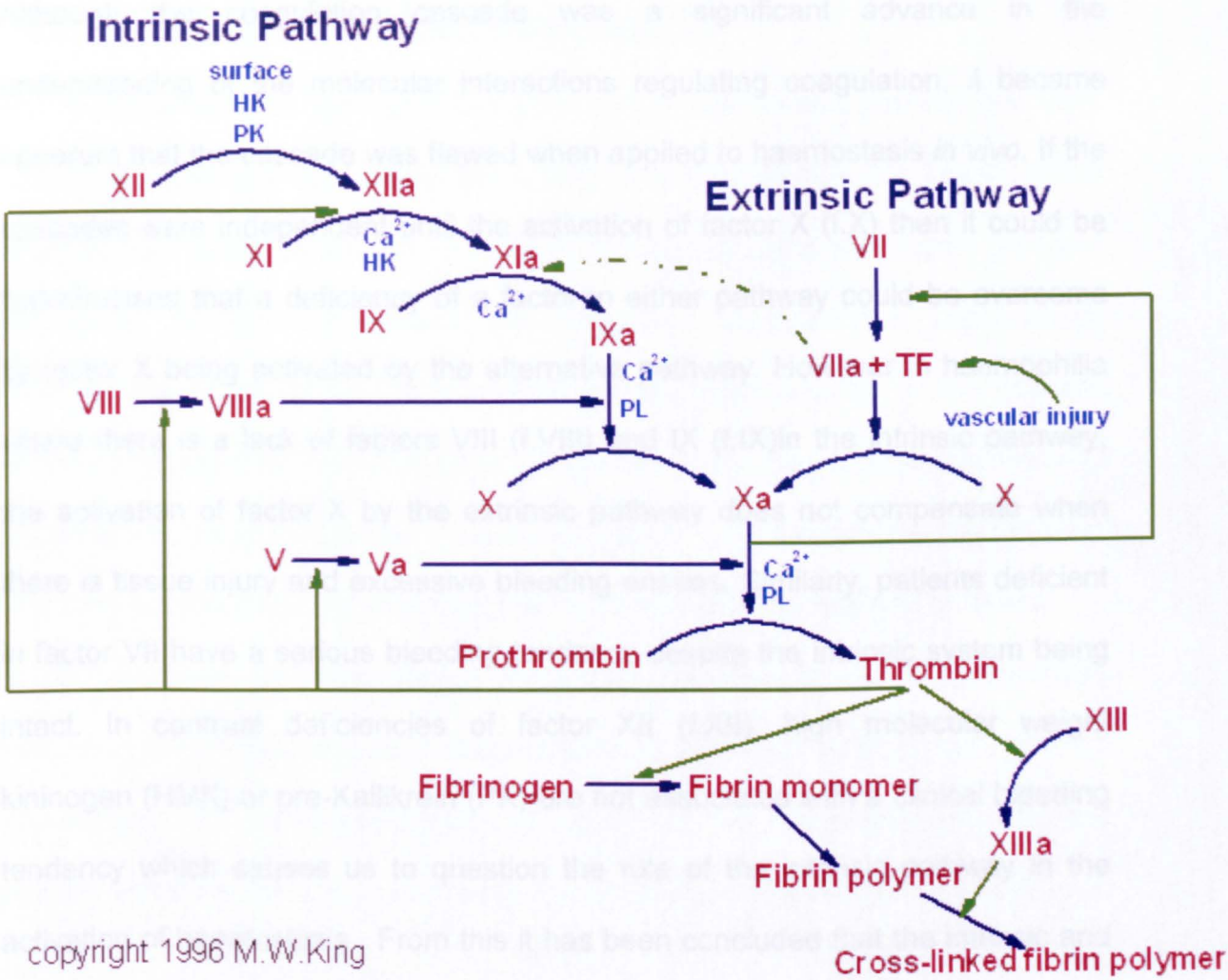

Fig 1.1 Coagulation Cascade

Although the coagulation cascade was a significant advance in the understanding of the molecular interactions regulating coagulation, it became apparent that the cascade was flawed when applied to haemostasis *in vivo*. If the cascades were independent until the activation of factor X (f.X) then it could be hypothesised that a deficiency of a factor in either pathway could be overcome by factor X being activated by the alternative pathway. However in haemophilia where there is a lack of factors VIII (f.VIII) and IX (f.IX)in the intrinsic pathway, the activation of factor X by the extrinsic pathway does not compensate when there is tissue injury and excessive bleeding ensues. Similarly, patients deficient in factor VII have a serious bleeding tendency despite the intrinsic system being intact. In contrast deficiencies of factor XII (f.XII), high molecular weight kininogen (HMK) or pre-Kallikrein (PK) are not associated with a clinical bleeding tendency which causes us to question the role of the intrinsic pathway in the activation of haemostasis. From this it has been concluded that the intrinsic and extrinsic pathways must be interconnected at other key points in the cascade and this has lead to a new model of *in vivo* coagulation, the cell based model of coagulation. A pivotal step in understanding how the extrinsic and intrinsic pathways may be interdependent in vivo was the recognition that the f.VIIa/TF complex can activate f.IX as well as f.X[3]. In addition it was noted that thrombin could directly activate f.XI on a charged surface[4]. In turn this offered an explanation as to why f.XII, HMK and PK may not be essential to haemostasis.

The cell based model of coagulation.

Haemostasis requires the rapid formation of an impermeable platelet and fibrin plug at the site of vessel injury, but it is also necessary that the factors which promote clot formation remain localised to the site of injury and do not disperse throughout the vascular system. The control of coagulation is achieved by confining the clotting reactions to specific cell membranes which in turn possess specific procoagulant or anticoagulant properties. In the last 15 years the focus of research has been the cellular interactions essential for the coagulation process. The cells central to this process are Tissue Factor-bearing cells and platelets.

In 2001 Hoffman and Monroe proposed a cell-mediated model of haemostasis that occurs in 3 overlapping phases, initiation, amplification and propagation[5]. The initiation phase occurs locally at the site of the tissue damage, and the priming and amplification phases occur on the surface of activated platelets.

Initiation.

Tissue factor (TF) is a membrane-bound non-enzymic protein expressed on the surface of cells not usually in contact with the blood plasma (e.g. fibroblasts) and is the primary initiator of coagulation. Disease states such as inflammation and infection can up regulate the expression of TF by other cells such as monocytes and endothelial cells which would not usually express it. During the haemostatic process, a break in a vessel wall allows plasma to come into contact with TF-bearing extravascular cells (Fig 1.2). Factor VII in plasma binds tightly to cellular TF and is rapidly activated by coagulation and non-coagulation proteases[6]. The

f.VIIa/TF complex activates both f.IX and f.X. Any fX activated by the f.VIIa/TF complex that disassociates itself from the cell surface is rapidly inhibited by Tissue Factor pathway inhibitor (TFPI) or Antithrombin III (ATIII), thus limiting haemostasis to the site of injury. The f.Xa that remains on the cell surface can combine with f.Va to produce small amounts of thrombin[7], which plays an important role in subsequently activating platelets and f.VIII during the amplification phase.

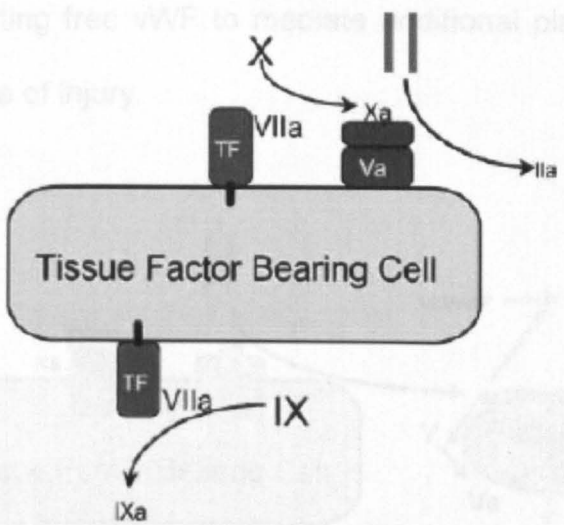

Fig 1.2 The cell-based model of coagulation: Initiation phase

(Image reproduced from Hoffman M, Monroe III DM. The cell-based model of haemostasis. *Thromb Haemost* 2001;85:958-965[5])

Amplification.

A breach in the integrity of a vessel wall will allow platelets and plasma to come into contact with extravascular tissues which then adhere to extra-vascular matrix components at the site of injury. This process activates platelets and localises them near to a site of TF exposure (Fig 1.3). The TF-bearing cells generate small amounts of thrombin which amplifies the procoagulants signal by enhancing platelet adhesion[8], fully activating platelets and activating factors V, VIII and XI[7]. In the process the fVIII/von Willebrand Factor (vWF) complex is dissociated, permitting free vWF to mediate additional platelet adhesion and aggregation at the site of injury.

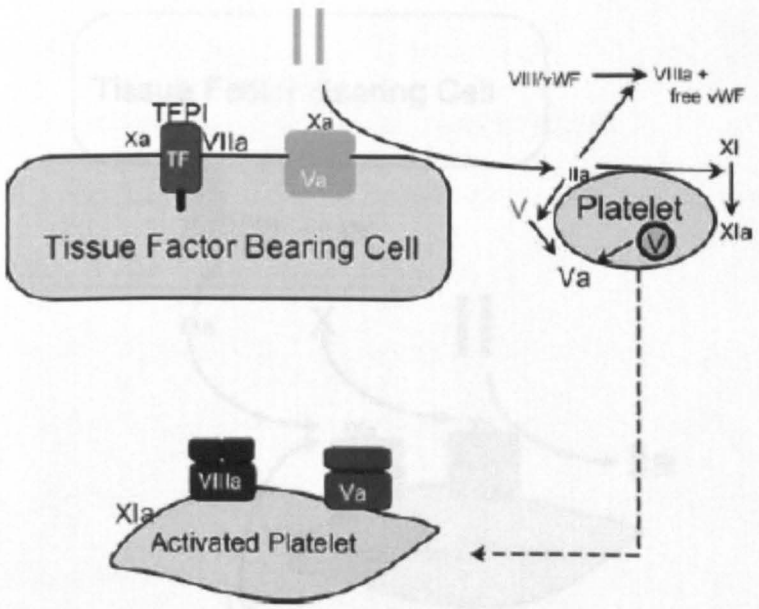

Fig 1.3 The cell-based model of coagulation: Amplification phase

(Image reproduced from Hoffman M, Monroe III DM. The cell-based model of haemostasis. *Thromb Haemost* 2001;85:958-965[5])

Propagation.

The propagation phase takes place on the surface of activated platelets and results in large-scale thrombin generation (Fig 1.4). The platelet surface expresses high affinity binding sites for f.IXa (produced in the initiation phase by TF/fVIIa complexes) which in turn binds with f.VIIIa (produced in the amplification phase) to form the tenase complex (f.VIIIa/IXa). Tenase complexes then activate f.X resulting in f.Xa which binds to f.Va on the surface of platelets forming the prothrombinase complex. The prothrombinase complex produces a burst of thrombin that not only converts soluble fibrinogen to insoluble fibrin but also activates fibrin stabilising f.XIII and thrombin activatable fibrinolysis inhibitor (TAFI) to form a stable insoluble clot.

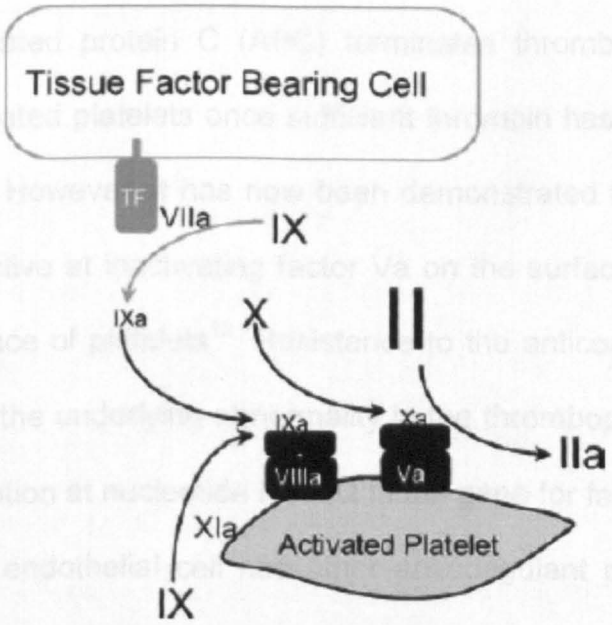

Fig 1.4 The cell-based model of coagulation: Propagation phase.

(Image reproduced from Hoffman M, Monroe III DM. The cell-based model of haemostasis. *Thromb Haemost* 2001;85:958-965[5])

Control of coagulation (natural anticoagulation system)

It is essential that the formation of a fibrin/platelet clot is confined to the site of injury to avoid occlusion by thrombus in neighbouring healthy tissue. If the coagulation mechanism was not tightly controlled a relatively small stimulus could result in extensive thrombosis of the vascular tree. Hoffman has described a mechanism by which this process is controlled involving protein C, protein S and thrombomodulin (TM)[9] The thrombin formed during the coagulation process can be swept downstream from the site of injury. When it reaches an intact endothelial cell, it binds to TM on the surface of the cell. The endothelial thrombin/TM complex then activates protein C, which binds to its co-factor protein S and inactivates factors Va, VIIIa and Xa. It has long been accepted that activated protein C (APC) terminates thrombin generation on the surface of activated platelets once sufficient thrombin has been generated to form a stable clot. However, it has now been demonstrated that APC/protein S is much more effective at inactivating factor Va on the surface of endothelial cells than on the surface of platelets[10]. Resistance to the anticoagulant effect of activated protein C is the underlying abnormality in the thrombophilia where there is a single point mutation at nucleotide R506Q in the gene for factor V (factor V Leiden).

The endothelial cell has other anticoagulant properties; Tissue factor pathway inhibitor (TFPI) is an efficient protease inhibitor, which rapidly inhibits procoagulant proteases, particularly fXa, on the surface of intact endothelium. In addition, endothelial cells also express an ADPase in their membrane which acts

to inhibit platelet aggregation[11]. The primary role of these mechanisms is to prevent thrombin generation on healthy endothelial cells.

Antithrombin III is also present in endothelial cells and is an important component of the endogenous anticoagulant system. Antithrombin III binds with thrombin to form a stable thrombin-antithrombin III complex, preventing conversion of fibrinogen to fibrin by thrombin. In addition antithrombin III directly binds to factors VIIa, IXa and Xa. Measurement of thrombin-antithrombin III complexes is used as a marker of *in vivo* thrombin generation. In addition Antithrombin III has a heparin binding site. When heparin is bound to antithrombin its anticoagulant activity is increased 1000-fold. The function of antithrombin III as a natural anticoagulant is highlighted by the 50-fold increased lifetime risk of thromboembolism in those individuals who have antithrombin III deficiency.

Fibrinolytic System.

The fibrinolytic system balances the effects of the coagulation system by preventing excessive fibrin formation and removing thrombus when damaged tissue has been repaired. Plasminogen is activated by plasma or tissue activators found in most tissues except the liver and placenta. The activators are synthesised by endothelial cells and concentrated in the walls of blood vessels. The active product, plasmin, is a proteolytic enzyme capable of degrading fibrin and disrupting the fibrin matrix.

Fibrinolysis is stimulated when plasminogen activator, plasminogen and fibrin are in close proximity. Plasminogen and its activators bind to fibrin as the clot forms.

Plasmin attacks fibrin at multiple sites resulting in fibrin fragments, some of which are still able to polymerise, thus preventing the destruction of the clot before the tissue is repaired. In addition these fibrin fragments are required for the binding of fibrin-bound plasminogen by plasminogen activators.

There are multiple inhibitors to the fibrinolytic system. Plasminogen activator inhibitors 1 and 2 (PAI-1, PAI-2) are efficient at preventing activation of plasmin and thrombin activatable fibrinolysis inhibitor (TAFI) interrupts the binding of plasminogen to fibrin. TAFI is activated by thrombin and in turn this depends on the co-factor thrombomodulin which is present on intact endothelial cells.

Coagulation changes in pregnancy.

During pregnancy many physiological adaptations are made and these include changes in haemostasis. Pregnancy is a hypercoagulable state associated with increased levels of procoagulant factors, enhanced thrombin generation and impaired fibrinolysis. It is reasonable to assume that these adaptations are to counteract the natural instability of the haemochorial circulation during placentation. The third stage of parturition with the separation of the placenta is a major challenge to the haemostatic system and to the pregnant woman. Haemorrhage is the leading cause of maternal death worldwide[12]. Contraction of the uterus is the primary mechanism which controls blood loss after delivery but the fact that patients with bleeding tendencies such as von Willebrand's disease are at increased risk of postpartum haemorrhage demonstrates that an intact clotting system is an essential physiological requirement. However, it is these very changes that may also predispose pregnant women to thromboembolic disease.

Pro-coagulant pathways.

During pregnancy the plasma concentration of a number of clotting factors increase. Factors VII[13,15], VIII[13,16,17] and X[13,16] increase steadily throughout pregnancy. Fibrinogen increases progressively with advancing gestation, a significant change is evident by the first trimester and there is an almost two-fold increase in levels by term[13,14].

Both factor VIII coagulant activity and von-Willebrand's factor antigen (factor VIII-related antigen) increase progressively throughout pregnancy[13,16]. Levels of Factor VIII-related antigen (an oligomer component of Factor VIII) are significantly higher than the non-pregnant state by 6 weeks gestation[17] and factor VIII coagulant activity is almost twice that of the nonpregnant state in the late 3rd trimester[16].

Increased activity of FXII has been noted in the third trimester with a marked decrease in levels at the time of delivery[16]. There is conflicting data regarding levels of factor V or prothrombin in pregnancy. Clark et al have demonstrated an increase of 29% from early to late pregnancy[18], however this was not supported by the most extensive study to date of clotting factors in pregnancy by Stirling[13] who reported a slight rise in the first trimester followed by a gradual decrease in the second and third trimester.

In contrast to the general increase of coagulation factors, factor IX has been shown to decrease as pregnancy advances, with average levels of between 60% and 70% near term[19]. Hellgren and Blomback have demonstrated a gradual fall of factor XI levels throughout pregnancy, reaching their lowest at term[16].

Studies of the prekalilrein/kalikrein system have demonstrated some interesting changes. Levels of prekalikrein rise during pregnancy[20] but at the onset of labour there is a sudden drop[20,21], which may stimulate further changes in the coagulation system. In contrast kallikrein levels remain at non-pregnant levels throughout pregnancy with a sudden rise just before labour[20].

Fletcher et al have demonstrated a rise in fibrin formation by measuring the levels of high-molecular weight fibrinogen complexes which reflect the rate of fibrin formation/fibrinolysis in vivo[14]. Their results suggest a threefold increase in fibrin formation at 8 weeks gestation compared to the nonpregnant state and a fivefold or greater increase in late pregnancy and the early puerperium.

D-dimer levels increase gradually, which indicates an increased fibrin deposition secondary to enhanced thrombin generation, and also a sustained plasmin generation and activity[22].

Measurement of thrombin-antithrombin III (TAT) complexes has been described as a sensitive marker of thrombin formation from prothrombin by the action of Factor Xa in vivo[23]. As thrombin is the end product of the coagulation system, measurement of its production provides information of the global effect that the changes in individual factors has on clot formation. There is a significant and progressive increase in TAT levels in normal pregnancy[22,24,25]. Bremme et al demonstrated these increases occurred early in pregnancy with 11 out of the 22 women having higher TAT levels compared with non-pregnant values in the first trimester($p<0.05$). By the second trimester all women had increased levels ($p<0.001$)[25].

The increase of procoagulant factors is partly due to hormonal effects. Raised levels of f.VII and f.X, and f.VII and fibrinogen are found in women taking combined oral contraceptives[26,27] and those taking hormone replacement therapy[28]. Animal studies have demonstrated increases in levels of clotting factors related to rising levels of 17β oestradiol in pregnant canine bitches[29] and pregnant calves[30]. However, the elevation of fibrinogen in pregnancy is much greater than that which occurs in women treated with high doses of oestrogen and progesterone[31], so the hormonal influence alone does not explain the huge increases in procoagulants during pregnancy.

Control of coagulation in pregnancy (natural anticoagulation system).

As described previously the body has a tight regulatory network to control clot formation. Protein S, thrombomodulin activated protein C and Antithrombin III are central components of this pathway. Clark et al have performed a methodological robust study in a normal pregnant population which demonstrated a fall in total and free protein S levels throughout pregnancy and no change in levels of protein C and antithrombin activity[18]. Cerneca et al have demonstrated by the 10th week of pregnancy the activity of protein S has been shown to be 60% of non-pregnant levels[32]. The hypothesis that decreasing levels of Antithrombin III in pregnancy may contribute to the increased incidence of thromboembolism has also been rebuked by a number of studies which demonstrate no significant change in antithrombin III throughout pregnancy[32-34]. Other studies have suggested that an acquired resistance to activated protein C may be a function of normal pregnancy in women with no evidence of factor V Leiden mutation[35,36], and the mechanism of this change has been attributed to a number of pathways. Acquired activated protein C resistance has been linked to an elevation of factor VIII in women using the oral contraceptive pill[37]. Clark et al demonstrated a fall in activated protein C-sensitivity ratio which correlates with the changes in factors V and VIII and protein S in pregnancy[18]. This results in a depression of the body's natural anticoagulant system and contributes to the net hypercoagulable state of pregnancy.

Fibrinolytic pathways in pregnancy.

A decrease in fibrinolytic activity (100/clot lysis time in hours) throughout pregnancy has long been recognised[38]. A fall in activity has already occurred by 11-15 weeks of pregnancy[13] and continues, with the lowest values in the third trimester[13,38]. Measurement of time taken to lyse a clot is a direct reflection of fibrinolytic activity and this has been shown to increase from 30 weeks of pregnancy to term[39]. There are two possible components which result in the depression of fibrinolysis, one is a decrease of activators and the other is an increase of fibrinolytic inhibitors.

There is conflicting data regarding the levels of tissue-plasminogen activator (t-PA) in pregnancy. Halligan et al report an increase in basal levels of t-PA in the 2nd and 3rd trimesters[40], but Astedt et al fail to replicate this reporting no change in t-PA levels during pregnancy[41].

Stegnar et al performed a study to observe the fibrinolytic response to a 20 minute venous occlusion in pregnancy and the puerperium by measuring the levels of tissue-plasminogen activator (t-PA) [42]. The increase in tissue-plasminogen activator antigen from the basal level stimulated by venous occlusion in the 2nd and 3rd trimester was the same as in the non-pregnant control population. However, 3 days after delivery the increase in t-PA antigen from the basal level was significantly enhanced (8.5 fold vs 3.7 fold $p<0.005$) thus demonstrating the inhibitory effect of pregnancy. A similar study has been conducted by Ballegeer et al and their findings were of a significant reduction in total t-PA release, with free t-PA remaining below the limit of detection following

occlusion and therefore they concluded that t-PA release is impaired in pregnancy and free t-PA is rapidly inhibited, in keeping with high levels of plasminogen activator inhibitors[43].

The reduction in fibrinolytic activity is contributed to by increased levels of plasminogen activator inhibitor (PAI) type 1 and more particularly the placentally derived PAI type 2[44,45]. PAI-1 increases throughout pregnancy and its activity is 8 times higher than non-pregnant levels at term[46]. PAI-2 is undetectable outside pregnancy with serum levels less than 10ng/ml, during pregnancy there is a steady rise with levels peaking in the 3rd trimester at 260ng/ml[46]. These changes in PAI-1 and PAI-2 may be important for stabilisation of the placental bed by local fibrin deposition. Levels of thrombin activatable fibrinolysis inhibitor (TAFI) increase steadily through pregnancy, again peaking at 35-39 weeks gestation[39,47], together with the required activator, thrombin and co-factor thrombomodulin.

The relative contributions of changes in plasminogen activator inhibitors (PAI) type 1 and 2 and thrombin activatable fibrinolysis inhibitor have been very elegantly explored by Mousa et al. They noted an increasing time for clots to lyse i.e., impairment of fibrinolysis, with increasing gestational age. When the effect of TAFI was eliminated with potato tuber carboxy peptidase inhibitor, the prolongation of clot lysis time was abolished which suggests that the impaired fibrinolysis effect occurring after week 30 of pregnancy is primarily due to increased levels and activity of TAFI[39].

Despite the mechanisms described above which inhibit fibrinolysis during pregnancy, increased levels of the fibrinolytic degradation product, D-dimer have been demonstrated in numerous studies[22,25,48]. It is likely that this reflects an increase in fibrin production rather than an enhancement of fibrinolysis.

Coagulation changes in labour and the puerperium

The adaptations in coagulation occurring during pregnancy are vital to reduce the risk of catastrophic haemorrhage during labour and delivery of the placenta. There are very few studies that have investigated the specific coagulation changes that occur during labour but it is widely accepted that huge activation of the coagulation system occurs at delivery in order to ensure maximum coagulation at the time of placental separation. The puerperium marks the time period were all the changes that occur during pregnancy revert to normal. There is a general consensus that coagulation returns to nonpregnant levels by 6 weeks postpartum but there are very few longitudinal studies performed in the puerperium to support this. The majority of studies addressing coagulation in the puerperium do so by studying at 5 days post partum and then again at 6 weeks post-partum with no data about the intervening period.

Bonnar et al[49] intensively studied labour and the early puerperium in 15 labouring women who had uncomplicated vaginal deliveries. He demonstrated increased levels of factors V and VIII, decreased plasma fibrinogen levels and a reduced clotting time at the time of placental separation. Similarly, Manning et al has

reported large drops in fibrinogen levels 30 minutes and 3 hours after normal vaginal delivery when compared to late third trimester levels ($p<0.05$)[50].

The most dramatic changes occur in the fibrinolytic pathway and these are initiated during labour and are evident in the first few hours after delivery. Gerbasi et al have performed a longitudinal study on 70 healthy pregnant women, obtaining blood samples at 5 time points during labour and up to 48 hours after normal vaginal delivery. They demonstrated a fall in antithrombin III levels during labour and reaching its lowest levels 3 hours after delivery before rising again[51]. They also report an increase in fibrinogen degradation products and D-dimer levels, peaking at 3 hours after delivery, which would suggest an increase in fibrinolytic activity. A methodologically similar study has been performed by MacKinnon et al and they demonstrate a rise in both t-PA levels and activity at 15 and 60 minutes post delivery compared with pre-delivery levels ($p<0.05$ and $p<0.001$ respectively) followed by a fall between 1 and 5 days postpartum[52]. These changes are similarly described by Kruithof [46] and Manning [50]

One hour after delivery, PAI-1 activity and PAI-1 antigen decrease sharply, PAI-2 levels remain constant. By the 3rd postpartum day the PAI-1 levels have returned to non-pregnant values[52]. The PAI-2 levels remain elevated but are falling[46] and become undetectable at 7 days postpartum[53]. Compared with the maximum mean TAFI level during pregnancy, there is a significant drop of TAFI antigen levels within 24 hours of delivery[39].

In the 5 days following delivery levels of f.VII fall sharply[13], f.X and f.XI have a secondary rise[54] and levels of f.V increase possible exceeding those in pregnancy[13,54]. Fibrinogen increases further after delivery and continues to do so for 5 days, delivery[13,14,54] before falling to non-pregnant levels by 4 weeks post-partum[14].

At 6 weeks postpartum levels of Fibrinogen, Factors VII and VIII[13,55] and II, IX, X and XII have returned to non-pregnant levels[13] but the exact timing of these changes has not been studied.

Venous thromboembolic disease in pregnancy.

Thromboembolic disease remains the leading cause of maternal mortality in the UK and the USA[56]. Data from the UK triennial report of maternal deaths show that 33% of all direct maternal deaths are due to thromboembolic disease[57]. In the puerperium women are more likely to die of this disease than any other. Many of the other serious complications of childbirth, such as haemorrhage and infection, which result in maternal deaths, have reduced in incidence over the past 25 years. Despite a very marked reduction in the number of maternal deaths due to thromboembolic disease since the 1950's the rate has remained static since the early 1980's. Fig 1.5. There are a number of demographic factors which may be contributing to the statistics. Maternal age is rising, as is maternal weight, women with coexisting medical problems e.g. diabetes, essential hypertension and renal disease are having increasing numbers of pregnancies and there is an increasing caesarean section rate. These demographic changes are likely to persist.

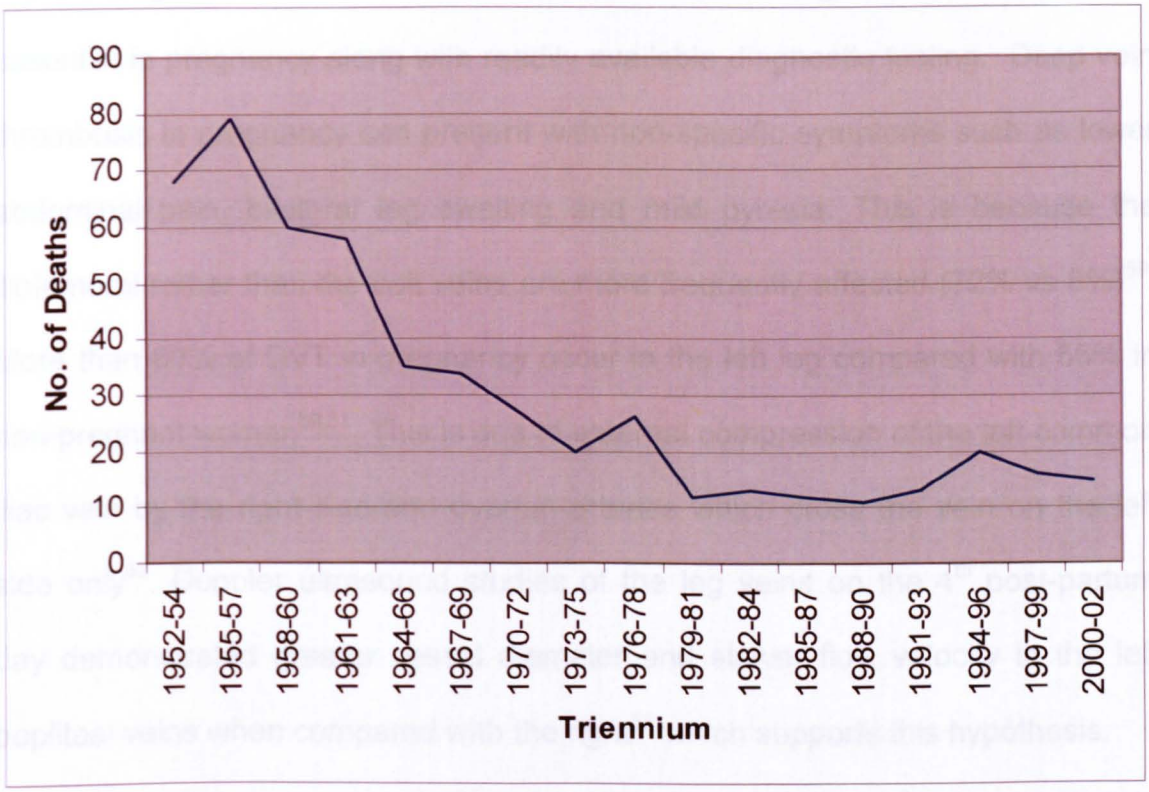

Fig 1.5 Number of maternal deaths due to thromboembolic disease.
Drife J, Lewis G editors. *Why Mothers Die 2000-2002: Sixth Report of the Confidential inquires into Maternal Deaths In the United Kingdom.* London: RCOG press 2004

As previously described, all pregnant women are rendered hypercoagulable by normal physiological adaptations to pregnancy. Other factors such as the increasing weight of the gravid uterus causes raised venous pressure and venous stasis and concomitant endothelial damage (occurring either during delivery or secondary to other conditions) may trigger thrombus formation. The combination of these factors, as described by Vichow's triad, result in a 5 fold increased risk for venous thrombosis in pregnancy which increases to 25 fold in the postpartum period[58].

Clinical diagnosis of deep vein thrombosis is difficult, a high index of suspicion is essential in pregnancy along with readily available diagnostic testing. Deep vein thrombosis in pregnancy can present with non-specific symptoms such as lower abdominal pain, bilateral leg swelling and mild pyrexia. This is because the iliofemoral rather than the calf veins are more frequently affected (72% vs 9%)[59]. More than 80% of DVT in pregnancy occur in the left leg compared with 55% in non-pregnant women[60,61]. This is due to external compression of the left common iliac vein by the right iliac and ovarian arteries which cross the vein on the left side only[62]. Doppler ultrasound studies of the leg veins on the 4th post-partum day demonstrated greater vessel diameter and slower flow velocity in the left popliteal veins when compared with the right[63] which supports this hypothesis.

Risk Factors for VTE

Maternal deaths due to thromboembolism antenatally and after normal delivery are rising which highlights the need for further investigation. One approach is to improve our ability to identify women at risk of thromboembolism and take measures to decrease the risk. Presently, our only method of doing this is by taking a personal and family history and as pregnancy may represent the first occasion that many women are challenged with the haemostatic changes of pregnancy, many women at risk will not be identified. Current recommendations are based on factors that are accepted to increase the risk of thromboembolism, but few of these factors have data to support the degree of increased risk associated with them.

Timing of VTE

Knowledge of the relative risk and incidence of thromboembolism during pregnancy and postpartum is important in identifying women who may benefit from prophylaxis. The reported incidence of VTE in pregnancy and the puerperium varies widely, rates range from 18 to 90 events per 100 000 woman-years during pregnancy and from 199 to greater than 1900 per 100 000 woman years in the post-partum period[64-68]. There are also marked differences in the reported incidence of thromboembolism by trimester and the timing of puerperal events[69-71]. These inconsistencies reflect the wide variations in the study designs. Patients were identified from a variety of sources including hospital discharge and maternity registers[66-67], hospital inpatient databases[64] and the National Health Service database[65]. If a patient re-presented in the postpartum period they may have been potentially overlooked by these studies and diagnostic criteria also varied between the studies. These factors combined may result in under or overestimation of the true incidence of this disease.

Ray and Chan have performed a meta-analysis of all published studies of deep vein thrombosis during pregnancy and the puerperium between 1966 and 1998. They calculated the estimated relative distribution of 100 deep vein thrombosis events during pregnancy and the puerperium as 0.23 per day during pregnancy, rising to 0.82 per day in the puerperium. This paper also draws attention to the fact that more than half of all deep vein thromboses in the antenatal period occur during the first and second trimesters[60]. In a 30 year population-based study Heit

et al have demonstrated that the highest risk time for a pulmonary embolism is in the first postpartum week when the risk is 25 times that in the third trimester[58].

Mode of delivery.

As previously discussed, the immediate post-partum period is the time when risk of a thromboembolic event is at its highest[60]. It is widely accepted that caesarean section increases the risk of thromboembolism, with the significant endothelial injury, prolonged immobility and reactive thrombocytosis[72] associated with surgery. This has been estimated to be between 4[67] and 20[64] times that following normal vaginal delivery. The medical response has been to introduce a range of interventions to reduce the risks of thromboembolism. The benefits of thromboprophylaxis have been demonstrated by the marked reduction in thromboembolic events following caesarean section after the introduction of guidelines for thromboprophylaxis[73] However, in 2001 the Confidential Inquiry into maternal deaths 1997-1999 noted that no impact had been made on the number of deaths following vaginal delivery. Only one of the ten deaths followed an operative vaginal delivery the remaining women had spontaneous vaginal deliveries, and it was noted that all women dying were either overweight or over the age of 35 years[74]. This has prompted the recommendation that all women in labour should be assessed for risk factors for thromboembolic disease. (Table 1). If two or more risk factors are present then the use of thromboprophylaxis in the form of Low- Molecular Weight Heparin should be considered for 3-5 days post-partum[75].

Table 1. Risk factors for venous thromboembolism in pregnancy and the puerperium[a]

Pre-existing	New onset or transient[b]
Previous VTE	Surgical procedure in pregnancy or puerperium, e.g. evacuation of retained products of conception, postpartum sterilisation
Thrombophilia	Hyperemesis
congenital	Dehydration
antithrombin deficiency	Ovarian hyperstimulation syndrome
protein C deficiency	Severe infection, e.g. pyelonephritis
protein S deficiency	Immobility (> 4 days bed rest)
Factor V Leiden	Pre-eclampsia
prothrombin gene variant	Excessive blood loss
acquired (antiphospholipid syndrome)	Long-haul travel
lupus anticoagulant	Prolonged labour[c]
anticardiolipin antibodies	Midcavity instrumental delivery[c]
Age over 35 years	Immobility after delivery[c]
Obesity (BMI > 30 kg/m2) either pre-pregnancy or in early pregnancy	
Parity > 4	
Gross varicose veins	
Paraplegia	
Sickle cell disease	
Inflammatory disorders e.g. inflammatory bowel disease	
Some medical disorders, e.g. nephrotic syndrome, certain cardiac diseases	
Myeloproliferative disorders, e.g. essential thrombocythaemia, polycythaemia vera	

[a] Although these are all accepted as thromboembolic risk factors, there are few data to support the degree of increased risk associated with many of them; [b] these risk factors are potentially reversible and may develop at later stages in gestation than the initial risk assessment or may resolve; an ongoing individual risk assessment is important; [c] risk factors specific to postpartum VTE only

Table 1: Royal College of Obstetricians and Gynaecologists. *Thromboprophylaxis during pregnancy, labour and after vaginal delivery.* Guideline No. 37. London: RCOG Press;2004

Thrombophilias.

Thrombophilias have been implicated in approximately 50% of episodes of venous thromboembolism in pregnancy and post partum women[59]. At least 15% of Western populations carry one or more thrombophilic mutations. A retrospective study by McColl et al of 72,000 pregnancies in which women with thromboembolism were assessed for thrombophilia and the underlying prevalence of these defects was known, demonstrates the significant effect of the thrombophilias on the risk of thromboembolism[76]. Among women with factor V Leiden the risk was 1:437, among those with protein C deficiency 1:113, among those with type I antithrombin deficiency 1:2.8 and among those with type II antithrombin deficiency 1:42[76,77].

Thrombophilic mutations have been implicated in other complications of pregnancy such as severe pre-eclampsia, placental abruption, fetal growth restriction and stillbirth[78-81].The results of studies are conflicting with some studies refuting a link. Many of the studies are small, definitions of pre-eclampsia and fetal growth restriction varied, a variety of ethnic groups were included but the difference in background incidence of thrombophilic mutations was not accounted for. Screening populations for thrombophilias is not currently recommended but increasingly individuals with poor obstetric outcomes are being investigated. Selective screening of women with a personal or family history of VTE may be of value as around 50% of such women will have an inheritable thrombophilia[82]. Clinicians are then often presented with a dilemma if

a thrombophilic defect is detected, as there is a lack of evidence regarding treatment with low-molecular-weight heparin or aspirin and improved pregnancy outcome. It is accepted that there are more thrombophilic defects to be described and these will influence yet further the debate regarding screening "at risk" or the general population.

Sequelae of venous thromboembolism.

In clinical obstetric practice the focus of attention is on the identification and treatment of thromboembolism but the long-term consequences of such an event are often not considered. There is little data on the sequelae but what there is demonstrates that the majority of women have some degree of post-thrombotic syndrome. Bergqvist et al found that only 22% of women with previous pregnancy-related VTE were asymptomatic for post-thrombotic syndrome at a median follow-up of 11 years, although few (4%) had severe symptoms[83]. When follow-up of patients with a DVT outside pregnancy is extended to 20 years 82% of patients demonstrate symptoms including itching, numbness, paraesthesia, skin darkening and hardening and severe pain, swelling and cramping[84]. Post thrombotic syndrome represents significant distress to patients – physically, psychologically and socially as symptoms may restrict their daily living, and they may be left with permanent disability. In addition, the risk of recurrence of VTE increases after just one venous thrombotic event and there may be as much as 25% risk of recurrence of DVT within 5 years[85].

Thromboelastography.

The use of Thromboelastography (TEG®) to assess whole blood coagulation was first described by Hartert in 1948[86]. The technique was mainly a research tool until being introduced to clinical practice in the setting of liver transplantation[87]and cardiac surgery in the 1980s[88].

Thromboelastography (TEG) is a test of the global assessment of haemostatic function from a single blood sample. The technique evaluates the viscoelastic properties of blood during coagulation and fibrinolysis. It is able to provide information relating to the cumulative effect of several components of coagulation at a given time point, and unlike conventional clotting tests is performed on whole blood, thus incorporating the contribution of platelets to coagulation.

Principles of Thromboelastography

Thromboelastography produces a graphic (visual) representation of clot formation and lysis. The thromboelastograph analyzer (Fig 1.6) consists of a prewarmed (37^0C) cup into which a small volume of blood (340µl) is placed. The cup rotates through 45^0 in either direction every 4.5 seconds. A pin is suspended in the cup and the movement of the pin is transmitted to the computer via an electrical transducer. Initially when no clot exists the movement of the cup does not affect the pin and a straight line is recorded. As the blood begins to clot the movement of the cup is transmitted to the pin and a characteristic thromboelastograph is produced (Fig 1.7).

Fig 1.6: Thromboelastography machine (Reproduced with permission form Haemoscope Corporation)

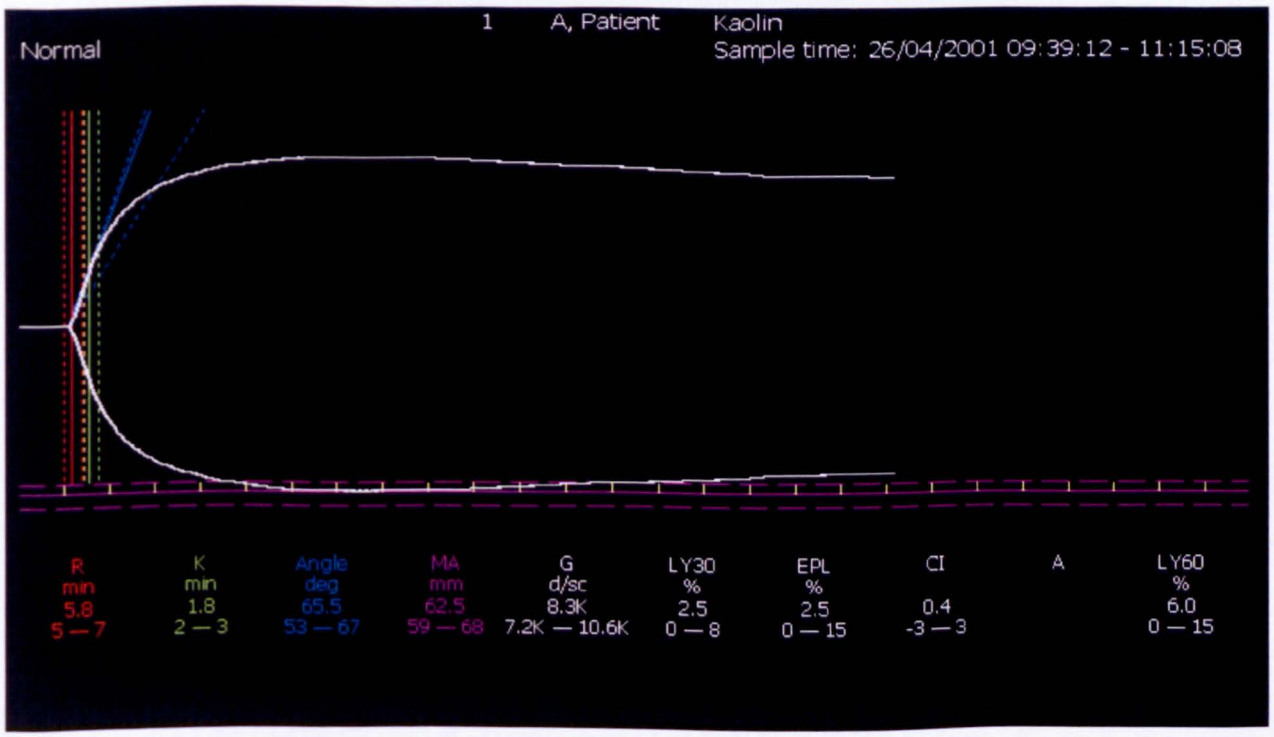

Fig 1.7. A characteristic thromboelastograph

A number of parameters describing clot formation and lysis are generated by computer analysis of the thromboelastograph (Fig 1.8).

R time (reaction time) is the time from placing the blood in the cup until the first significant levels of detectable fibrin formation. This is the point at which most traditional clotting assays, including prothrombin time and activated thromboplastin time, reach their end-points.

K time is the time for achievement of a defined level of clot "firmness". It is the time from R time (beginning of clot formation) until a fixed level of clot firmness is reached (amplitude=20mm). As such it is a measure of the velocity of clot formation.

α angle reflects the kinetics of clot development and the rate of polymerisation. The angle is more comprehensive than K time, since there are hypocoagulable conditions in which the final level of clot firmness does not reach an amplitude of 20mm, in which case K is undefined.

MA (maximum amplitude) is the greatest vertical amplitude of the TEG trace. It measures the maximum strength of the developed clot. Clot strength is the result of two components, the contribution of fibrin to clot strength and the much more significant contribution of the platelets.

LY30 and **LY60** are measures of percent lysis at 30 and 60 minutes after MA is reached. The LY30 and LY60 measurements are based on the reduction of the area under the TEG tracing from the time MA is measured until 30 (or 60) minutes later.

Coagulation Index describes the overall coagulation and is derived from R time, K time, α angle and MA of native or kaolin activated whole blood tracings. The equation is CI=-0.2454R + 0.0184K + 0.1655MA - 0.0241α - 5.022

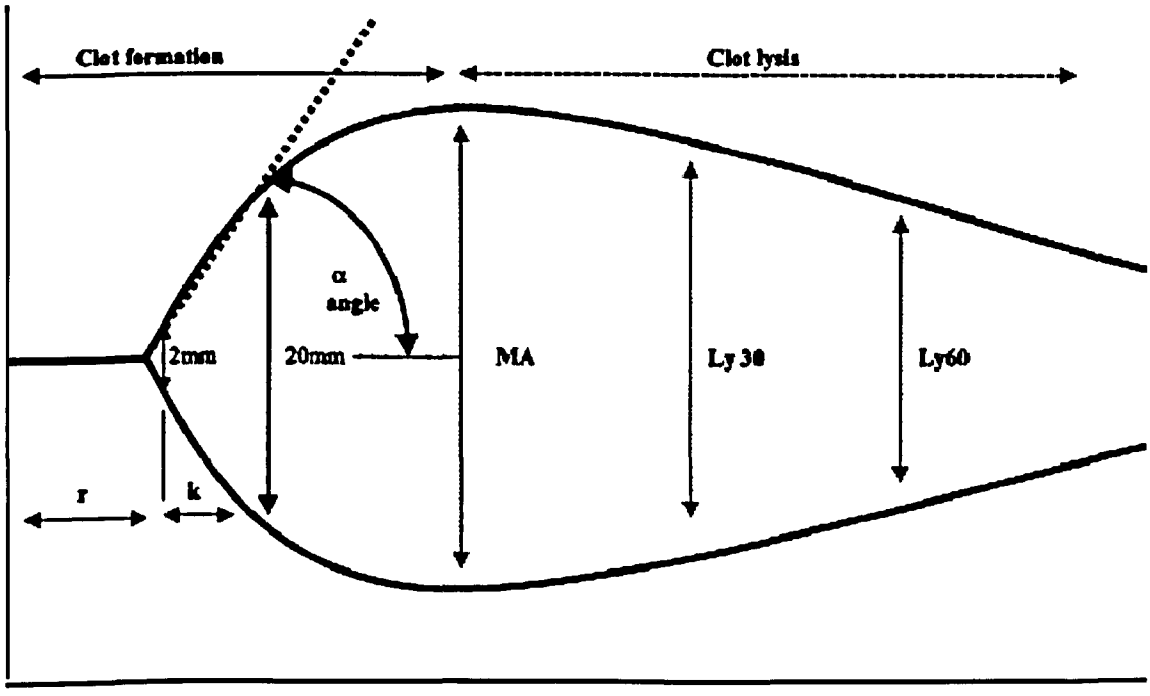

Fig 1.8. A schematic drawing of a thromboelastograph demonstrating the parameters measured.

(Image reproduced from Salooja N, Perry DJ. Thromboelastography. *Blood Coagulation and Fibrinolysis* 2001;12:328)

Thromboelastography and the hypocoagulable state.

Thromboelastography was incorporated into clinical practice in the field of liver transplantation. Disordered coagulation is universal in this patient group due to deficiencies of clotting factors synthesised in the liver, enhanced fibrinolysis and frequently a low platelet count due to depressed marrow production or sequestration by the enlarged spleen. Excessive blood loss during such procedures results in high levels of morbidity and mortality postoperatively. Transfusion of whole blood and blood products is almost inevitable. Conventional coagulation tests, prothrombin time and activated partial thromboplastin time and platelet counts were used to guide administration of blood products but there has been criticism that obtaining results perioperatively can be slow and possibly delay administration of the most appropriate product. Kang et al pioneered the use of thromboelastography in 1985[87]. A TEG-guided algorithm was developed to guide blood product administration: an r time of more than15 min was treated with 2 units of fresh frozen plasma, a MA less than 40mm was treated with 10 units of platelets. Six units of cryoprecipitate was given if these measures did not improve coagulation or when the angle was less than 45^{o}. Using this algorithm in 66 patients undergoing liver transplantation resulted in an increase in the transfusion of platelets and cryoprecipitate but a 33% reduction in blood and fluid replacement overall.

Thromboelastography has been similarly used to guide blood product administration in the setting of cardiac surgery. Patients undergoing such surgery are also at risk of haemorrhage which is usually due to inadequate surgical haemostasis. There are also underlying defects in haemostasis which contribute to the increased risk of bleeding. These patients are given heparin to prevent coagulation on the surface of the extracorporeal circuit. The contact of blood on the foreign surface of the circuit stimulates fibrinolysis. Platelet number and function is disrupted by the hypothermia, which is induced to reduce neuronal damage and the cellular damage resulting from the pumps in the circuit.

TEG monitoring has also been used to identify patients at risk of postoperative haemorrhage[88,89] and to contribute to the assessment of surgical versus haemostatic bleeding postoperatively[90].

Thromboelastography and the hypercoagulable state.

There are five main coagulation tests that are used in current clinical practice to screen for abnormalities in coagulation, haematocrit, platelet count, prothrombin time, activated partial thromboplastin time and fibrinogen. These tests represent separate albeit linked components of the coagulation process. Zuckermann et al[91] have compared thromboelastography with conventional clotting tests in a group of 141 normal volunteers and 121 patients with malignancies which they referred to as the "hypercoagulable" group. The TEG variables correctly classified 96.7% of the hypercoagulable group compared with 72.3% by the standard tests. The authors concluded that the additional information provided by thromboelastography from initiation of clotting, strength and stability of the developed clot to clot lysis and retraction made TEG a more sensitive test in detecting hypercoagulability.

Thromboelastography has been used in a narrow range of clinical settings to explore hypercoagulability and its relation to the pathogenesis of thrombotic complications. Thrombotic events in the post-operative period are a common cause of morbidity and mortality which has stimulated the generation of guidelines to assess the risk of such complications and in turn recommendations to reduce the occurrence through prophylactic measures. There is however a lack of knowledge about the underlying mechanisms which result in the relative hypercoagulability and subsequent thrombus formation. This is due to the fact that measuring levels of individual procoagulant and fibrinolytic factors does not reflect the dynamic *in vivo* process of coagulation

which includes the interaction between fibrin and platelets and individual activity of procoagulant factors and platelets. There are several studies that demonstrate a postoperative rise in procoagulant factors and an associated fall in anticoagulant and fibrinolytic factors[92-95] but the net *in vivo* effect of these changes can only be hypothesised. The study performed by Gibbs et al[92] investigates patients undergoing elective abdominal aortic surgery. They demonstrate large increases in the procoagulants, fibrinogen, factor VIII, von Willebrand factor and α_1-antitrypsin over the first 6 days postoperatively, with maximum changes on the 2nd and 4th day. Over the same time there were significant decreases in the naturally occurring anticoagulants protein C and Antithrombin III. Despite these changes in individual factors there were no significant changes outside normal range in prothrombin time, partial thromboplastin time or thrombin clotting time. Mahla et al[96] have performed a methodologically similar study but have used thromboelastography to monitor the coagulation state of 20 patients undergoing major abdominal surgery. Thromboelastography was performed prior to surgery and up to the 7th postoperative day. The study demonstrates a substantial postoperative hypercoagulability lasting for at least 7 days after major abdominal surgery. The hypercoagulability comprises an accelerated clot formation with a shortened R time, and an increase in the clot strength with a continuous increase of MA. This is the most comprehensive study of postoperative hypercoagulability to date.

Other studies have used thromboelastography to demonstrate changes consistent with hypercoagulability but these are limited to the immediate postoperative period. Caprini et al[97] demonstrated hypercoagulability with a significant reduction in R and K times on the first postoperative day following laparoscopic cholecystectomy although the other aim of the study, to correlate TEG changes with thrombotic complications was thwarted by only 1 of the 100 subjects developing such a complication.

As experience of thromboelastography in the hypercoagulable state has grown studies are now aiming to correlate specific TEG parameters with thrombotic events. A study by Traverso et al[98] included 100 patients undergoing elective abdominal surgery. Patients were randomised to receive postoperative heparin thromboprophylaxis or to receive no prophylaxis. In the group that did not receive heparin prophylaxis MA value showed the ability to predict the occurrence of DVT with a sensitivity of 72.2% and a specificity of 69%. The population undergoing emergency orthopaedic surgery are at particularly high risk of venous thromboembolism. Wilson et al[99] performed a study on 250 patients having surgery for repair of proximal femoral fractures. Thromboelastography was performed pre-operatively, and on days 1,3,5 and 7 and 7 weeks postoperatively. Despite administration of low-molecular weight heparin for the duration of the hospital stay the incidence of DVT formation was 28%. When the patients with DVT were compared with those without they had significantly greater coagulation index on post-operative days 1-7 ($p<0.005$).

McCrath et al[100] performed a study on 240 patients undergoing elective major, non-cardiac surgery to explore the hypothesis that a postoperative hypercoagulable state as determined by thromboelastography is associated with an increased likelihood of postoperative thrombotic complications including myocardial infarction. Thromboelastography was performed 2 hours after the completion of surgery. The upper limit of normal for MA in celite-activated TEG as used in this study is 68mm and this value was used to divide patients into control (MA≤68mm) and hypercoagulable (MA>68mm) groups. The incidence of thrombotic complications in the hypercoagulable (MA>68mm) group was significantly higher (8.4%vs1.4% p=0.016). More specifically the incidence of postoperative MI was increased in the hypercoagulable group (6.3%vs0% p=0.004). The authors propose that identifying those at increased risk of thrombotic complications in the immediate postoperative period offers an opportunity to decrease this risk by administration of aspirin or anticoagulants.

Thromboelastography in Obstetrics.

The use of thromboelastography in obstetrics to date is limited. The main focus of interest has been directed at the use of thromboelastography to guide the safe use of regional anaesthesia in women who may be hypocoagulable, either due to thrombocytopenia related to pre-eclampsia or the use of thromboprophylaxis in individuals at risk of thromboembolism. The hypercoagulable state of pregnancy has been demonstrated by thromboelastography in the late stages of the third trimester and in the early postpartum period. Thromboelastography has also been used to explore possible exaggerated hypercoagulability in recurrent miscarriage.

Gorton et al[101] conducted a study on three groups of 50 subjects, men, nonpregnant and pregnant women presenting at term for elective caesarean section. The results demonstrated a significant trend of increasing coagulability from men, through nonpregnant women to pregnant women. There were statistically significant differences in R time, K, α and MA among male, nonpregnant and pregnant subjects ($p<0.01$). This is the first study to identify sex-related differences in TEG parameters and demonstrates the importance of using a control group comprising entirely of women for any studies in pregnancy. Sharma et al conducted a similar study but extended the study period into the 24 hours after delivery[102]. The control population were 17 nonpregnant women, the 134 pregnant women were again presenting for elective caesarean section and the 69 postpartum women were presenting for

tubal ligation 12-24 hours after delivery. This study again demonstrated significant changes in TEG parameters in pregnancy compared to the nonpregnant state with a reduction in R and K time and an increase in α, MA and coagulation index. These changes persist in the first 24 hours postpartum. Neither of these studies describes the indication for caesarean section. The incidence of caesarean section is increased in women with coexisting medical conditions such as diabetes, obese women and women over the age of 35 years and these demographics may influence thromboelastographic profiles. The timing of the blood sample implies that it was after a period of fasting pre-operatively that may result in relative dehydration. Despite these factors both studies claim that their pregnant samples are representative of a pregnant population as a whole, but the authors acknowledge the need for specific reference ranges to be determined before thromboelastography can be used further in pregnancy and the puerperium.

Specific coagulation disorders can be a feature of pre-eclampsia. Thrombocytopenia is present in an estimated 10% of women with severe pre-eclampsia[103] and may be associated with other biochemical abnormalities that combine to present as HELLP (haemolysis, elevated liver enzymes and low platelets) syndrome. In severe cases a low platelet count may be associated with other haematological aberrations including an increase in fibrin degradation products, D-dimer, thrombomodulin, thrombin-antithrombin complexes and a decrease in circulating antithrombin III, fibrinogen and protein C activity. The clinical manifestation of such abnormalities is disseminated

intravascular coagulation (DIC). Sibai et al[104] defined DIC as evidence of excessive bleeding with thrombocytopenia (<100,000/mm^3), plasma fibrinogen level less than 300/mg/dL and fibrin-split products more than 40μg/mL. Using this strict definition they reported a 38% incidence of DIC in the setting of HELLP syndrome. Many of the tests to demonstrate the abnormalities in coagulation are expensive, time consuming and not universally available in the clinical setting. Orlikowski et al[105] conducted a study of 49 women with pre-eclampsia and eclampsia exploring the use of thromboelastography alongside conventional coagulation tests. Seven patients had mild pre-eclampsia (systolic pressure >140mmHg, diastolic >90mmHg and proteinuria >0.3g/l or 1+,2+ on dipstick testing), 33 had severe pre-eclampsia (systolic pressure >160mmHg, diastolic >110mmHg and proteinuria >5g/l or 3+,4+ on dipstick testing) and 9 with eclampsia. Of the 49 patients, 18(37%) had a platelet count ≤$150x10^9$ $litre^{-1}$ and 7 of these ≤$100x10^9$ $litre^{-1}$. Maximum amplitude was reduced in 4 patients, 3 of which had the lowest platelet counts in the series, 2 of the 4 patients had a slightly prolonged prothrombin time but a normal APTT. One patient had a platelet count of $30x10^9$ $litre^{-1}$ with prolonged R and k times; reduced MA but PT and APTT were normal. The authors site this as an illustration of thromboelastography reflecting the interdependence of platelets and clotting factors in *in vivo* coagulation, whereas PT and APTT measure isolated aspects of coagulation in platelet poor plasma and do not provide this information. An MA value of 53mm was used as the lower limit for normal pregnancy and it is

likely that this was defined from Sharma's study using women presenting for elective caesarean section[102].

Sharma et al performed a study using thromboelastography in women with pre-eclampsia. As a control population they used a group of 52 healthy women presenting in labour. Their definitions of mild and severe pre-eclampsia were the same as Orlikowski et al[105]. The incidence of thrombocytopenia was 2% in the healthy pregnant controls, 3% in mild preeclamptic women and 30% in severe preeclamptic women. This study demonstrated in mild pre-eclampsia MA was significantly hypercoagulable compared to healthy pregnant women and women with severe pre-eclampsia. All thromboelastography parameters were significantly hypocoagulable in severe pre-eclamptic women with platelet counts $<100,000/mm^3$ when compared to normal pregnant women, mild preeclamptic women and severe preeclamptic women with a platelet count $\geq 100,000/mm^3$. Of the women with severe pre-eclampsia and a platelet count $\leq 100,000/mm^3$ 29% (10/34) had a MA <54mm (lower limit of normal) whereas only 14% had an abnormal coagulation profile (defined as at least one abnormal value in PT, APTT and fibrinogen). The authors conclude that thromboelastography provides a better assessment of whole blood coagulability than routine coagulation profiles.

The safety of regional anaesthetic techniques in the management of labour in women who have platelet counts $\leq 100x10^9$ litre^{-1} is a frequent clinical dilemma. Many units will deny regional anaesthesia to such women but the cut-off of $100x10^9$ litre^{-1} is arbitrary and is not supported by evidence. There is a growing

body of work using thromboelastography in such situations to guide the use of regional anaesthesia[106-108]. These case reports contain very few patient numbers and as the incidence of epidural haematoma is low it is not surprising that no complications occurred in such few cases.

There is evidence to suggest that some cases of recurrent miscarriage are the result of an exaggerated haemostatic response during pregnancy[109,110]. Rai and Regan have explored the use of thromboelastography in the investigation of 494 women with recurrent miscarriage (three or more consecutive miscarriages at <12 weeks gestation) in whom 108 pregnancies subsequently occurred and 55 parous women who had never experienced a pregnancy loss[111]. The MA was significantly higher and the LY30 (percentage clot lysis at 30 minutes) significantly lower amongst nonpregnant women with a history of recurrent early miscarriages compared with parous controls. In addition, in the prospective pregnancy study the MA was significantly higher amongst women who subsequently miscarried compared with those whose pregnancy resulted in a live birth.

The evidence surrounding thrombophilias and adverse pregnancy outcomes such as pre-eclampsia and intrauterine growth restriction is conflicting as discussed earlier in this chapter. However, there remains convincing evidence from large, methodologically robust studies that there is an increased incidence of inherited thrombophilic defects in patients with such pregnancy complications[65]. Miall et al[112] performed a large prospective study in an

unselected group of 600 women in early pregnancy. A number of investigations were performed on the single blood sample obtained including PT, APTT, plasma Antithrombin level, thromboelastography and thrombophilia tests. The R time was significantly shorter and the coagulation index significantly higher in the 7 women who experienced mid trimester loss (fetal death between 12-23 weeks gestation) compared to the women who had a normal pregnancy outcome. Statistical analysis comparing TEG parameters in the 95 women who experienced one or more pregnancy complication with the 495 women who had normal pregnancies revealed no significant differences. This was confirmed by the analysis of the individual complications. There was no correlation of TEG parameters with the women with or without thrombophilic mutations. There are however, some concerns with the methodology of this study. The mean gestation at which the samples were obtained was 13.6 weeks (SD 3.8), but the range was from 6 to 38 weeks gestation. The authors however do acknowledge that this could be a potential flaw by performing further statistical analysis with the exclusion of all women recruited over 20 weeks. Also thromboelastography was performed 20 minutes after obtaining a citrated blood sample. There is evidence to suggest that activation is still occurring at this time and thromboelastography should be performed at least 30 minutes after a citrated blood sample is obtained[113]. The importance of timing and methodology when performing TEG analysis is discussed further in the next chapter.

Hypothesis and Aims.

Changes in thromboelastography parameters occur in a pregnant population. The aim of this thesis is to describe the timing of these changes during normal pregnancy and the resolution after delivery.

Many complications of pregnancy are linked to an exaggeration of the normal hypercoagulability of pregnancy. I will explore the hypothesis that women who develop complications in pregnancy may have changes in thromboelastography parameters before clinical disease is evident.

Delivery by caesarean section is associated with an increased risk of thromboembolic complications. I will explore the hypothesis that women who are delivered by caesarean section are relatively more hypercoagulable than women who have normal vaginal deliveries, and the resolution of the hypercoagulable state is prolonged after caesarean section.

CHAPTER 2.

MATERIALS AND METHODS.

All of the study protocols contained in this thesis received approval by the Leicestershire, Northampton and Rutland Research Ethics Committee.

Patients were recruited when attending the Leicester Royal Infirmary for maternity care and written; informed consent was obtained after each patient had received the patient information leaflet relevant to the study in which they were participating.

Laboratory techniques.

A variety of differing methodologies are represented in the studies previously discussed. In the earlier studies thromboelastography was performed on whole native whole blood collected in glass or polypropylene vials with analysis commencing 4 minutes after venepuncture[86-88,91,97,99-102,105]. However, this is not practical for testing remote from the TEG machine due to the activation of blood when stored, therefore recalcification of citrated blood has now been introduced[96,111,112]. This stabilises the blood allowing for transport and processing up to 3 hours after venepuncture. In addition a number of activators have also been used, celite, kaolin and tissue factor with the aim of reducing variation in results and allowing for a more rapid analysis[96,111]. The evolution of TEG analytical techniques has served to make the current test clinically robust as well as being "point of care".

Vig et al have conducted a rigorous study investigating the methodology of thromboelastography. They examined the effects of storage of citrated blood demonstrating a shortening in reaction time between venepuncture and 30

minutes, but all TEG parameters remained stable between 30 and 90 minutes after venepuncture[113]. Zambruni et al extended the time period investigated and demonstrated stability of TEG parameters between 30 and 120 minutes after venepuncture[114]. Both authors demonstrated that repeated sampling of citrated blood led to an increasing trend towards hypercoagulability and therefore demonstrated stability of the citrated blood by collecting a number of samples in different vials at venepuncture and using a different vial for each time point[114,115]. In both of these studies samples were maintained at room temperature as there is strong scientific evidence demonstrating excessive cooling can induce aggregation and irreversibly damage platelets[115].

A number of studies have investigated the comparability of native and recalcified citrated blood and the consensus of the results indicates that there are significant differences in all TEG parameters and therefore specific reference ranges should be used for citrated blood[113,114,116,117].

The methodology employed in this thesis has been defined after an extensive review of the various methods employed in the literature and as a result of a number of in-house validation studies performed and detailed in chapter 3 of this thesis.

Blood Sampling.

Venepuncture was performed with a 21-gauge needle using minimum stasis into two 3ml Sarstedt Monovett bottles, containing 0.3ml 3.2% sodium citrate. The bottles were not pre-vacuumed; a conventional syringe technique was used. The

first sample was discarded to avoid contamination with tissue thromboplastin and the second was used for analysis by thromboelastography.

If venepuncture was performed at a time when additional blood samples were required for antenatal or postnatal care these samples were obtained first via a 21-gauge needle and the 3ml citrated sample was obtained last with the conventional syringe technique.

The blood was maintained at room temperature until analysed by thromboelastography.

Thromboelastography.

All the samples collected for the studies in this thesis were analysed using a TEG® 5000 Haemostasis Analyzer (Haemoscope Corporation, Illinois, USA).

Daily quality assurance checks were performed ensuring the instrument was level using the leveling bubble and that the baselines of each channel were within range. To ensure quality and calibration of the thromboelastograph , every month biological control samples were run, Level I (normal control) and Level II (abnormal control) samples supplied by Haemoscope Corporation. When reconstituted, these controls provide standard tracings which are reproducible. Every six months routine maintenance and calibration was performed by Haemoscope Corporation trained technicians.

Kaolin Activation of Citrated Whole Blood.

Blood samples were maintained at room temperature and analysed by thromboelastography between 30 and 120 minutes after venepuncture. A disposable cup and pin was loaded into each channel, prewarmed to 37°C and 20µl of 0.2M calcium chloride added. Patient details were added to the database. The blood samples were inverted 5 times to ensure mixing of blood components. One ml of blood was pipetted into a room temperature vial containing kaolin, supplied by Haemoscope Corporation, and mixed by inversion 5 times. 340µl of the kaolin activated blood was pipetted into the cup containing the calcium chloride, the cup was elevated to the pin, locked into position and analysis commenced.

Kaolin, also known as hydrated aluminium silicate, is a coagulation activator which acts by stimulating the intrinsic pathway via factor XII.

All samples in this thesis were activated with kaolin and analysed in this way except the samples collected on the 71 women recruited for a longitudinal study of women after normal vaginal delivery which is described in Chapter 8 of this thesis. The samples collected for this study were not activated by kaolin and were performed on native citrated whole blood.

Native Citrated Whole Blood.

The methodology is similar to that outlined above but 340µl of citrated whole blood was added to the cup rather than 340µl of kaolin activated blood. This change in methodology is reiterated in Chapter 8.

Statistical Analysis.

A variety of statistical methods have been employed to analyse the data in this thesis. They are detailed in each chapter of the thesis alongside the precise methodology and design of each study.

The work contained in this thesis was funded by TNO, Netherlands and I am extremely grateful for their support.

CHAPTER 3.

STANDARDISATION AND REPRODUCIBILITY OF THE THROMBOELASTOGRAPH.

Standardisation of automated TEG analyser.

Aims.

The aims of this study are to validate the two channel TEG machine used in the analysis of samples for this thesis and to demonstrate the stability of native and kaolin-activated citrated blood samples between 30 and 120 minutes after venepuncture.

Methods.

a) *Intra-channel variation*:

Two citrated blood samples were obtained at one sitting from each of 20 subjects recruited, 10 nonpregnant subjects and 10 pregnant women. TEG analysis was performed simultaneously on both samples from the same subject on each of the 2 channels of the TEG machine.

The samples obtained from the 10 nonpregnant subjects were analysed according to the native citrated whole blood method described in chapter 2, and the samples obtained from the 10 pregnant women was analysed according to the kaolin activated citrated whole blood method.

b) *Stability of blood samples over time*: Four citrated blood samples were obtained at one sitting from 20 nonpregnant subjects. TEG analysis was performed at 30, 60, 90, and 120 minutes after collection.

The samples obtained were divided randomly into 2 groups of 10. Ten subjects' samples were analysed according to the native citrated whole blood method

described in chapter 2, and the remaining 10 subjects' samples were analysed according to the kaolin activated citrated whole blood method.

Statistical Analysis.

Statistical analysis was performed using MINITAB version 15 (Minitab Inc.,PA, USA).

a) *Intra-channel variation*: The data was analysed by one-way ANOVA and percentage coefficients of variation were generated for each TEG parameter

b) *Stability of blood samples:* A repeated measures analysis of variation was performed and each time interval was compared in pairs by Fisher's least significant difference method.

Results.

a) *Intra-channel variation*: Analysis of variation between the 2 channels showed that there were no significant differences between the channels with p values all greater than 0.05 (Table 3.1). Kaolin activation of citrated blood reduces the percentage coefficient of variation of all four TEG parameters (Table 3.1).

b) *Stability of blood samples over time:* When stability of the samples was studied, all TEG parameters were stable between 30 and 120 minutes for the native and kaolin-activated samples. This is represented by p values of greater than 0.05 at all time points when the data is analysed by repeated measures ANOVA (Table 3.2) and the confidence intervals generated by Fisher's least

significant difference all containing zero for all parameters between 30 and 120 minutes (Table 3.3).

Discussion.

The findings of these two validation studies form the basis of the methodology employed throughout this thesis. The results demonstrate excellent reproducibility of all four TEG parameters on each channel of the TEG machine. The reduction of the percentage coefficient of variation as a result of kaolin activation supports the change in methodology from using native citrated samples to kaolin activation of all citrated samples. These results also indicate that citrated blood remains stable for processing between 30 and 120 minutes post venepuncture. This is an important finding which increases the potential of using this technique as a point of care test in the clinical setting. Based on these findings the standard operating procedure described in Chapter 2 is supported.

	Analysis of Variation between channels 1&2				Coefficient of Variation %	
TEG Parameter	Native		Kaolin		Native	Kaolin
R Time	-2.57- 4.69	p=0.55	-0.53- 0.23	p=0.42	7.7	4.6
K Time	-2.00- 2.04	p=0.98	-0.49- 0.07	p=0.13	10.3	7.8
α angle	-11.52- 11.72	p=0.99	-1.15- 4.81	p=0.21	6.9	1.9
Maximum Amplitude	-5.88- 3.28	p=0.56	-3.16- 6.52	p=0.48	2.1	2.9

Table 3.1. Statistical analysis by ANOVA analysis and Percentage Coefficient of Variation of repeatability between 2-channels of a TEG machine in native citrated and kaolin activated citrated samples

Data shown as 95% Confidence Intervals for pairwise comparisons between channels 1 and 2 in the ANOVA analysis.

	Analysis of Variation between 30, 60, 90, and120 mins	
	Native citrated samples	Kaolin activated citrated samples
R Time	p=0.859	p=0.427
K Time	p=0.986	p=0.488
α Angle	p=0.980	p=0.320
Maximum Amplitude	p=0.983	p=0.994

Table 3.2. Statistical analysis by ANOVA of effect of storage of native citrated samples and kaolin activated samples. Data shown as p values.

	Native citrated samples				Kaolin activated citrated samples			
mins	R Time	K Time	α Angle	MA	R Time	K Time	α Angle	MA
30 vs 60	-3.31- 3.33	-1.51- 1.91	-12.43- 8.20	-6.29- 6.25	-1.78- 0.56	-0.82- 0.28	-2.71- 9.49	-3.02- 3.26
30 vs 90	-2.14- 4.50	-1.25- 2.17	-13.48- 7.15	-5.59- 6.95	-1.48- 0.86	-0.83- 0.27	-2.22- 9.97	-3.11- 3.17
30 vs 120	-1.91- 4.73	-1.64- 1.78	-12.83- 7.80	-5.54- 6.99	-2.10- 0.24	-0.97- 0.13	-0.52- 11.68	-2.75- 3.53
60 vs 90	-2.07- 4.41	-1.41- 1.93	-11.09- 8.99	-5.40- 6.80	-0.87- 1.47	-0.56- 0.54	-5.62- 6.58	-3.23- 3.05
60 vs 120	-1.84- 4.64	-1.80- 1.54	-10.44- 9.64	-5.35- 6.85	-1.49- 0.85	-0.70- 0.40	-3.91- 8.29	-2.87- 3.41
90 vs 120	-3.01- 3.46	-2.06- 1.28	-9.39- 10.69	-6.05- 6.15	-1.79- 0.55	-0.69- 0.41	-4.39- 7.81	-2.78- 3.50

Table 3.3. Statistical analysis of effect of storage of native citrated samples and kaolin activated samples by Fisher's least significant differences. Data shown as 95% Confidence Intervals for pairwise comparisons among sample times.

CHAPTER 4.

A REFERENCE RANGE FOR THROMBOELASTOGRAPHIC PARAMETERS IN NORMAL PREGNANCY.

Introduction.

The use of thromboelastography in obstetrics is in its infancy. There are various methodologies employed for which a variety of "normal ranges" have been adopted. Currently, reference ranges are extrapolated from non-pregnant populations or from pregnant cohorts without consideration to the effect that gestation may have on TEG parameters. In order to use thromboelastography as a technique to explore the hypercoagulable state of pregnancy, it is essential that gestation-specific reference ranges are defined using a standardised methodology.

Aim.

To define gestation specific 95% reference ranges for 4 thromboelastographic (TEG) parameters in uncomplicated pregnancy.

Methods.

This was a prospective cross sectional study of women attending Leicester Royal Infirmary for antenatal care or ultrasound examination. After approval of the protocol by the Leicestershire, Northampton and Rutland Research Ethics Committee, each woman attending was invited to participate if they had a singleton pregnancy with no complications in the index pregnancy and any previous pregnancies had been uncomplicated and resulted in the delivery of a baby weighing more than 2.5kg at more than 37 weeks gestation. Women were not recruited if they had a personal history of hypertension, diabetes, significant past medical history or if they had a personal or family history of venous

thromboembolic disease. Written informed consent was obtained from 245 women to provide a citrated blood sample and examination of their notes after delivery.

Blood sampling and analysis.

Citrated blood samples were obtained and analysis by thromboelastography was performed between 30 and 120 minutes after venepuncture in accordance with the study protocol outlined in chapter 2. All citrated blood samples were activated with kaolin.

The samples were maintained at room temperature. Twenty microlitres of 0.2mol/l of calcium chloride were pipetted into a disposable plastic cup, which had been loaded in a prewarmed thromboelastography® machine. The citrated blood sample was inverted 5 times to ensure mixing of the sample and then 1000µl of citrated blood was pipetted into a room temperature vial containing kaolin. This vial was inverted five times and 340µl of kaolin activated citrated blood was added to the cup.

Four thromboelastographic parameters were analysed, R time, k time, α angle, and Maximum Amplitude.

Statistical Analysis.

The general approach of Altman & Chitty (1994) was followed[136]. Outliers were detected by distributional plots and scatter diagrams. Appropriate transformations of the measures were investigated using the Box-Cox method, with gestational age as a predictor. Power-Normal models were chosen for R, K,

Angle and Maximum Amplitude. These were then fitted using maximum likelihoods[137,138]. The final models fitted were in each case quadratic in the M (median) term, linear in S (Coefficient of variation or standard deviation) and constant in G (shape parameter) (Table 4.1). All analysis was conducted in the statistical package Stata, Version 9.2 (StataCorp, College Station, Texas).

Results.

Two hundred and forty-five women each had a single blood sample taken to establish the reference ranges for the TEG parameters in uncomplicated pregnancy.

Twenty women had abnormal pregnancy outcomes and were excluded from the study. Two women developed pre-eclampsia, (Blood pressure greater than 140/90mmHg on two occasions greater than 4 hours apart and proteinuria ≥0.5g/24 hours), seven women developed non-proteinuric gestational hypertension, (blood pressure greater than 140/90mmHg and proteinuria <0.3g/24 hours), three women delivered at a gestation of less than 36 completed weeks with no other antenatal problem, seven women delivered a baby weighing less than the 10th customised birth weight centile with no coexisting antenatal complication and one women developed obstetric cholestasis at 33 weeks gestation.

Of the 225 women remaining who had an uncomplicated pregnancy, 77 were sampled in the first trimester, 66 in the second and 81 in the third. Their average age was 29 years (range 18-40), 88 were nulliparous and the maximum parity

was three. Ninety-one percent were of Caucasian European origin, 4% Asian, 1.5% Afro-Caribbean and 3.5% mixed ethnic origin.

Gestation specific reference ranges with 3rd, 50th and 97th centiles are shown for R time, K time, angle and maximum amplitude in Fig. 4.1, 4.2, 4.3 and 4.4 respectively and in Table 4.2.

R Time.

The 3rd centile remains stable throughout pregnancy but there is a fall in the 50th and 97th centiles as pregnancy progresses reflecting the shortening of the time taken to initialise clot formation.

K Time.

There is a steady fall in all centiles with advancing gestation. The greatest decrease is evident in the 97th centile. This reflects a shortening of the time taken to reach a defined level of clot firmness and an increased velocity of clot formation as pregnancy progresses.

α Angle.

There is a steady increase in all centiles with advancing gestation, the greatest rise is seen in the 3rd centile. This represents increasing velocity of clot formation as pregnancy progresses.

Maximum Amplitude.

The pattern of change differs with this parameter. All three centiles are stable until 24 weeks. There is then a rapid increase in maximum amplitude with advancing gestation. This reflects increasing firmness of the clot.

Discussion.

Although thromboelastography has been used to investigate changes in coagulation in pregnancy complications such as miscarriage and pre-eclampsia, it has been without the use of a consistently defined reference range. Many authors have used a control population of women presenting at term for elective caesarean section[105,135] or the mean of samples obtained from a population whose gestational age ranged from 6 to 38 weeks[112].

The longitudinal study of thromboelastography in pregnancy described in the next chapter will demonstrate that hypercoagulable changes occur early in pregnancy and are progressive. The significance of these changes will be discussed at length.

This study is the first description of a reference range for TEG parameters in pregnancy from a cross sectional study of a well defined low-risk normal population. The methodology employed in this study, namely kaolin activation of citrated whole blood, is easily reproduced in a clinical setting to provide a point of care assessment. It is immediately evident that the hypercoagulable state is progressive with advancing gestation, thus to employ this technique in further studies of the obstetric population it is essential that this is taken into account.

With this it will now be possible to explore the use of thromboelastography in a variety of pathologies which may have a basis in exaggerated or disordered coagulation.

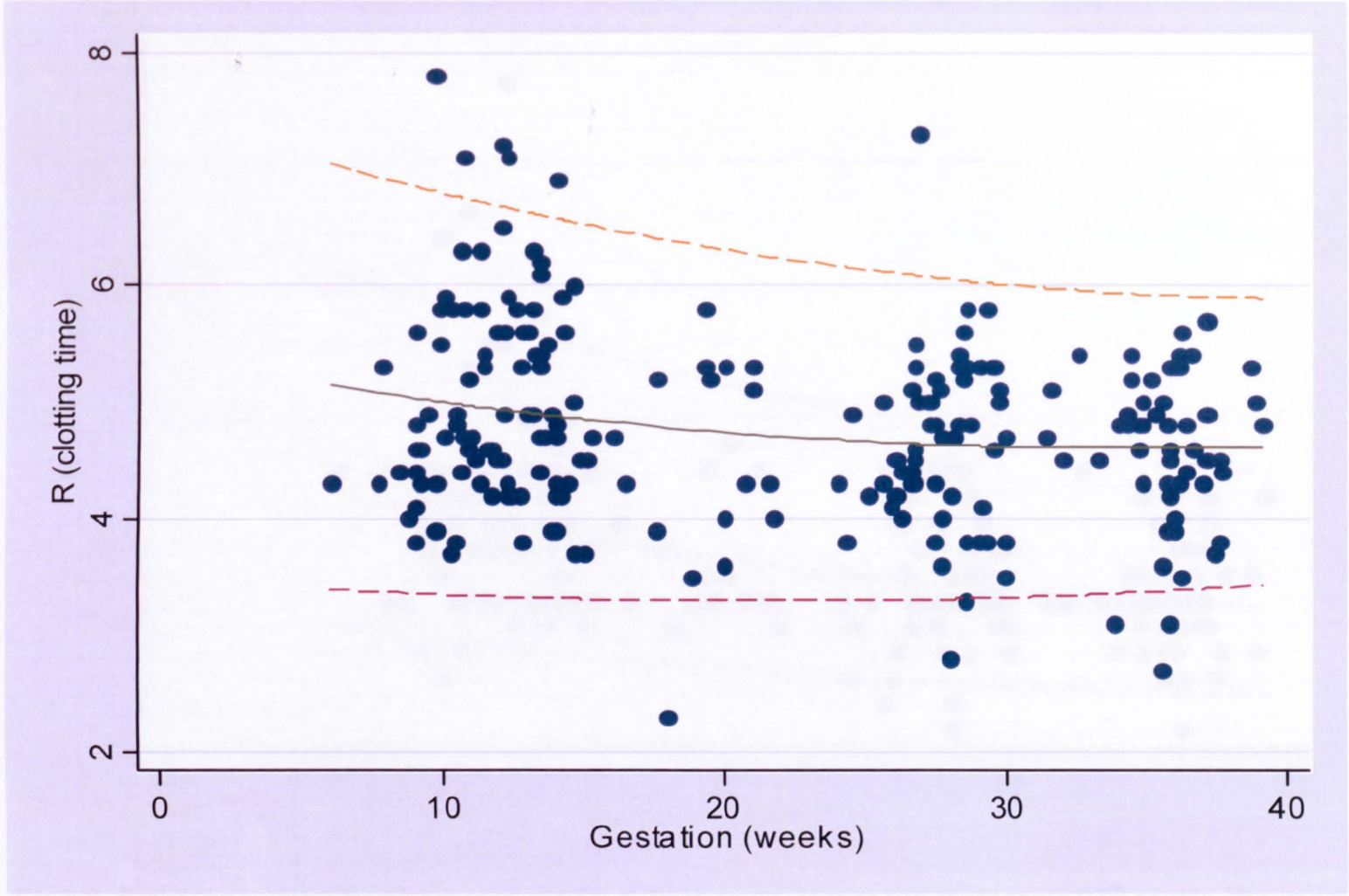

Fig 4.1. Reference range by gestational age for R Time (min). Upper line represents 97th centile, the middle line the 50th and the lower line the 3rd.

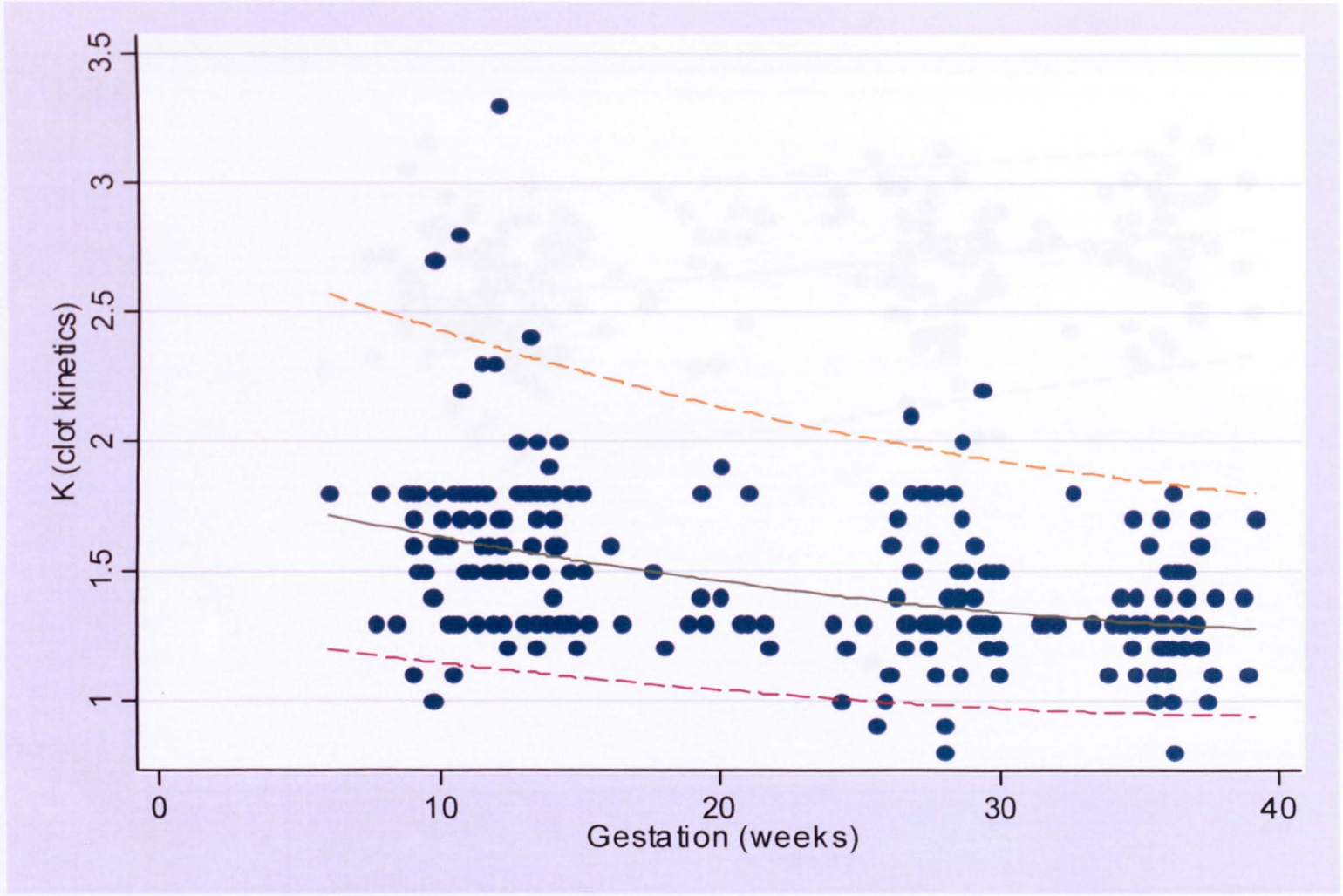

Fig 4.2. Reference range by gestational age for K Time (min). Upper line represents 97th centile, the middle line the 50th and the lower line the 3rd.

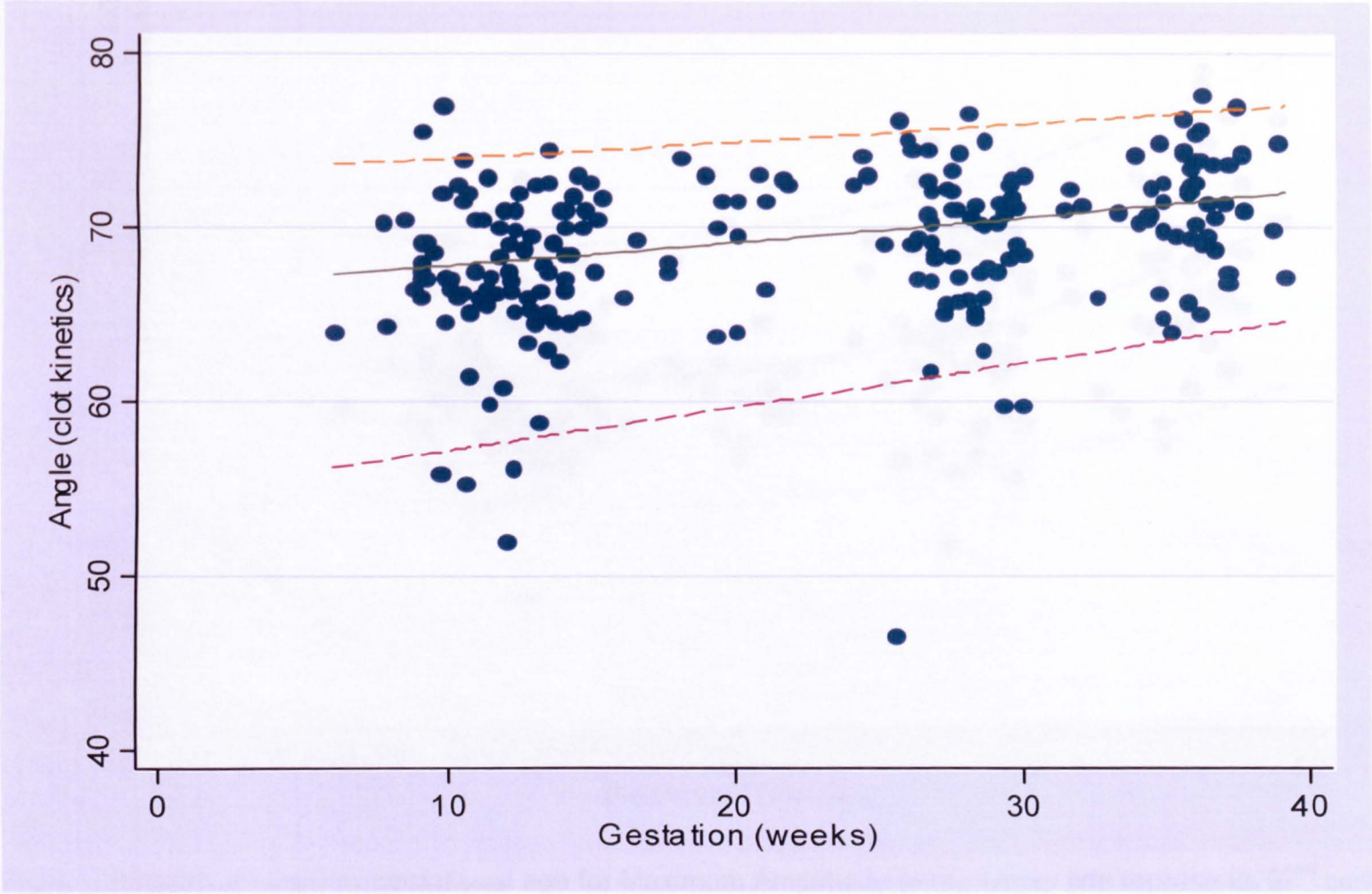

Fig 4.3. Reference range by gestational age for Angle (deg). Upper line represents 97th centile, the middle line the 50th and the lower line the 3rd

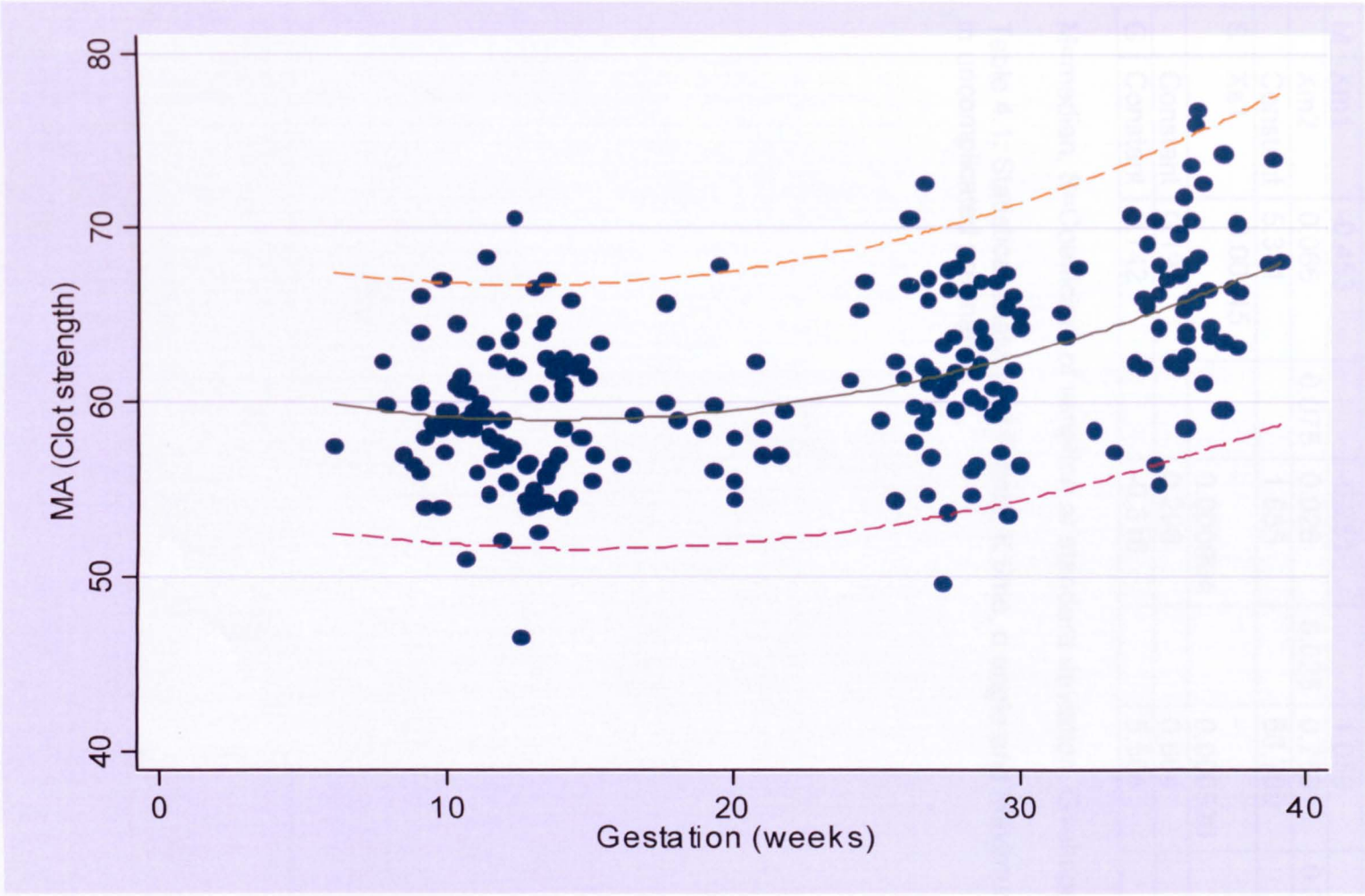

Fig 4.4. Reference range by gestational age for Maximum Amplituide (mm). Upper line represents 97th centile, the middle line the 50th and the lower line the 3rd.

		R Time		K Time		Angle		Maximum Amplitude	
		Coefficient	SE	Coeff	SE	Coeff	SE	Coeff	SE
M	Xm1	-0.453		-0.251		1.059		-3.8.5	
	Xm2	0.066	0.075	0.026	0.025	0.753	0.311	1.391	0.429
	Constant	5.381		1.855		66.700		61.598	
S	Xs1	-0.00145		-0.000904		-0.000526		0.000255	
	Constant	0.197		0.208		0.064		0.646	
G	Constant	0.742		-0.316		5.501		0.630	

M=median, S=Coefficient of variation or standard deviation, G=shape parameter.

Table 4.1. Statistical analysis of R time, K time, α angle and Maximum Amplitude in uncomplicated pregnancy.

	R Time			K Time			Angle			Maximum Amplitude		
Weeks	3rd	50th	97th	3rd	50th	97th	3rd	50th	97th	3rd	50th	97th
10	3.4	5.0	6.8	1.1	1.6	2.4	57.2	67.8	74.0	51.9	59.2	66.8
11	3.3	5.0	6.7	1.1	1.6	2.4	57.4	68.0	74.0	51.8	59.1	66.8
12	3.3	4.9	6.7	1.1	1.6	2.4	57.7	68.1	74.1	51.7	59.0	66.7
13	3.3	4.9	6.6	1.1	1.6	2.3	57.9	68.2	74.2	51.6	59.0	66.7
14	3.3	4.9	6.6	1.1	1.6	2.3	58.2	68.3	74.3	51.6	59.0	66.7
15	3.3	4.9	6.5	1.1	1.5	2.3	58.4	68.5	74.4	51.6	59.0	66.8
16	3.3	4.8	6.5	1.1	1.5	2.2	58.7	68.6	74.5	51.6	59.1	66.9
17	3.3	4.8	6.4	1.1	1.5	2.2	58.9	68.7	74.6	51.7	59.1	67.0
18	3.3	4.8	6.4	1.1	1.5	2.2	59.2	68.9	74.7	51.7	59.3	67.2
19	3.3	4.8	6.3	1.0	1.5	2.2	59.4	69.0	74.8	51.8	59.4	67.3
20	3.3	4.7	6.3	1.0	1.5	2.1	59.7	69.1	74.9	51.9	59.6	67.5
21	3.3	4.7	6.3	1.0	1.4	2.1	59.9	69.3	75.0	52.1	59.7	67.8
22	3.3	4.7	6.2	1.0	1.4	2.1	60.2	69.4	75.1	52.2	60.0	68.1
23	3.3	4.7	6.2	1.0	1.4	2.1	60.4	69.5	75.2	52.4	60.2	68.4
24	3.3	4.7	6.2	1.0	1.4	2.0	60.7	69.7	75.3	52.6	60.5	68.7
25	3.3	4.7	6.1	1.0	1.4	2.0	60.9	69.8	75.4	52.9	60.8	69.1
26	3.3	4.6	6.1	1.0	1.4	2.0	61.2	70.0	75.5	53.1	61.1	69.5
27	3.3	4.6	6.1	1.0	1.4	2.0	61.4	70.1	75.6	53.4	61.5	69.9
28	3.3	4.6	6.1	1.0	1.4	2.0	61.7	70.3	75.7	53.7	61.8	70.4
29	3.3	4.6	6.0	1.0	1.3	1.9	62.0	70.4	75.8	54.0	62.3	70.9
30	3.3	4.6	6.0	1.0	1.3	1.9	62.2	70.6	75.9	54.4	62.7	71.4
31	3.3	4.6	6.0	1.0	1.3	1.9	62.5	70.7	76.1	54.8	63.2	72.0
32	3.3	4.6	6.0	1.0	1.3	1.9	62.7	70.9	76.2	55.2	63.7	72.6
33	3.4	4.6	6.0	1.0	1.3	1.9	63.0	71.0	76.3	55.6	64.2	73.2
34	3.4	4.6	5.9	1.0	1.3	1.9	63.2	71.2	76.4	56.0	64.7	73.9
35	3.4	4.6	5.9	0.9	1.3	1.8	63.5	71.3	76.5	56.5	65.3	74.6
36	3.4	4.6	5.9	0.9	1.3	1.8	63.8	71.5	76.6	57.0	65.9	75.3
37	3.4	4.6	5.9	0.9	1.3	1.8	64.0	71.6	76.8	57.5	66.6	76.1
38	3.4	4.6	5.9	0.9	1.3	1.8	64.3	71.8	76.9	58.1	67.2	76.9
39	3.4	4.6	5.9	0.9	1.3	1.8	64.5	72.0	77.0	58.6	67.9	77.7

Table 4.2. The gestation specific 3rd, 50th and 97th centiles for R time, K time, α angle and Maximum Amplitude.

CHAPTER 5

A LONGITUDINAL STUDY OF HAEMOSTATIC FUNCTION IN WOMEN DURING PREGNANCY: a) NORMAL PREGNANCY

Introduction.

The hypercoagulable state of pregnancy has been extensively investigated by many authors. Most of these studies however, focus on individual aspects of the haemostatic system such as the increasing levels of coagulation factors[13], changes in the natural anticoagulant system including Proteins C and S[18,32,35,36] and inhibition of the fibrinolytic pathways[13,38,39,44-46,47]. The net global effects of the changes are merely hypothesised rather than demonstrated.

The changes that occur to the haemostatic mechanisms in pregnancy have been implicated in the development of maternal and fetal disorders such as thrombosis[59,61,69] pre-eclampsia[22,23] and growth retardation[54]. An improved understanding of this system may elucidate the pathophysiology involved in these conditions and then may lead to the development of therapeutic interventions.

Aims.

The aims of this study are to explore the sequential changes in global blood coagulation in pregnancy as measured by thromboelastography in a tightly defined low risk population and to investigate the potential that the technique may offer in identifying women at risk of adverse pregnancy outcomes.

Methods.

Approval of the study protocol was obtained from the Leicestershire, Northampton and Rutland Research Ethics Committee. This was a prospective

longitudinal study of women attending Leicester Royal Infirmary for pregnancy care. They were recruited when they first attended for a booking ultrasound in the first trimester of pregnancy. The hand-held antenatal records were inspected and those who met the recruitment criteria as detailed in table 5.1 were invited to participate in the study. Written informed consent was obtained from 284 women for serial blood sampling, on three occasions in pregnancy and once in the postnatal period and examination of their notes after delivery. Individual customised birth weight centiles were generated following each delivery using software from the Perinatal Institute (www.gestation.net/birthweight_centiles).

All women completed a questionnaire at recruitment in order to elicit relevant past medical, past obstetric and family histories and to gain demographic data including ethnicity, age, body mass index and current medications such as folic acid and iron supplements.

A nonpregnant control group of 108 women was also recruited. These women were healthy volunteers, between 18 and 41 years of age who had no personal or family history of thromboembolism, were not taking any regular medication or the combined oral contraceptive pill. A single blood sample was taken from this group.

Blood sampling and analysis.

Blood samples were obtained in accordance with the study protocol outlined in chapter 2. Analysis by thromboelastography was performed between 30 and 120 minutes after venepuncture. The samples were maintained at room temperature. Twenty microlitres of 0.2mol/l of calcium chloride were pipetted into a disposable

plastic cup, which had been loaded in a prewarmed thromboelastography® machine. The citrated blood sample was inverted 5 times to ensure mixing of the sample and then 1000µl of citrated blood was pipetted into a room temperature vial containing kaolin. This vial was inverted five times and 340µl of kaolin activated citrated blood was added to the cup.

Six thromboelastographic parameters were analysed, R time, k time, α angle, Maximum Amplitude, coagulation index and LY30.

Statistical Analysis.

Statistical analysis was performed using MINITAB version 15 (Minitab Inc.,PA, USA). The data was tested for normality and the 1 sample t-test was used to generate means and 95% confidence intervals. The 2 sample t-test was the performed to compare the individual parameters at different time points. *P*-values of <0.05 were taken as statistically significant.

Results.

Two hundred and eighty-four women were recruited to the study and a blood sample was obtained at recruitment. All 3 antenatal blood samples were obtained from 219 women. Thirty seven women provided 2 antenatal blood samples and 28 women provided 1 blood sample. Of the 28 women from whom only 1 sample was obtained 8 women chose to withdraw from the study, 7 women were unable to be contacted, 5 women did not attend 2 or more appointments, 2 women developed mental health problems which required medication and therefore

discontinued the study, 3 women moved away from the area, 1 women had a miscarriage at 14 weeks, another at 21 weeks and one women terminated the pregnancy due to trisomy 21. Of the 37 women from whom 2 samples were obtained 9 of these delivered prematurely, the remaining women failed to attend two appointments. Postnatal samples were obtained from 161 women (mean 30 weeks postpartum, range 8-58 weeks). At the time of recall 4 women were pregnant again. A number of women failed to respond to 2 invitations to attend the hospital, 12 had moved away from the area and a greater number were unable to be contacted at the addresses they had been discharged from hospital to.

Completed questionnaires were obtained from 253 women.

Demographic Characteristics.

The ethnic origin of the subjects was recorded, 89.8% were of European Caucasian origin, 4.5% were of Indo-Asian origin, 1.5% Afro-Caribbean origin, 0.4% non-Caucasian European origin and 3.8% were of mixed ethnic origin. The mean age of the subjects was 28.8 years (range 18-41 years). The mean booking body mass index was 23.7 (range 15-42). One hundred and twenty-two subjects were nulliparous, 115 had a parity of one, 35 had a parity of two, 7 had a parity of 3 and parity was not recorded in 5 subjects. The smoking status of subjects prior to and during the index pregnancy was recorded. Of the 249 women who answered, 31.7% of subjects smoked before pregnancy, reducing to 12.6% during pregnancy. Folic acid supplements were taken by 92.4% of the

249 subjects who responded to this question. None of the subjects had a personal or family history of venous thromboembolic disease. (Table 5.1)

Pregnancy Outcomes.

Of the 284 women recruited to the study 232 (81.7%) had a normal antenatal course and delivered a baby after 36 completed weeks of pregnancy that was of normal birth weight. There were a total of 52 women with adverse outcomes who were excluded from the longitudinal analysis of normal pregnancy. Detailed analysis of this group is presented in the second part of this chapter.

Nineteen women delivered a baby weighing less than the 10^{th} customised birth weight centile with no coexisting antenatal complication.

Fifteen women developed non-proteinuric gestational hypertension, (blood pressure greater than 140mmHg systolic and/or 90mmHg diastolic and proteinuria <0.3g/24 hours) all of these delivered at term, 4 delivered babies weighing less than the 10^{th} customised birthweight centile.

Eight women developed pre-eclampsia, (Blood pressure greater than 140/90mmHg on two occasions greater than 4 hours apart and proteinuria ≥0.5g/24 hours) 5 of these women delivered preterm and 6 of the 8 babies weighed less than the 10^{th} customised birthweight centile.

Five women delivered spontaneously at a gestation of less than 37 completed weeks with no other antenatal problem.

One women had a miscarriage at 15 weeks gestation, another women terminated the pregnancy at 18 weeks due to Trisomy 21, one women developed obstetric cholestasis at 34 weeks gestation, one women had an intrapartum

placental abruption and one women delivered a baby at 39 weeks gestation who required admission to the neonatal unit with respiratory distress syndrome.

Longitudinal Study: Normal outcomes.

After exclusion of the women who had adverse pregnancy outcomes, 232 women were included in the analysis.

R Time.

The R time is significantly reduced in the first trimester of pregnancy compared with the nonpregnant female control population (4.91 min vs 6.03 min $p<0.0001$) (Table 5.2, Fig. 5.1). The R time reduces further in the second trimester (4.55 min vs 4.91 min $p<0.0001$) and there is no significant change between the 2^{nd} and 3^{rd} trimesters. In the postpartum samples the R time resolves to the nonpregnant level.

K Time.

The K time is significantly reduced in the first trimester of pregnancy compared with the nonpregnant female control population (1.88 min vs 1.66 min $p<0.0001$) (Table 5.2, Fig. 5.2). There is a further reduction in the second trimester (1.66 min vs 1.38 min $p<0.0001$). There is no further change as pregnancy progresses and the resolution to nonpregnant levels is apparent in the postpartum samples.

α Angle.

A dramatic increase in α angle is apparent in the first trimester compared with the nonpregnant female control population (66.91° vs 63.58° $p<0.0001$) and there is a further increase in the second trimester (69.81° vs 66.91° $p<0.0001$) (Table 5.2, Fig. 5.3). There is no further change as pregnancy progresses and the resolution to nonpregnant levels is apparent in the postpartum samples.

Maximum Amplitude.

In the first trimester the maximum amplitude does not differ from the nonpregnant population (58.47mm vs 57.25mm) (Table 5.2, Fig. 5.4). In the second trimester the maximum amplitude is significantly larger than in the first trimester (60.65mm vs 58.47mm $p<0.005$) and this is followed by an even greater increase in the third trimester (64.86mm vs 60.65mm $p<0.0001$). This is followed by resolution to nonpregnant levels in the postpartum samples.

Coagulation Index.

The coagulation index is increased in the first trimester compared to nonpregnant values (0.54 vs -0.59 $p<0.0001$) (Table 5.2, Fig. 5.5) and continues to increase progressively throughout pregnancy peaking in the third trimester.

LY30.

The LY30 is decreased in the first trimester compared with nonpregnant values (3.11% vs 5.23% $p<0.0001$) (Table 5.2, Fig. 5.6) but then remains stable throughout pregnancy with no further changes.

6-9 weeks gestation.

On further analysis of 22 women who were recruited early in pregnancy between 6 and 9 weeks gestation significant changes are observed in four parameters compared to non pregnant controls. R and K times are reduced ($p<0.0001$ and $p=0.001$), α angle is increased ($p<0.0001$) and LY30 is reduced ($p<0.0001$).

Discussion.

Although there have been many previous studies observing and quantifying variations in the individual components of the haemostatic system in pregnancy this study, using thromboelastography, demonstrates the global effect of these changes on coagulation. Highly significant changes have occurred by 6-9 weeks gestation with a reduction in the time taken to initialise clot formation (R time), increasing velocity of clot formation (α angle) and reduction in the lysis of the formed clot (LY30). There is very little published data concerning this very early stage of pregnancy, Cernecea et al report an increase in fibrinogen levels in the 10^{th} week of pregnancy, together with a 40% reduction in protein S activity compared to nonpregnant controls in 117 healthy pregnant women[32]. These isolated findings provide some information suggesting that hypercoagulable

changes have begun but these are only two components of the complex haemostatic mechanism and conclusions cannot be drawn. In contrast Fletcher et al studied fibrinogen levels in 18 women between 6-10 weeks of pregnancy but failed to show any increase until 16 weeks of pregnancy[14]. Stirling et al conducted what is generally accepted as being the most extensive longitudinal study of haemostasis in pregnancy[13]. In her study the earliest sampling occurred between 11-14 weeks at which time she suggests a decrease in fibrinolytic activity has occurred, however she has extrapolated pre-pregnancy levels from postpartum data on which to base this conclusion.

Such extensive changes in TEG parameters in the early first trimester demonstrate that significant hypercoagulable changes have already occurred in the low risk population studied which has implications in the management of women at increased risk of thromboembolism. James et al have performed a prospective study of women with a confirmed deep vein thrombosis during pregnancy and the puerperium[118]. Forty-four percent of the antenatal DVTs occurred at less than 14 weeks gestation. This finding has been replicated in a retrospective study by Gherman et al with 50% of the antenatal cases of DVT detected before 15 weeks gestation[71]. The results presented in this chapter demonstrate that the hypercoagulable changes occur at a very early stage of pregnancy, 6-9 weeks gestation, which suggest that if thromboprophylaxis is indicated in pregnancy this should be started as soon as the pregnancy is diagnosed.

The changes in the TEG parameters that occur in the 2nd and 3rd trimesters demonstrate a further evolution of a progressively more hypercoagulable state. A point of interest is that each parameter undergoes its most significant changes at different stages of pregnancy. The time to initialise clot formation (R Time) undergoes its most significant reduction in the 1st and 2nd trimesters with no further change in the 3rd trimester. The α angle representing the velocity of clot formation follows a similar pattern, increasing in the 1st and 2nd trimesters and with no further increase in the 3rd trimester. In contrast the maximum amplitude which represents the strength of the formed clot does not change in the 1st trimester. In the 2nd trimester the maximum amplitude increases but it is not until the 3rd trimester that the most significant increase occurs. Clot lysis as demonstrated by LY30, decreases in the first trimester and then remains stable throughout pregnancy. It is difficult to relate these changes exactly to the published data on levels of individual clotting factors and components of the fibrinolytic pathway but trends are echoed. Cernecea et al observed the most significant increase in procoagulant factors occurred up to 20 weeks gestation and from the 30th week onwards they remained stable until delivery[32], this is reflected in the changes that occur in R time and α angle. Inhibition of clot lysis is attributed to a number of factors, the reduction in Protein S which occurs in the first trimester[32], an acquired resistance to protein C[35,36] and a decrease in fibrinolysis. A significant fall in fibrinolytic activity has been demonstrated at 11-15 weeks gestation[13] and continues with the lowest values in the 3rd trimester[13,38]. Cernecea et al demonstrated an increase in PAI-1 levels from 31

weeks gestation, rising to term and levels of another inhibitor thrombin activatable fibrinolytic inhibitor increase through pregnancy, peaking at 35-39 weeks gestation[32]. These changes offer some explanation for the increase in maximum clot strength that occurs between the 2nd and 3rd trimesters.

Implantation of a fertilised egg into the endometrium is a time associated haemorrhage and subsequent miscarriage. I would hypothesis that the shortening of the time to initialise clot formation is a natural adaptation to maximise the stability of the implanting pregnancy. As parturition approaches the coagulation system has evolved to develop clots that develop rapidly but also have increased strength and stability to cope with the challenge of placental separation. These physiological adaptations are an essential part of normal pregnancy.

Inclusion Criteria	Exclusion Criteria
• Primigravida or multigravida with normal outcome to previous pregnancies • Normotensive at booking • Singleton pregnancy	• Age <18 and >41 years • Personal or family history of thromboembolism • Previous delivery <37 weeks • Previous delivery of SGA baby <2500g • Previous history of pre-eclampsia • Diabetes • Previous gestational diabetes • Essential hypertension • Taking regular medication other than folic acid and iron supplements.

Table 5.1. Recruitment criteria.

		R min mean	R min 95% CI	K min mean	K min 95% CI	Angle deg mean	Angle deg 95% CI	MA mm mean	MA mm 95% CI	Coag Index mean	Coag Index 95% CI	LY30 % mean	LY30% 95% CI
Control	N=108	6.03	5.84-6.21	1.93	1.85-2.01	63.56	62.46-64.65	57.25	56.15-58.34	-0.59	-0.89--0.30	5.23	4.30-6.16
Weeks 6-9	N=22	4.77[a]	4.38-5.15	1.62[b]	1.47-1.77	67.93[a]	66.07-69.79	59.19	57.71-60.67	0.9[a]	0.38-1.42	3.4	1.76-5.04
Trimester													
1	N=177	4.91[c]	4.77-5.05	1.66[c]	1.61-1.72	66.91[c]	66.20-67.61	58.47	57.55-59.39	0.54[c]	0.29-0.79	3.11[c]	2.71-3.51
2	N=179	4.55[d]	4.39-4.69	1.38[d]	1.34-1.43	69.81[d]	69.09-70.52	60.65[e]	59.56-61.74	1.49[d]	1.32-1.67	3.97	2.46-5.49
3	N=248	4.73	4.53-4.92	1.41	1.34-1.49	70.30	69.43-71.15	64.86[f]	64.09-65.63	1.88[g]	1.66-2.11	3.22	2.16-4.28
Postnatal	N=128	5.96[h]	5.79-6.14	1.88[h]	1.78-1.98	63.72[h]	62.74-64.70	56.87[h]	55.30-58.44	-0.51[h]	-0.78--0.25	4.86[i]	4.02-5.70

Table 5.2. TEG parameters for 232 women with normal pregnancy outcomes.

The above data has been analysed by a series of t-tests described as follows:
Control vs Weeks 6-9: a=$p<0.0001$; b=$p<0.005$
Control vs Trimester 1: c=$p<0.0001$
Trimester 1 vs Trimester 2: d= $p<0.0001$; e=$p<0.005$
Trimester 2 vs Trimester 3: f=$p<0.0001$; g=$p<0.05$
Trimester 3 vs Postnatal: h=$p<0.0001$; i=$p<0.005$

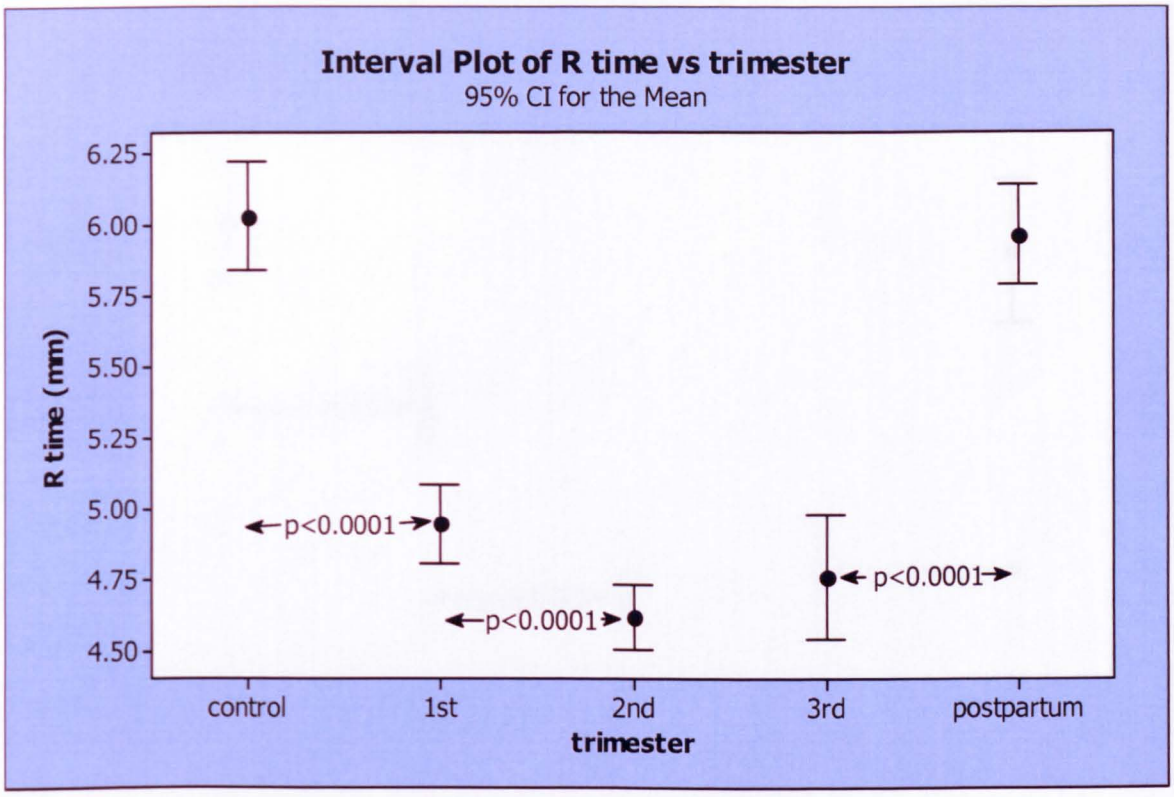

Fig. 5.1 Interval plot of R time vs control population, 1st 2nd and 3rd trimesters and postpartum in uncomplicated pregnancy population

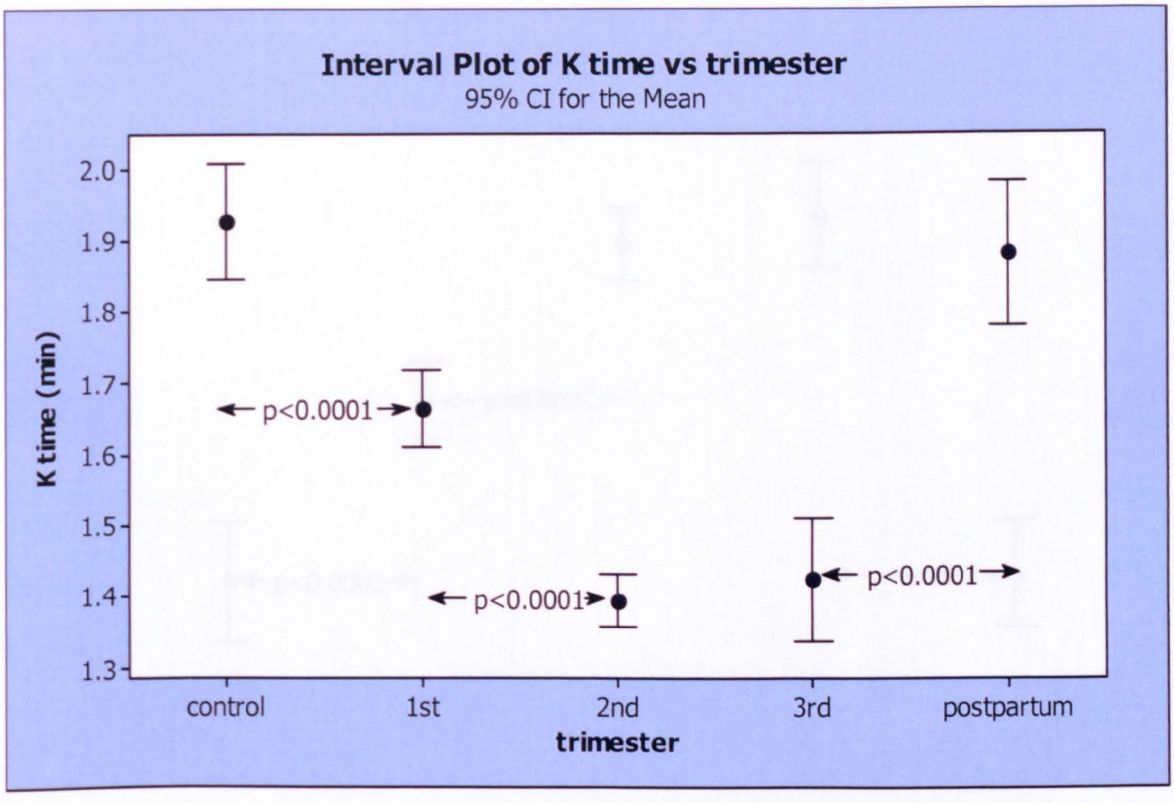

Fig. 5.2 Interval plot of K time vs control population, 1st 2nd and 3rd trimesters and postpartum in uncomplicated pregnancy population

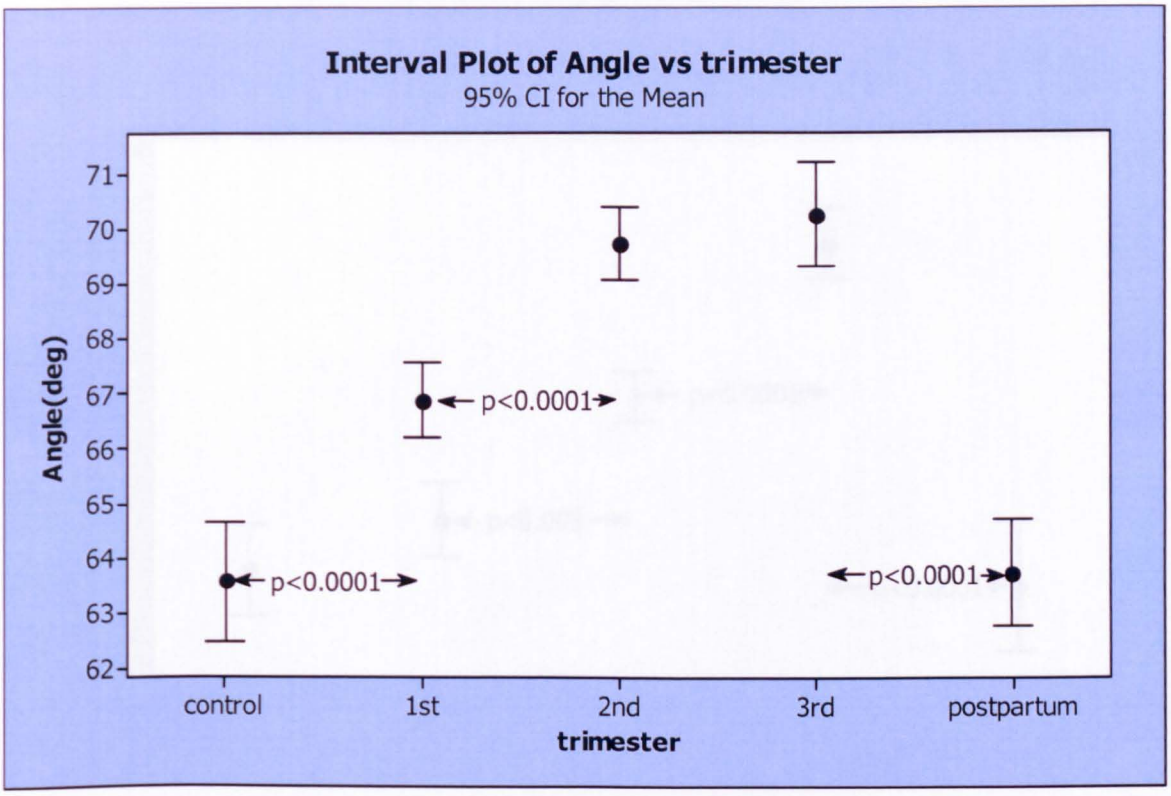

Fig. 5.3 Interval plot of α angle vs control population, 1st 2nd and 3rd trimesters and postpartum in uncomplicated pregnancy population

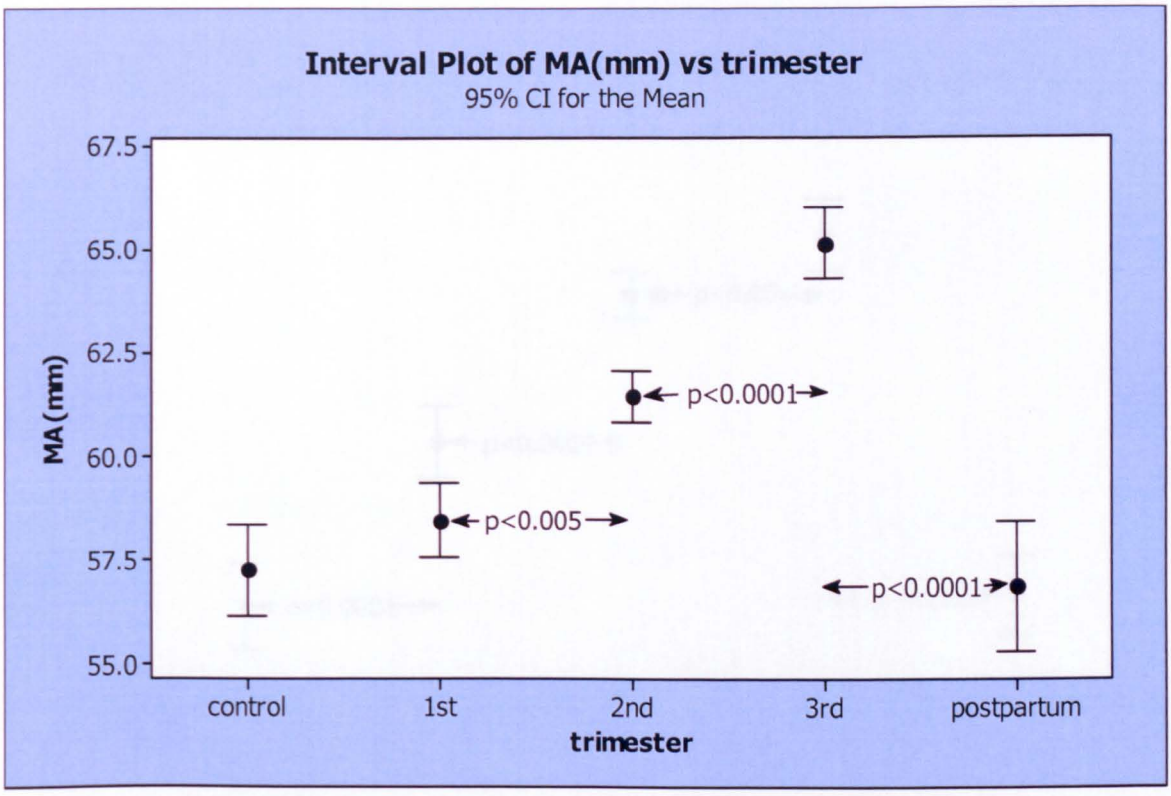

Fig. 5.4 Interval plot of Maximum Amplitude vs control population, 1st 2nd and 3rd trimesters and postpartum in uncomplicated pregnancy population

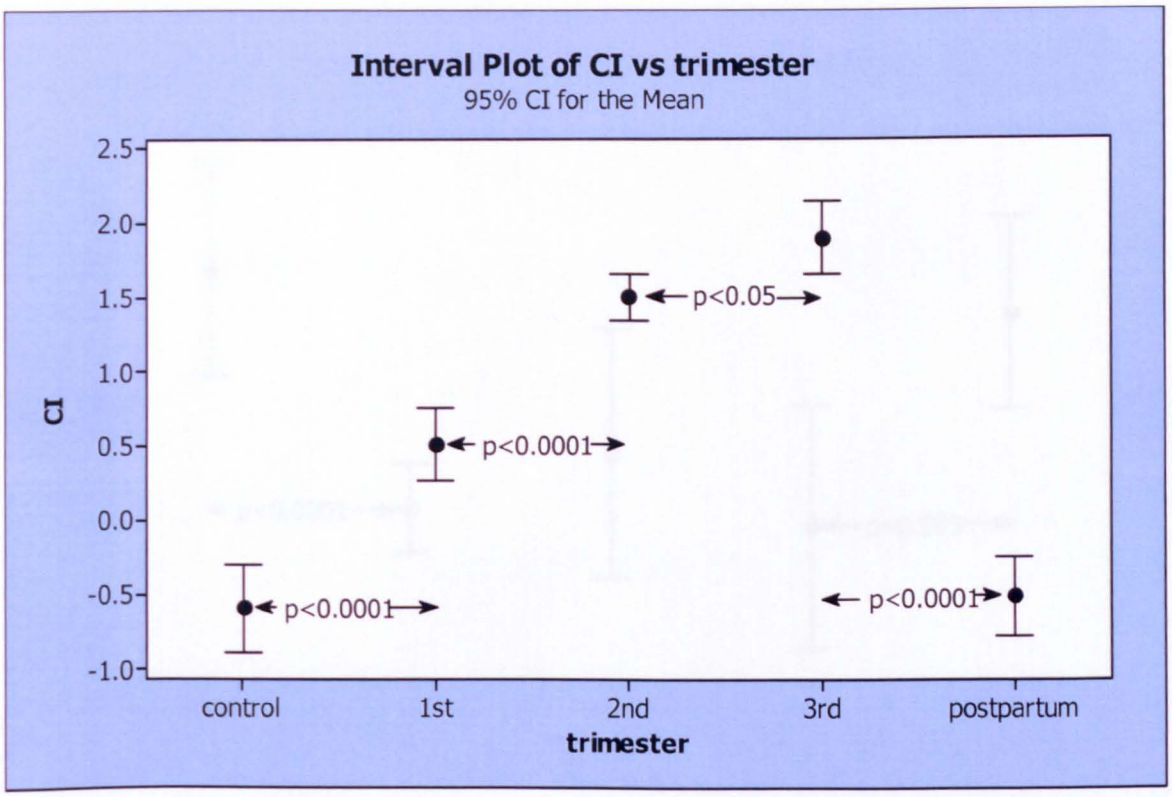

Fig. 5.5 Interval plot of Coagulation Index vs control population, 1st 2nd and 3rd trimesters and postpartum in uncomplicated pregnancy population

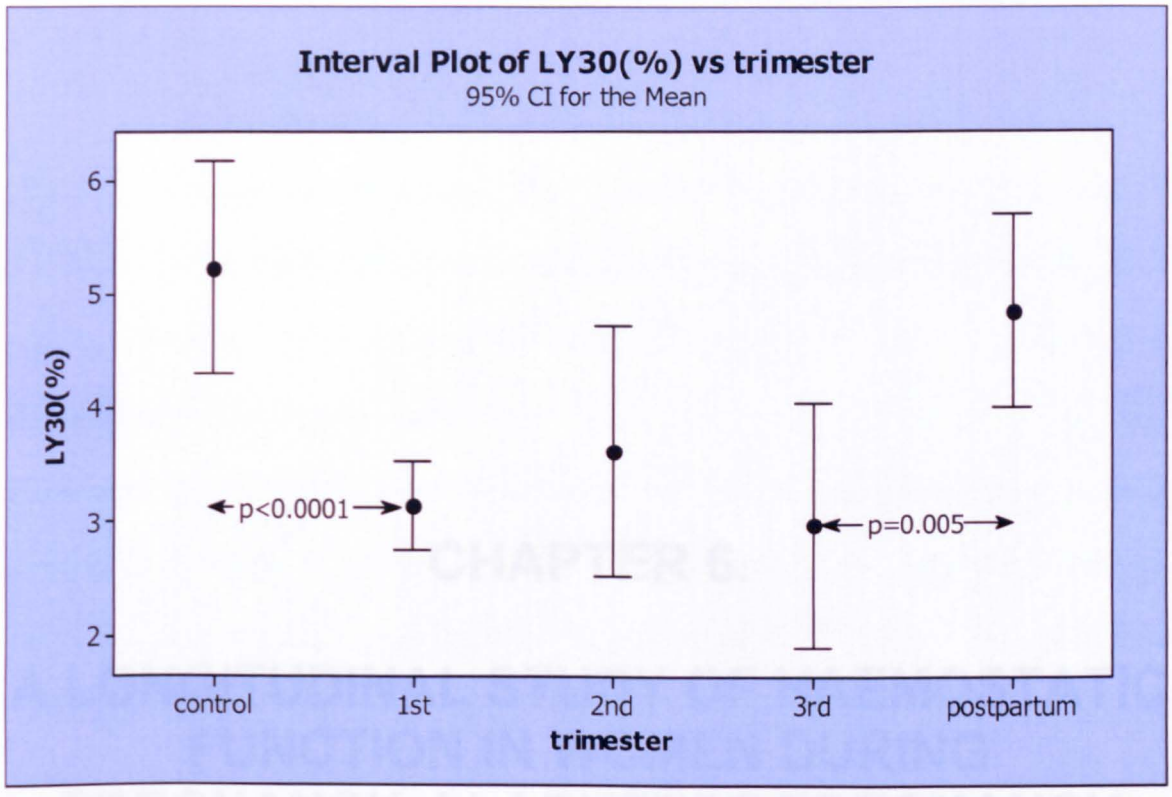

Fig. 5.6 Interval plot of LY30 vs control population, 1st 2nd and 3rd trimesters and postpartum in uncomplicated pregnancy population

CHAPTER 6.

A LONGITUDINAL STUDY OF HAEMOSTATIC FUNCTION IN WOMEN DURING PREGNANCY: b) ADVERSE PREGNANCY OUTCOME.

Introduction.

The haemostatic system is a focus of investigation in the pathophysiology of many pregnancy complications. The possible contribution of thrombophilic mutations in the development of severe pre-eclampsia, placental abruption, fetal growth restriction and stillbirth[78-81] has highlighted the role of haemostatic disturbance in abnormal pregnancy.

Many studies have described an exaggeration of the hypercoagulable state in pregnancy-induced hypertension and pre-eclampsia by exploring various components of the haemostatic mechanism[49,119,120]. In pre-eclampsia microthrombi formation and excess fibrin deposition affects multiple maternal organs, including the placenta, and contributes to the multisystem dysfunction that characterises the clinical syndrome[121,122,123].

Increased levels of markers of thrombin activity including TAT complex[22,23,124], D-dimers[124,125] and prothrombin fragments 1+2[22,125] in pregnancies complicated by pre-eclampsia are widely described.

Increased levels of t-PA antigen have also been reported in pre-eclampsia[40,126,127]. This is likely to be a compensatory response to the increased intravascular coagulation occurring. In addition, endothelial cells produce t-PA and injury of these cells has been implicated in the disease process of pre-eclampsia[128,129]. There are changes in fibrinolytic mechanisms with elevated levels of PAI-1 [40,130], and a reduction in PAI-2 levels in established disease which is thought to reflect the placental insufficiency associated with pre-eclampsia and PIH [40,130-132].

In the natural anticoagulant system, there is a reduction in antithrombin III levels [40,132,133] due to the intravascular consumption during thrombin mediated fibrin generation.

Thromboelastography has been used to study possible coagulation changes in women with established pre-eclampsia. In established disease a hypocoagulable state resulting from thrombocytopenia is the most common coagulation disorder but there is also much evidence demonstrating changes in platelet function. Excessive platelet activation by dysfunctional endothelium, as a result of abnormal nitric oxide, prostaglandin and endothelin release and metabolism, accounts for increased platelet turnover, and ultimately, reduced numbers Whigham et al noted that women with both mild and severe pre-eclampsia had disordered platelet function having undergone aggregation and disaggregation despite normal platelet counts[134].

Sharma et al have used thromboelastography to investigate mild and severe pre-eclampsia[135]. They described an increased MA in patients with mild pre-eclampsia and in more severe disease with platelet counts <100,000mm^3 all TEG parameters became hypocoagulable.

Thromboelastography has been compared to bleeding time in patients with pre-eclampsia to guide the administration of regional anaesthesia. Orlikowski et al demonstrated normal TEG parameters in women with pre-eclampsia and platelet counts >100,000mm^3 despite twenty percent of the cohort having prolonged bleeding times[105]. Although thromboelastography offers the advantage of using whole blood and thus including the role of platelets in the coagulation process, it

is not possible to replicate the interaction that occurs *in vivo* between damaged vascular endothelium and platelets which is an important process in the pathology of pre-eclampsia.

Aims.

To investigate the hypothesis that women who develop complications in pregnancy may demonstrate differences in TEG parameters before clinical disease is apparent, specifically an exaggeration of the hypercoagulable state.

To investigate the effect of defined demographic characteristics on pregnancy outcome.

Methods.

This chapter represents a detailed analysis of the women recruited to the longitudinal study of haemostatic function in pregnancy who had adverse pregnancy outcomes. The methodology detailing recruitment criteria, blood sampling and analysis has been described in chapter 5.

Statistical Analysis.

Statistical analysis was performed using MINITAB version 15 (Minitab Inc.,PA, USA). The relationships between demographic characteristics and pregnancy outcome were analysed by Chi-Square tests using Pearson Chi Square and Fisher's exact test with a $p<0.05$ judged to demonstrate significance. The

influence of maternal age, body mass index (BMI) at booking, parity, smoking status and taking of folic acid on pregnancy outcome were analysed.

The data of the TEG parameters was tested for normality and the 1 sample t-test was used to generate means and 95% confidence intervals. Generalised Linear Model ANOVA analysis with Tukey's pairwise comparisons were used to compare the women in the three major adverse outcome groups, pre-eclampsia, gestational hypertension and birth weight less that 10^{th} centile, with the 232 women who had a normal pregnancy outcome.

Results.

Of the 284 women enrolled in the study, 42 developed a complication in their pregnancy which is widely considered to be "placental" in origin, namely gestational hypertension, pre-eclampsia and delivery of a low birth weight infant. Nineteen women delivered a baby weighing less than the 10^{th} customised birth weight centile with no coexisting antenatal complication.

Fifteen women developed gestational hypertension, (blood pressure greater than 140mmHg systolic and/or 90mmHg diastolic with proteinuria<0.3g/24 hours) all of these delivered at term (more than 37 weeks gestation), 4 delivered babies weighing less than the 10^{th} customised birthweight centile.

Eight women developed pre-eclampsia, (blood pressure greater than 140/90mmHg and proteinuria >0.5g/24 hours) 5 of these women delivered preterm and 6 of the 8 babies weighed less than the 10^{th} centile.

Five women had spontaneous preterm deliveries at a gestation of less than 36 completed weeks with no other antenatal problem and were not part of this analysis due to the great diversity of aetiological factors involved in spontaneous preterm labour.

The number of adverse outcomes was small but representative of the low risk population that was recruited to the study.

Demographic characteristics.

The demographic characteristics for the women in the adverse outcome groups and the low-risk control group are described in table 6.1. Questionnaires were completed from 253 women. In addition some missing information was available on review of the maternity notes and pregnancy outcome data was available for all women.

Analysis by Pearson Chi-Square test and Fisher's exact test demonstrated that development of pre-eclampsia was associated with a higher body mass index at booking ($p<0.05$) and smoking during pregnancy ($p<0.05$). Smoking during pregnancy was also associated with delivery of a baby $<10^{th}$ birthweight centile ($p<0.05$) (Table 6.1).

Pre-eclampsia.

There were no differences in the TEG parameters between the women who developed pre-eclampsia and the women who had a normal pregnancy outcome in the first, second or third trimesters (Table 6.2).

Gestational Hypertension.

There were no differences in the TEG parameters between the women who developed gestational hypertension and the women who had a normal pregnancy outcome in the first, second or third trimesters (Table 6.3).

Birthweight <10th individualised birthweight centile.

There were no differences in the TEG parameters between the women who delivered a baby weighing less than the 10th customised birth weight centile and the women who had a normal pregnancy outcome in the first or third trimesters (Table 6.4). In the 2nd trimester the maximum amplitude was greater in the group that delivered a baby weighing less that the 10th centile (MA 65.31mm vs 61.46mm $p<0.05$) compared to the women with a normal pregnancy outcome.

Postnatal Samples.

There were no differences in the TEG parameters of women in the three adverse outcome groups and the women with normal pregnancy outcomes when the women were sampled in the postnatal period. All samples were obtained more than 12 weeks after delivery.

Discussion.

This study did not demonstrate any changes in thromboealstographic parameters for women affected by pre-eclampsia or gestational hypertension and I would propose a number of reasons for this. Firstly the numbers of women who developed these complications were small, 8 women developed pre-ecalmpsia and 15 gestational hypertension. Two women developed severe pre-eclampsia at an early stage of pregnancy and required delivery at 29 and 31 weeks gestation. These two women were therefore not sampled in the third trimester thus reducing the numbers further. The average gestational age at delivery of the women affected by gestational hypertension was 39.9 weeks which suggests that the disease was mild in the majority of those affected. The studies that have described TEG changes in pre-eclampsia have all studied women with established disease whereas, in this study all women were sampled before pre-eclampsia was clinically detectable. In this study women were sampled at three intervals in pregnancy and it is likely that this sampling frequency is insufficient to detect changes. The time between obtaining a blood sample and development of clinically detectable disease was often many weeks. Pre-eclampsia is often fulminant and progresses rapidly. These cases are missed by routine antenatal care as well[138].

Further studies are warranted on a population at risk of pre-eclampsia to evaluate fully any changes in TEG parameters and to explore further the potential of changes in TEG parameters prior to the development of clinically detectable disease.

	Low-Risk Controls n=232		Pre-eclampsia n=8		Gestational BP n=15		Birth Weight <10th Centile n=19	
	mean	**range**	**mean**	**range**	**mean**	**range**	**mean**	**range**
Age (yrs)	28.8	18-41	26.0	19-35	30.1	20-36	29.2	22-36
	n	**%**	**n**	**%**	**n**	**%**	**n**	**%**
Parity: P0	90	38.8	6	75.0	10	66.6	11	57.9
P1	101	43.5	2	25.0	3	20.0	7	36.8
P2	28	12.1			2	13.3		
P3	5	2.2						
Smoking before pregnancy	60	29.4	5	62.5	5	33.3	7	36.8
Smoking during pregnancy	19	9.4	4*	50.0	3	20.0	4*	21.1
Folic Acid 1st trimester	188	81.0	8	100.0	13	86.7	17	89.5
	mean	**SD**	**mean**	**SD**	**mean**	**SD**	**mean**	**SD**
BMI at booking	23.7	4.11	29.1*	5.82	23.7	5.03	24.4	3.57
Gestational Age at delivery (wks)	39.59	1.26	35.25	4.4	39.9	1.17	37.23	3.99
Birth Weight (grams)	3486	471	2112	981	3273	455	2462	786

Table 6.1. Demographic characteristics for the low-risk control group and the 3 adverse outcome groups.

* = $p<0.05$

		R time (min)	K time (min)	Angle (deg)	MA (mm)	Coagulation Index
PET n=8	1st trim	4.76 ± 0.57	1.56 ± 0.28	67.63 ± 4.14	60.69 ± 7.13	1.09 ± 1.02
	2nd trim	5.01 ± 0.89	1.49 ± 0.16	66.88 ± 5.34	58.49 ± 7.79	0.6 ± 1.32
	3rd trim n=6	3.96 ± 1.52	1.22± 0.24	72.22 ± 4.73	63.40 ± 8.23	2.42 ± 1.36
Control n=232	1st trim	4.94± 0.94	1.88 ± 2.12	66.87 ± 4.71	58.45 ± 6.21	0.51 ± 1.69
	2nd trim	4.62 ± 0.82	1.39 ± 0.27	69.73 ± 4.84	61.46 ± 4.65	1.51 ± 1.15
	3rd trim	4.76 ± 1.63	1.43 ± 0.64	70.27 ± 6.93	65.18 ± 6.33	1.90 ± 1.80

Table 6.2. TEG parameters for subjects with pre-eclampsia and low-risk controls in 1st, 2nd and 3rd trimesters.

Data are mean ± SD.

		R time (min)	K time (min)	Angle (deg)	MA (mm)	Coagulation Index
GBP n=15	1st trim	4.94 ± 0.75	1.62 ± 0.25	67.68 ± 3.44	59.91 ± 3.59	1.05 ± 0.62
	2nd trim	5.04 ± 1.44	1.52 ± 0.21	68.55 ± 2.29	60.64 ± 3.82	0.97 ± 0.76
	3rd trim	4.19 ± 0.66	1.19 ± 0.16	73.13 ± 2.26	63.42 ± 6.18	2.35 ± 1.26
Control n=232	1st trim	4.94± 0.94	1.88 ± 2.12	66.87 ± 4.71	58.45 ± 6.21	0.51 ± 1.69
	2nd trim	4.62 ± 0.82	1.39 ± 0.27	69.73 ± 4.84	61.46 ± 4.65	1.51 ± 1.15
	3rd trim	4.76 ± 1.63	1.43 ± 0.64	70.27 ± 6.93	65.18 ± 6.33	1.90 ± 1.80

Table 6.3. TEG parameters for subjects with gestational hypertension and low-risk controls in 1st, 2nd and 3rd trimesters.

Data are mean ± SD.

		R time (min)	K time (min)	Angle (deg)	MA (mm)	Coagulation Index
<10th centile n=19	1st trim	4.98 ± 0.33	1.65 ± 0.25	67.19 ± 3.14	58.72 ± 5.56	0.74 ± 0.87
	2nd trim	4.53 ± 0.70	1.26 ± 0.22	71.61 ± 3.45	65.31* ± 5.96	2.20 ± 1.31
	3rd trim	4.84 ± 0.65	1.26 ± 0.25	71.46 ± 6.39	69.14 ± 7.50	2.49 ± 1.30
Control n=232	1st trim	4.94± 0.94	1.88 ± 2.12	66.87 ± 4.71	58.45 ± 6.21	0.51 ± 1.69
	2nd trim	4.62 ± 0.82	1.39 ± 0.27	69.73 ± 4.84	61.46 ± 4.65	1.51 ± 1.15
	3rd trim	4.76 ± 1.63	1.43 ± 0.64	70.27 ± 6.93	65.18 ± 6.33	1.90 ± 1.80

Table 6.4. TEG parameters for subjects with birth weight <10th centile and low-risk controls in 1st, 2nd and 3rd trimesters.

Data are mean ± SD.
* = $p<0.05$ compared with low-risk controls

CHAPTER 7.

THROMBOELASTOGRAPHY AND PERIPARTUM COAGULATION PROFILES ASSOCIATED WITH CAESAREAN SECTION DELIVERY.

Introduction.

The period following operative delivery has the highest thromboembolic risk. There are a number of interventions within the caesarean section process that may be responsible for this peak in risk. Such factors include pre-operative fluid restriction, the surgery itself (blood loss and damage to vascular endothelium), prolonged immobility following anaesthesia, and a reactive thrombocytosis[72]. Previous estimates have put the incidence of thromboembolism following caesarean section as being between 4 and 20 times that succeeding spontaneous vaginal deliveries[64,67].

After a formal risk assessment, thromboprophylaxis is currently advocated in the post-partum period for women delivered by caesarean section who are judged to be at moderate or high risk of thromboembolism[73]. In these patients prophylaxis is continued for a minimum of 5 days post-partum.

Aims.

To describe, using TEG, the changes in the global haemostatic function in women undergoing elective caesarean section and to assess whether demographic stratification of low, moderate and high thromboembolic risk sub-groups, with subsequent thromboprophylaxis, relates to TEG parameters.

Methods.

After approval of the study protocol was obtained from the Leicestershire, Northampton and Rutland Research Ethics Committee, informed consent was

obtained from women undergoing elective caesarean sections between March and April 2004. Women were provided with written information about the study in the ante-natal clinic when booking for their caesarean section. Women in any of the thromboembolic risk categories were recruited (fig 7.1).

Prospective blood samples were taken at the following times through the caesarean section process:

stage (i) A week prior to surgery at caesarean section booking in the ante-natal clinic, routine pre-operative blood samples were also taken.

stage (ii) On the day of surgery following overnight 'nil-by-mouth' and moments before administration of a combined spinal epidural.

stage (iii) Immediately following wound closure.

stage (iv) Four hours later in recovery.

stage (v) Twenty-four hours post operatively.

Other operative variables such as intra-operative fluid volumes, estimated blood loss, surgical time, time of thromboprophylaxis administration and fluid given in theatre and recovery were all noted. This blinded observational study was pragmatic and thromboprophylaxis regimes were unaltered by the TEG results.

RCOG Risk assessment profile for thromboembolism in caesarean section

Low risk: Early mobilisation and hydration

Elective caesarean section: uncomplicated pregnancy and no other risk factors.

Moderate risk: Consider one of a variety of prophylactic measures

- Age > 35 years
- Obesity (> 80 kg)
- Parity 4 or more
- Labour 12 hours or more
- Gross varicose veins
- Current infection
- Pre-eclampsia
- Immobility prior to surgery (> 4 days)
- Major current illness (e.g. heart or lung disease, cancer, inflammatory bowel disease, nephrotic syndrome)
- Emergency caesarean section in labour.

High risk: Heparin prophylaxis with or without leg stockings

- A woman with three or more moderate risk factors from above
- Extended major pelvic or abdominal surgery (e.g. caesarean hysterectomy)
- Women with personal or family history of deep venous thrombosis, pulmonary embolism or thrombophilia, paralysis of lower limbs
- Women with antiphospholipid antibody (cardiolipin antibody or lupus anticoagulant).

Fig 7.1. Royal College of Obstetricians and Gynaecologists. *Report of the RCOG Working Party on Prophylaxis Against Thromboembolism in Gynaecology and Obstetrics*. London: RCOG; 1995.

Blood sampling and analysis.

Citrated blood samples were obtained and analysis by kaolin-activated thromboelastography was performed between 30 and 120 minutes after venepuncture in accordance with the study protocol outlined in chapter 2.

Statistical Analysis.

Two analyses took place and a p value of <0.05 was considered significant. In the longitudinal analysis data from all 54 women were combined and analysed by General Linear Model ANOVA. Separate analyses of the low, moderate and high risk sub-groups were performed using ANOVA and independent sample T-tests.

Results.

Longitudinal Analysis

For the longitudinal group analysis 54 women (14 low risk, 32 moderate risk and 8 high risk) were recruited. Demographic data for this group is shown in Table 7.1.

The indications for the caesarean section included: previous caesarean section (36), breech presentation (8), previous perineal tear (3), placenta previa (3), fibroids (1), avoidance of instrumental delivery (1), congenital malformation (1) and macrosomia (1). The average period of 'nil-by-mouth', was 12hrs 50mins (range: 6hrs 0min – 18hrs 40min) and the average surgical time, first incision to closure, in this series of patients was 36mins (range: 15mins-75mins).

R time.

Analysis shows that pre-operative fluid restriction [stage (i) – stage (ii),], does not influence R time. The surgical procedure [stage (ii) – stage (iii)], (4.16min vs 3.55min $p<0.01$), significantly reduces the time taken for clot formation to commence and thus renders the mother hypercoagulable (Table 7.2, Fig. 7.2). This clot formation time then increases 24hrs after the procedure, approaching pre-operative levels [stage (iii) – (v)], (3.55min vs 3.83min $p<0.0003$).

K Time.

Pre-operative starvation alone [stage (i) – stage (ii)], does not influence K time. The cumulative effect of pre-operative startvation and surgery results in a reduction in K time [stage (i) – stage (iii), 1.43min vs 1.08min, $p=0.005$], and this persists at 4 hours post-operatively [stage (i) – stage (iv) 1.43min vs 1.13 min $p<0.05$] (Table 7.2, Fig. 7.3).

α Angle.

Pre-operative starvation alone [stage (i) – stage (ii)], does not influence α angle. The cumulative effect of pre-operative startvation and surgery results in an increase in α angle [stage (i) – stage (iii), 65.64° vs 73.06°, $p<0.005$], and this persists at 4 hours post-operatively [stage (i) – stage (iv) 65.64° vs 71.59° $p<0.05$] and at 24 hours post-operatively [stage (i) – stage (v), 65.64° vs 71.77°, $p<0.05$] (Table 7.2, Fig. 7.4).

Maximum Amplitude.

Analysis shows a hypercoagulable picture pre-operatively with starvation significantly increasing MA values (62.9mm vs 68.2mm $p<0.005$) (Table 7.2, Fig. 7.5). MA values remain significantly high after surgery [stage (iii)] ($p<0.0005$), at 4hours [stage (iv)] ($p<0.005$) and at 24hours post-operatively [stage (v)] ($p<0.005$) when compared to pre-admission [stage (i)]. Thus, unlike the R and K time, MA values do not approach pre-operative levels at 24hrs post-operatively.

Coagulation Index.

Coagulation index is increased by pre-operative starvation [stage (i) – stage (ii) 1.28 vs 2.77 $p=0.002$] and rises further immediately after surgery [stage (ii – stage (iii) 2.77vs 3.45 $p<0.02$] (Table 7.2, Fig. 7.6). Coagulation index remains elevated at 4 hours [stage (iv) 3.16, $p<0.00001$] and at 24 hours post-operatively [stage (v) 2.81, $p<0.00001$] when compared to pre-admission [stage (i)].

Longitudinal Sub-group Analysis.

For the sub-groups comparative analyses between the combined high and moderate TE risk groups and the low TE risk group were performed pre-operatively [Stage (ii)] and at 24hrs post operatively [Stage (v)]. Demographic data for the low, moderate and high TE risk women is given in Table 7.1.

R Time.

In the pre-operative samples [stage (ii)] the low TE risk women were less coagulable than the combined high and moderate TE risk women (4.66 vs. 4.03), ($p=0.03$). No significant differences were seen between the two groups at 24hrs post operatively, when the high and moderate risk women had received thromboprophylaxis (Table 7.3, Figs. 7.7 and 7.8).

K Time, α Angle, MA and Coagulation Index.

Pre-operative and 24hrs post-operative analyses did not achieve significant differences between the two groups. However, despite this there are consistent trends between the two groups with all TEG parameters being more hypercoagulable in the mixed group pre-operatively and all parameters being more hypercoagulable in the low risk group post-operatively.

Discussion.

Although it has been possible to speculate, no studies to date have been able to identify what aspect of the caesarean section process creates or triggers the high increase in TE risk associated with this procedure. In this study TEG® demonstrated that; (a) pre-operative 'nil-by-mouth' and (b) regional anaesthesia and surgical intervention render a woman hypercoagulable. Although it is widely assumed that dehydration is a risk factor for the development of venous TE disease, the literature does not reveal any large studies investigating this. However, one study of hydration following acute ischaemic stroke, concluded that dehydration is strongly independently associated with venous TE disease[140].

Nil-by-mouth is requested in patients undergoing surgery to avoid anaesthetic complications and for elective caesarean section patients this is requested from midnight before surgery. In reality, however, most patients are nil-by-mouth much earlier than this. Furthermore, it is easily possible for a woman to have waited in excess of 12 hours before pre-operative IV fluids are administered, as was the case in our study.

Thromboelastography has been used to assess the effect on coagulation of regional and general anaesthesia for caesarean section. Gorton et al demonstrated that venous cannulation and anticipation of surgery alone induced hypercoagulable changes in R and K times and MA[141], highlighting the effect of stress and the associated release of catecholamines which have a stimulatory effect on platelet aggregation[142]. When the effects of general and regional

anaesthesia were compared R and K times significantly decreased ($p<0.05$), and the values for α angle ($p<0.05$) and CI ($p<0.01$) significantly increased in the general anaesthesia group when compared to the spinal anaesthesia group[143]. Tracheal intubation has been associated with increased stress and higher levels of cathecholamine release. The use of regional anaesthesia has been associated with a reduced incidence of thromboembolic events[144,145] and is widely accepted in obstetric practice.

Hypercoagulable changes in TEG parameters have been widely described after a variety of surgical interventions, repair of femoral fracture[146], laparoscopic cholecystectomy[147]and major abdominal surgery[148]. In addition large increases in the procoagulants, fibrinogen, Factor VIII and α_1-antitrypsin together with decreases in the naturally occurring anticoagulants, protein C and antithrombin III have been demonstrated[149]. The increased incidence of thrombotic complications that occur postoperatively is the clinical manifestation of these changes, however in assessing patients for the risk of these complications we rely on demographic characteristics rather than direct information about blood coagulability.

This study demonstrates that women who are high and moderate risk for TE disease, as per the TE risk categories described in the RCOG guideline[73], are relatively hypercoagulable compared to those at low risk. In addition, the findings confirm the efficacy of heparin thromboprophylaxis in reducing the hypercoagulability of the high and moderate risk groups to that of the low risk group, as demonstrated by TEG®. Thromboelastography shows an ability to

identify hypercoagulable states and potentially has a role in the screening of patients pre-operatively to assess the risk of thrombotic complication. Given the significant hypercoagulability resulting from fluid restriction we would suggest early hydration for women on the elective caesarean list as a possible intervention to reduce thromboembolic risk.

Risk Group	Low TE		Moderate TE		High TE	
Characteristic	**Mean**	**Range**	**Mean**	**Range**	**Mean**	**Range**
Age	28.4	19-34	32.5	23-41	30.1	27-35
BMI (kg/m^2)	24.0	21-30	27.3	21-42	29.9	20-40
Gestation (weeks)	39.1	38-41	38.7	32-42	38.1	34-40

Table 7.1. Demographic data for the 14 low TE risk women, the 32 moderate TE risk and 8 high TE risk women.

	R Time (min)		K Time (min)		Angle (deg)		MA (mm)		Coagulation Index	
Stage	Mean	95% CI	Mean	95% CI	Mean	95% CI	Mean	95% CI	Mean	95% CI
(i)	4.44	4.00-4.89	1.43	1.31-1.54	65.64	60.38-70.90	62.9	59.4-66.5	1.28	0.31-2.25
(ii)	4.16	3.94-4.37	1.22	1.15-1.29	71.00	69.37-72.64	68.2	67.0-69.4	2.77	2.48-3.05
(iii)	3.55	3.30-3.80	1.08	1.02-1.13	73.06	71.79-74.33	69.6	68.2-71.1	3.45	3.19-3.72
(iv)	3.83	3.61-4.06	1.13	1.06-1.20	71.59	70.10-73.08	69.0	67.8-70.2	3.16	2.87-3.44
(v)	4.32	4.04-4.60	1.20	1.11-1.30	71.77	70.29-73.26	68.5	66.8-70.1	2.81	2.47-3.15

Table 7.2. Mean R time, K time, α angle, Maximum Amplitude and Coagulation Index values at each stage, with 95% confidence intervals, for the longitudinal analysis.

Stage	TEG parameter	Low TE Risk n=14	Moderate & High TE Risk n=40
(ii)	R Time (min)	4.66*	4.03*
	K Time (min)	1.30	1.20
	Angle (deg)	69.92	71.37
	Maximum Amplitude (mm)	68.14	68.20
	Coagulation Index	2.40	2.89
(v)	R Time (min)	4.10	4.39
	K Time (min)	1.14	1.22
	Angle (deg)	73.72	71.16
	Maximum Amplitude (mm)	68.63	68.43
	Coagulation Index	3.10	2.71

Table 7.3. Mean R time, K time, Angle, Maximum Amplitude and Coagulation Index for the low TE risk group and the combined moderate and high TE risk group.

* = p=0.03 2-sample t-test.

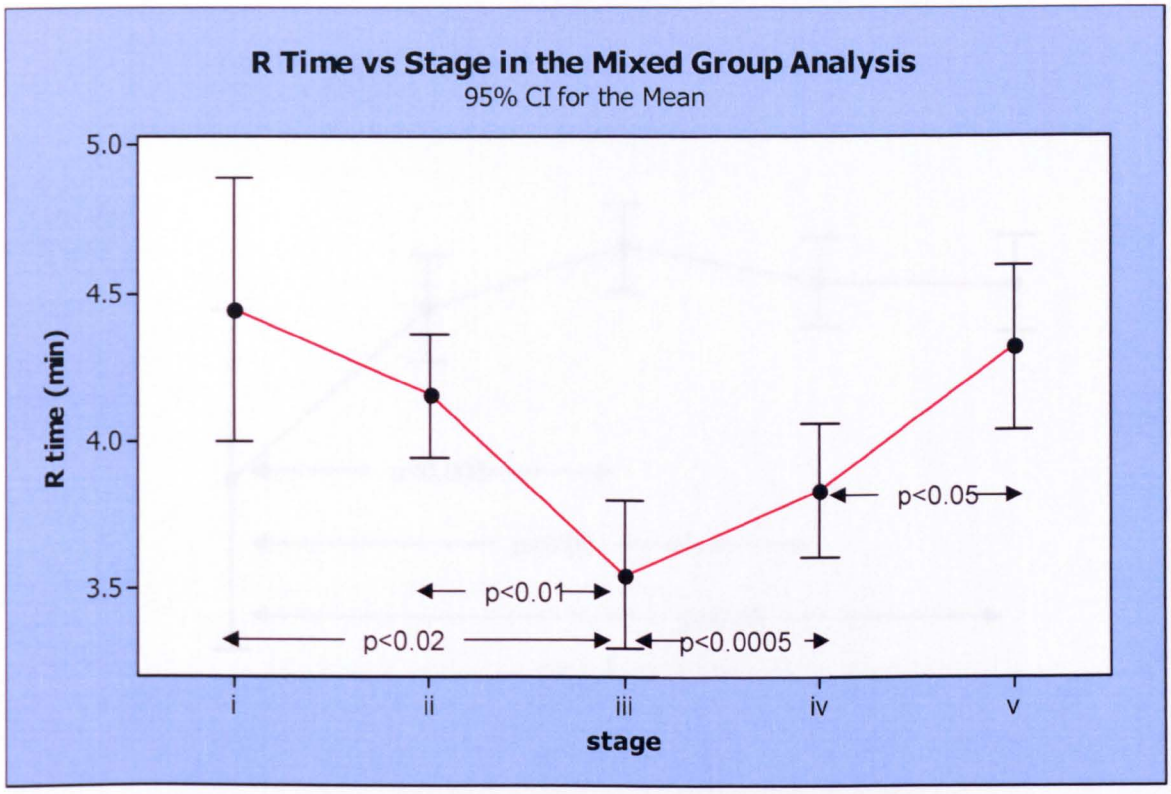

Fig. 7.2 Interval plot of R time (min) vs stage

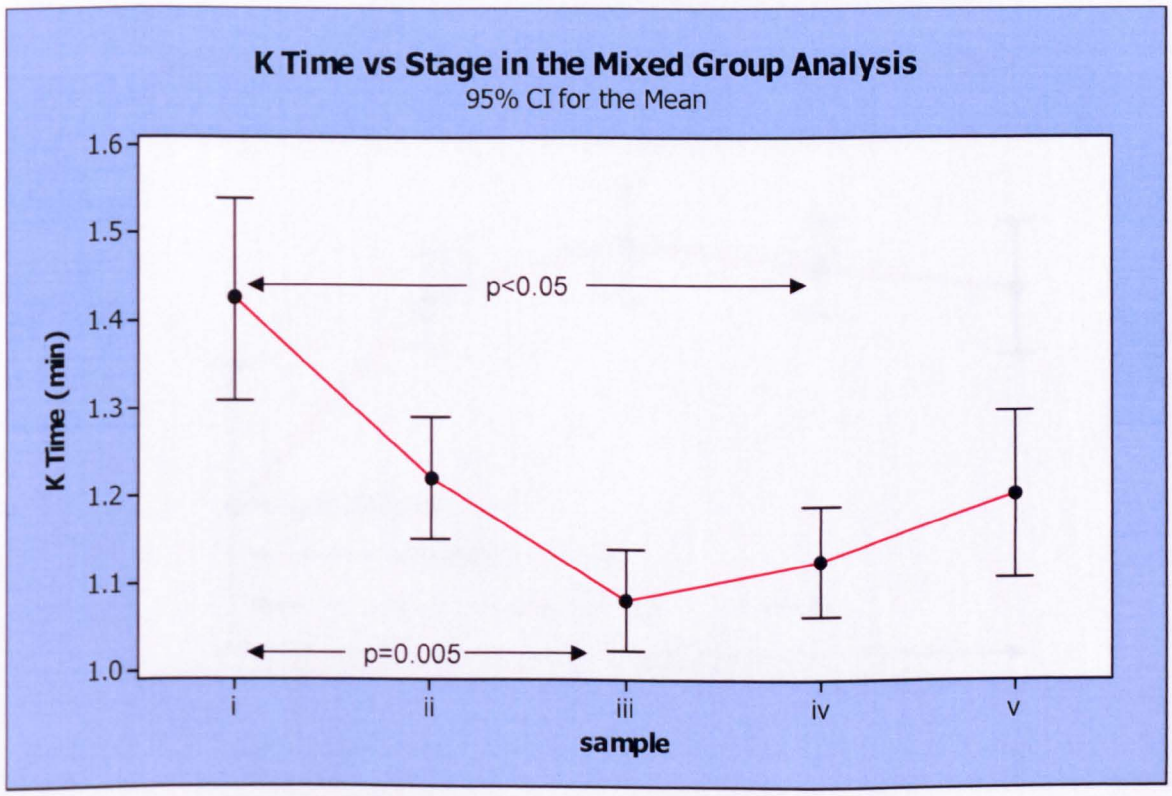

Fig. 7.3 Interval plot of K time (min) vs stage

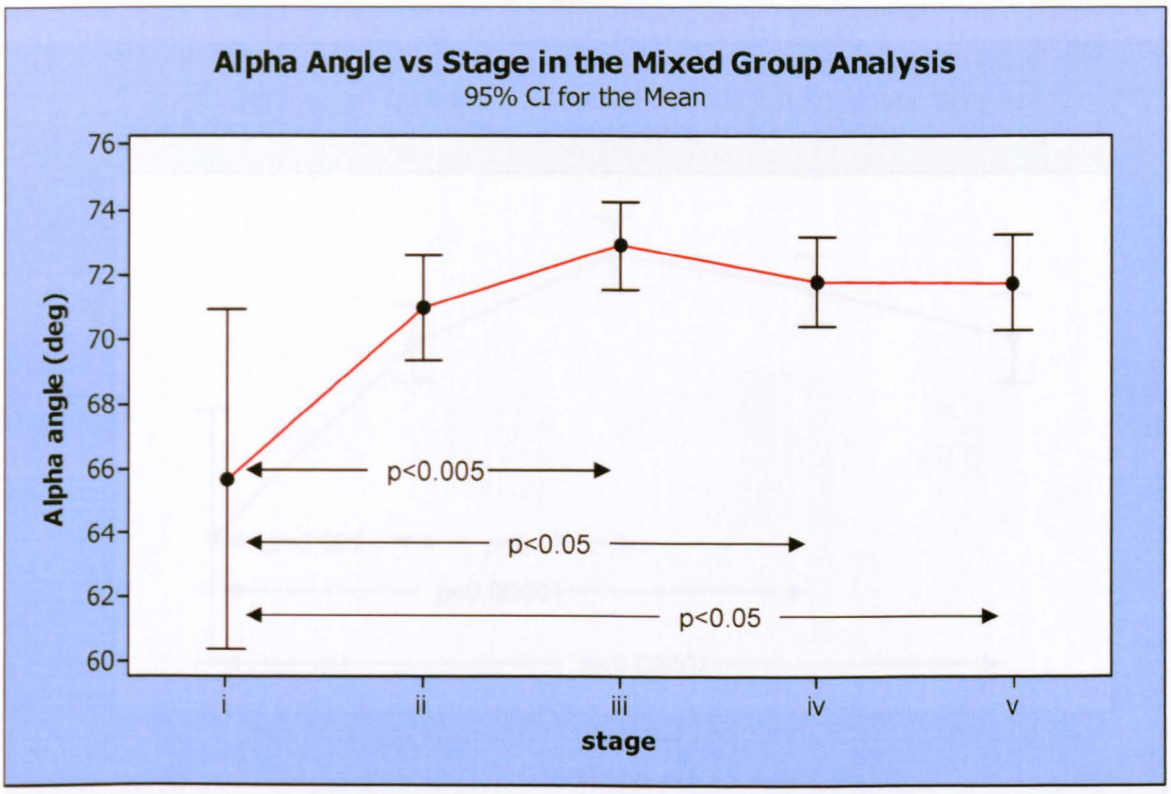

Fig. 7.4 Interval plot of α angle (deg) vs stage

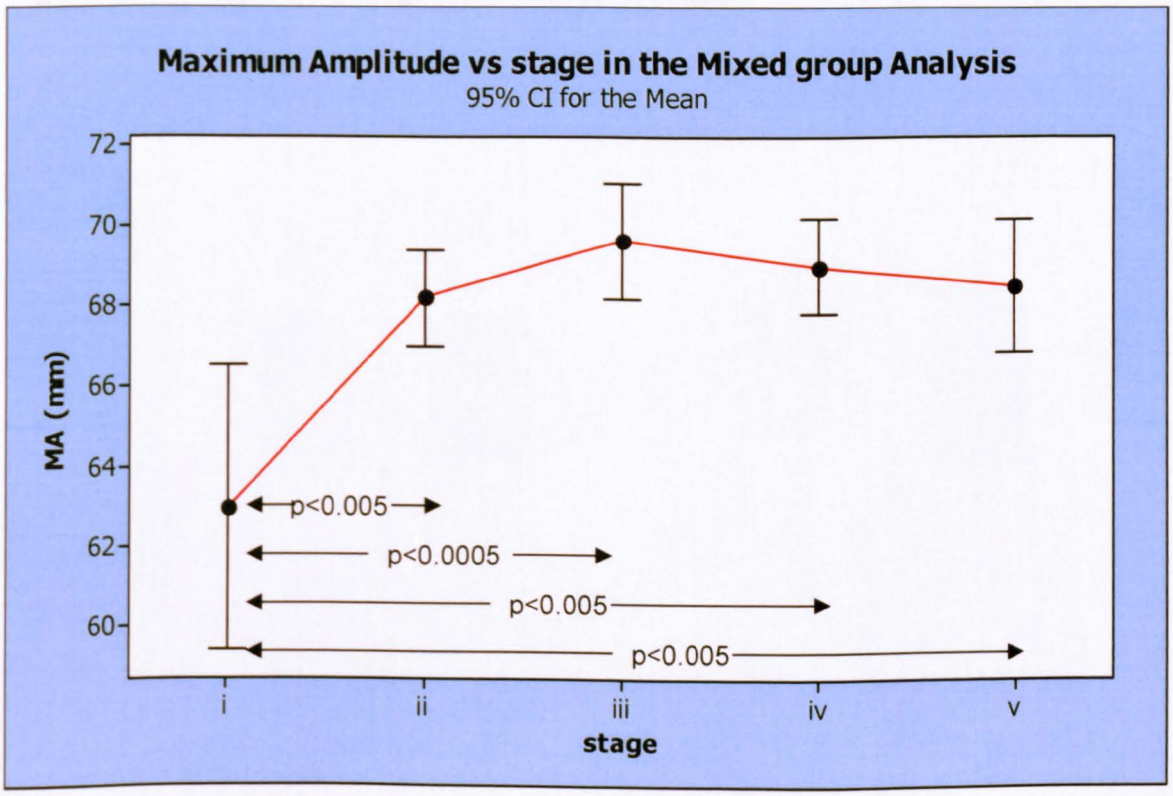

Fig. 7.5 Interval plot of Maximum Amplitude (mm) vs stage

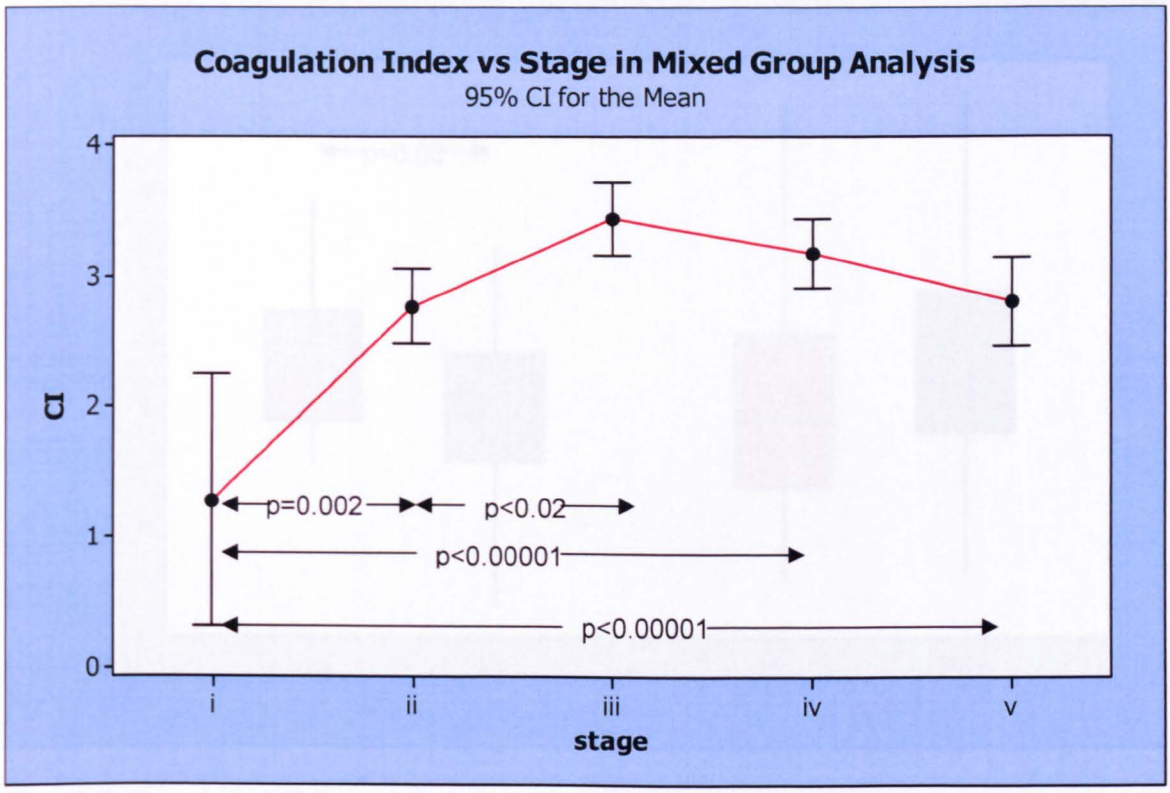

Fig. 7.6 Interval plot of Coagulation Index vs stage

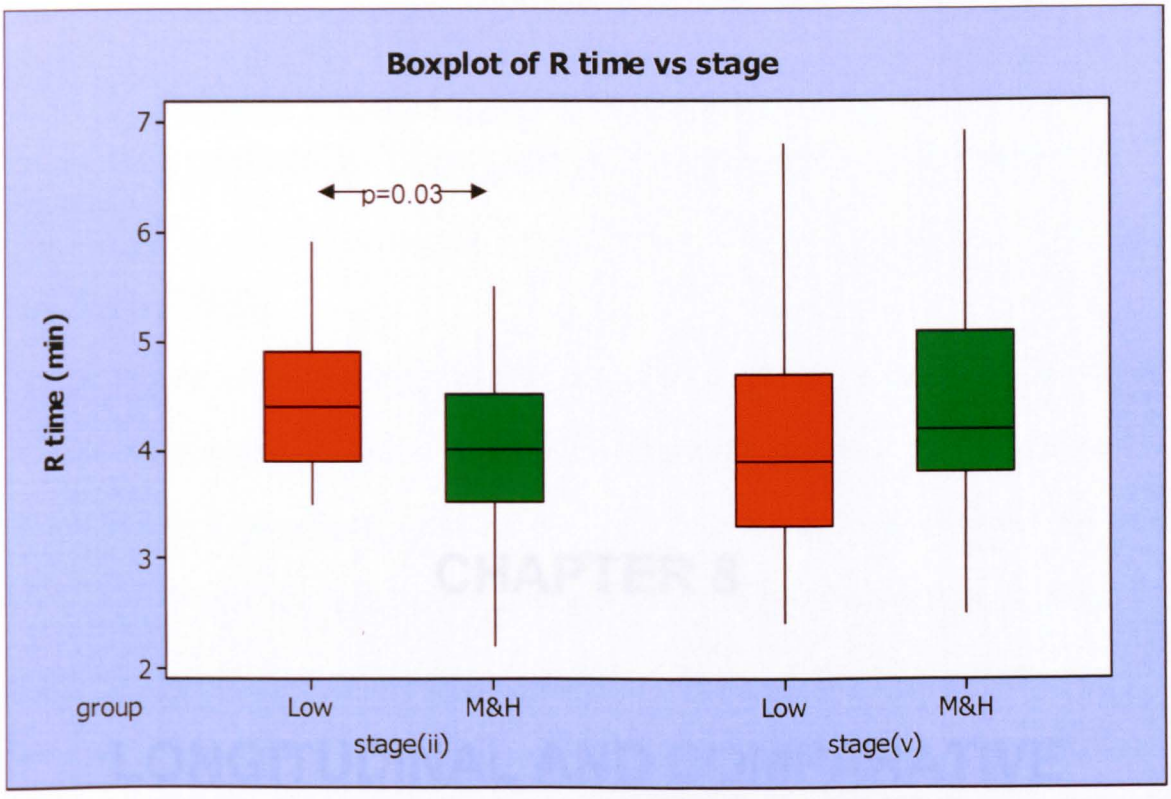

Fig. 7.7. Boxplot of R time (min) vs stage (ii) and stage (v)

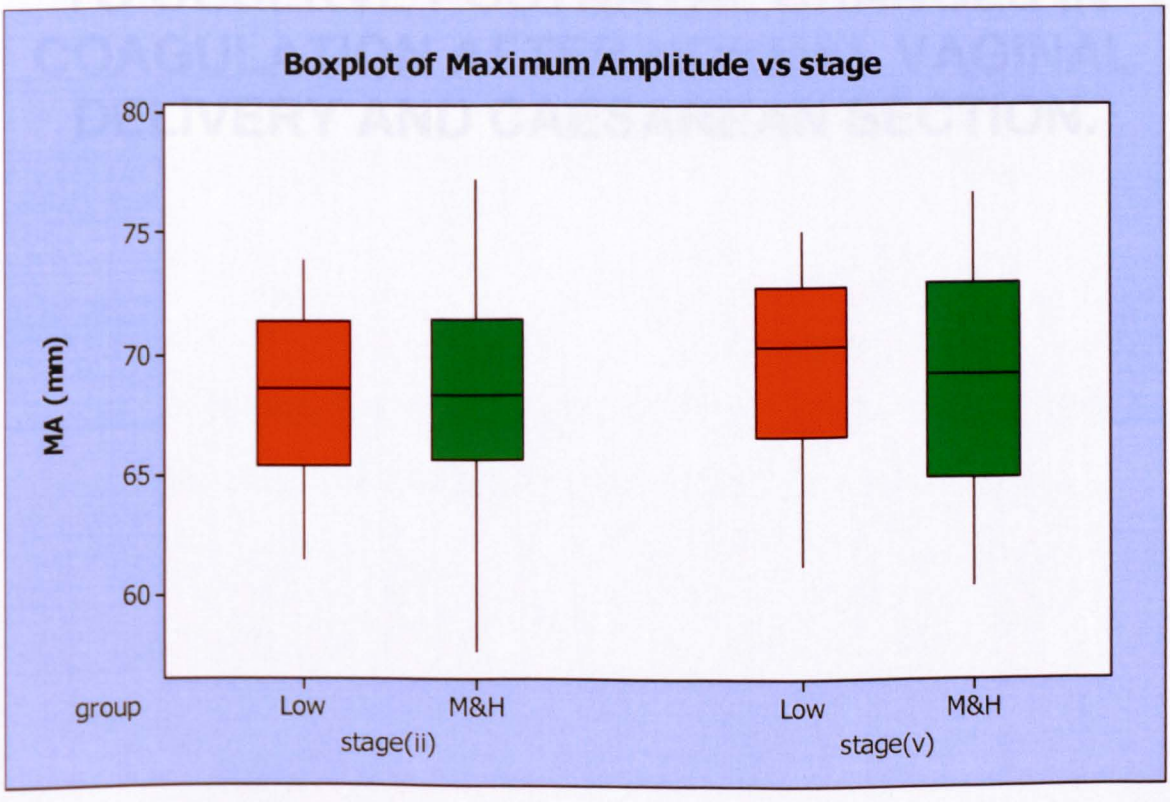

Fig. 7.8 Boxplot of Maximum Amplitude (mm) vs stage (ii) and stage (v)

CHAPTER 8

LONGITUDINAL AND COMPARATIVE STUDIES USING THROMBOELASTOGRAPHY TO OBSERVE POSTNATAL CHANGES IN COAGULATION AFTER NORMAL VAGINAL DELIVERY AND CAESAREAN SECTION.

A longitudinal study using thromboelastography to observe postnatal changes in coagulation after normal delivery.

Introduction.

The widely accepted assumption that coagulation returns to non-pregnant levels at 6 weeks postnatal is based on very little scientific evidence. There is a paucity of studies that investigate coagulation in postpartum women between the very early puerperium, the first 5 days, and 6 weeks which is the standard timing for a postpartum examination. Understanding the rate of change after delivery and the time at which coagulation returns to non-pregnant levels in a population of healthy women who have uncomplicated vaginal deliveries will aid the understanding of normal physiological changes, which in turn, will provide a standard against which we can compare women who are at higher risk of thromboembolic disease e.g. women delivered by caesarean section.

Aims.

The aim of this study was to investigate global coagulation changes in healthy women after uncomplicated vaginal delivery using thromboelastography.

Methods.

Subjects.

The study protocol was approved by Leicestershire, Northamptonshire and Rutland Ethics Committee. Women who had uncomplicated vaginal deliveries after a normal antenatal period were approached to take part in the study. Exclusion criteria were multiple pregnancies, delivery of baby weighing less than 2500g, blood pressure > 140/90 mmHg, past personal or family history of thromboembolism, currently taking regular medication and blood loss at delivery exceeding 400 mls. Women were sampled weekly for up to 10 weeks post delivery. 71 women were recruited and gave informed consent.

A control group of 50 nonpregnant women was recruited. These women were healthy volunteers, between 18 and 40 years of age who had no personal or family history of thromboembolism, not taking any regular medication or the combined oral contraceptive pill. A single blood sample was taken from this group.

Blood Sampling and Analysis.

The blood samples were obtained according to the protocol detailed in chapter 2. The analysis by thromboelastography differs from the other studies within this thesis in that the citrated blood was not activated by kaolin. The method employed uses native citrated blood.

Twenty microlitres of 0.2mol/l of calcium chloride were pipetted into a disposable plastic cup, which had been loaded in a prewarmed thromboelastography machine. The citrated blood sample was inverted 5 times to ensure mixing of the sample and then 340µl of native blood was added to the cup.

Five thromboelastographic parameters were analysed, R time, k time, α angle, MA and coagulation index.

Justification for change of methodological technique.

Despite being presented in the later stages of this thesis, this longitudinal study of postnatal changes in coagulation after normal delivery was the first to be performed. In planning the subsequent studies two considerations were applied. The first was that the method used should have the potential to provide rapid results in a clinical setting and secondly the large number of samples obtained in subsequent studies necessitated a rapid turnaround in sample processing time. Therefore, in all other studies the citrated blood was activated with kaolin (hydrated aluminium silicate) which reduces the percentage coefficient of variation, as demonstrated in Chapter 3, and shortens the running time by maximally activating the coagulation pathway by Factor XII.

Statistical Analysis.

Statistical analysis was performed using MINITAB version 15 (Minitab Inc.,PA, USA). To determine the time point at which coagulation in the post-partum women returned to that of the non-pregnant controls non-parametric, 2-

independent sample Mann-Whitney U tests were performed. p-values of <0.05 were taken as statistically significant.

Results.

The demographic characteristics of the 71 women recruited and details of labour and delivery were recorded. The mean age of subjects was 28.3 years (range 17-41yr), mean weight at booking 67.7kg (range 46.5-118kg). The mean length of labour was 8.05 hours (range 1.08-22.4 hrs) and mean blood loss 253.0mls (range 100-400mls). The epidural usage rate was 30% with 21 of the 71 women choosing this form of analgesia. 37 (52%) of the women were primiparous. The mean age of the control group was 29.5 years (range 18-40 yrs).

Only one sample was obtained from 10 women due to difficulty sampling (2), unable to contact (3) or the women choosing to withdraw (5). Of the 61 women remaining 2 had 2 samples taken, 7 had 3 samples taken, 17 had 4 samples taken, 9 had 5 samples taken, 6 had 6 samples taken, 13 had 7 samples taken and 7 had 8 samples taken.

R Time.

The R time is significantly shorter in the first postpartum week (9.6min vs 13.9min $p<0.0001$) and remains shortened in the second week (12.2min vs 13.9min $p<0.05$) (Table 8.1, Fig. 8.1). By the third week postpartum there is no significant difference when compared to the nonpregnant controls.

K Time.

The K time shows similar changes when comparing the postnatal women with a nonpregnant population. In the first week postpartum the time taken to reach a defined clot strength is shortened (2.3min vs 4.4min $p<0.0001$) and although this lengthens in the second week it is still significantly shorter than the nonpregnant controls (3.8min vs 4.4min $p<0.05$) (Table 8.1, Fig. 8.2). By the third postpartum week the K time does not differ from the nonpregnant control population.

α angle.

The α angle is increased in the first week postpartum (59.1 vs 43.3 $p<0.0001$) but returns to nonpregnant values by the second postpartum week (Table 8.1, Fig. 8.3).

Maximum Amplitude.

The maximum amplitude is the only parameter which differs from the nonpregnant population for 3 weeks after delivery. The MA is maximally increased in the first week postpartum (67.1mm vs 52.7mm $p<0.0001$) (Table 8.1, Fig. 8.2). In the second week the MA reduces (61.2mm vs 52.7mm $p<0.0001$) and there is a further reduction in the third week (56.6mm vs 52.7mm $p<0.05$) but the mean MA remains significantly elevated compared to the nonpregnant controls.

Coagulation Index.

This parameter is increased during the first 3 weeks after delivery and shows a similar trend to maximum amplitude. The first postpartum week demonstrates the greatest increase (2.33 vs -0.67 $p<0.0001$) (Table 8.1, Fig. 8.6). In the second postpartum week the coagulation index remains increased but not to the level of the first week (1.04 vs -0.67 $p<0.0001$), in the third postpartum week there is a further reduction in coagulation index but the level remains significantly elevated compared to a nonpregnant population (0.05 vs -0.67 $p<0.05$).

By the fourth postpartum week none of the parameters differ from the nonpregnant control population that defines baseline blood coagulability, I therefore conclude that the hypercoagulable state of pregnancy has resolved and postpartum women have similar blood coagulability as a nonpregnant female population.

		R min mean	R min 95% CI	K min mean	K min 95% CI	Angle deg mean	Angle deg 95% CI	MA mm mean	MA mm 95% CI	Coag Index mean	Coag Index 95% CI
Controls	N=50	13.9	12.9-15.0	4.4	4.0-4.8	43.3	40.1-46.5	52.7	50.6-54.8	-0.67	-1.14--0.20
Week											
1	N=102	9.6*	9.1-10.5	2.3*	2.1-2.5	59.1*	56.9-61.4	67.1*	65.8-68.3	2.33*	2.08-2.58
2	N=34	12.2$^{\Phi}$	11.3-13.2	3.8$^{\Phi}$	3.2-4.5	46.7	41.9-51.5	61.2*	58.2-64.2	1.04*	0.51-1.58
3	N=36	13.4	12.3-14.4	4.3	3.7-4.8	44.4	41.1-47.8	56.6$^{\Phi}$	53.7-59.4	0.05$^{\Phi}$	-0.45-0.56
4	N=26	13.9	12.9-15.0	4.5	4.0-5.0	41.8	38.4-45.2	52.2	49.6-54.8	-0.73	-1.17--0.29
5	N=36	13.4	12.4-14.3	3.8	3.5-4.2	44.5	41.7-47.3	53.2	50.9-55.4	-0.47	-0.88--0.06
6	N=31	13.9	12.8-14.9	4.5	3.9-5.0	41.8	38.1-45.6	51.2	48.7-53.6	-0.84	-1.32--0.36
7-9	N=26	15.1	13.1-17.2	4.9	3.7-6.0	42.5	37.6-47.4	57.3$^{\Phi}$	54.5-60.0	-0.19	-0.80-0.42
10-12	N=41	15.3	14.1-16.4	4.6	4.1-5.2	42.5	39.3-45.7	53.1	51.0-55.1	-0.93	-1.31--0.54

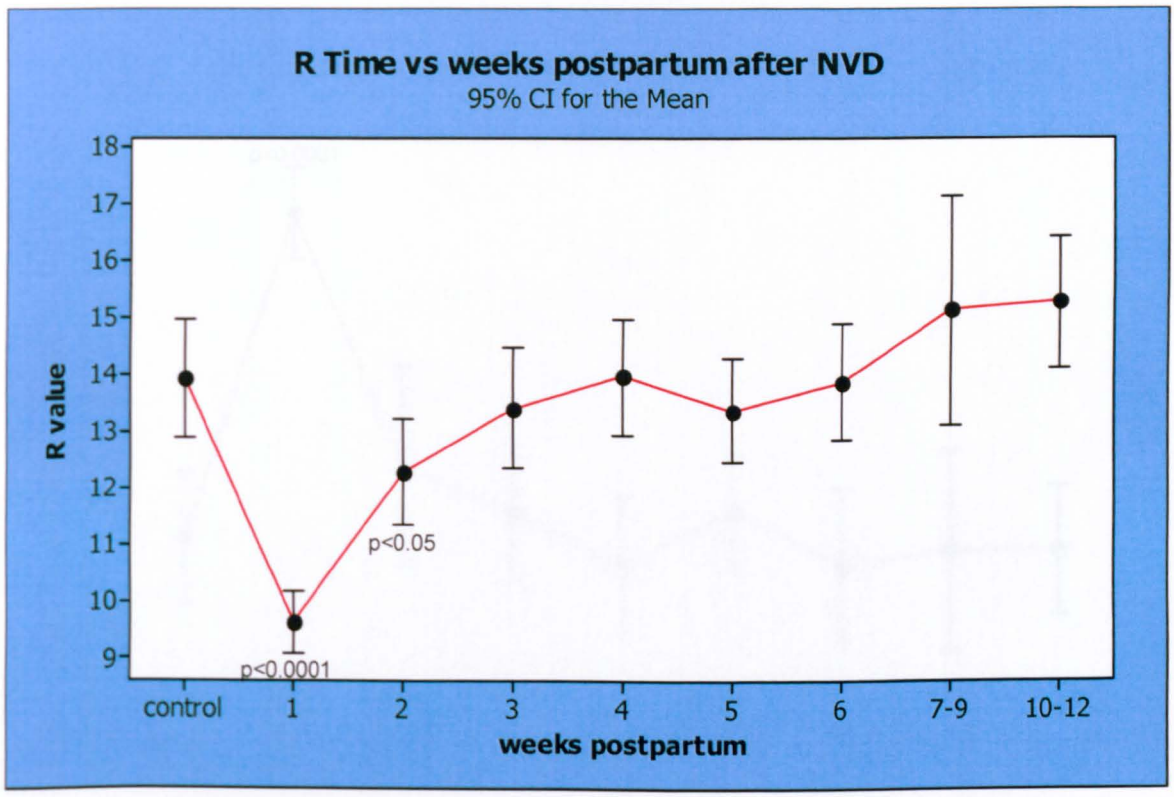

Fig. 8.1. Interval plot of R time vs weeks postpartum after normal delivery

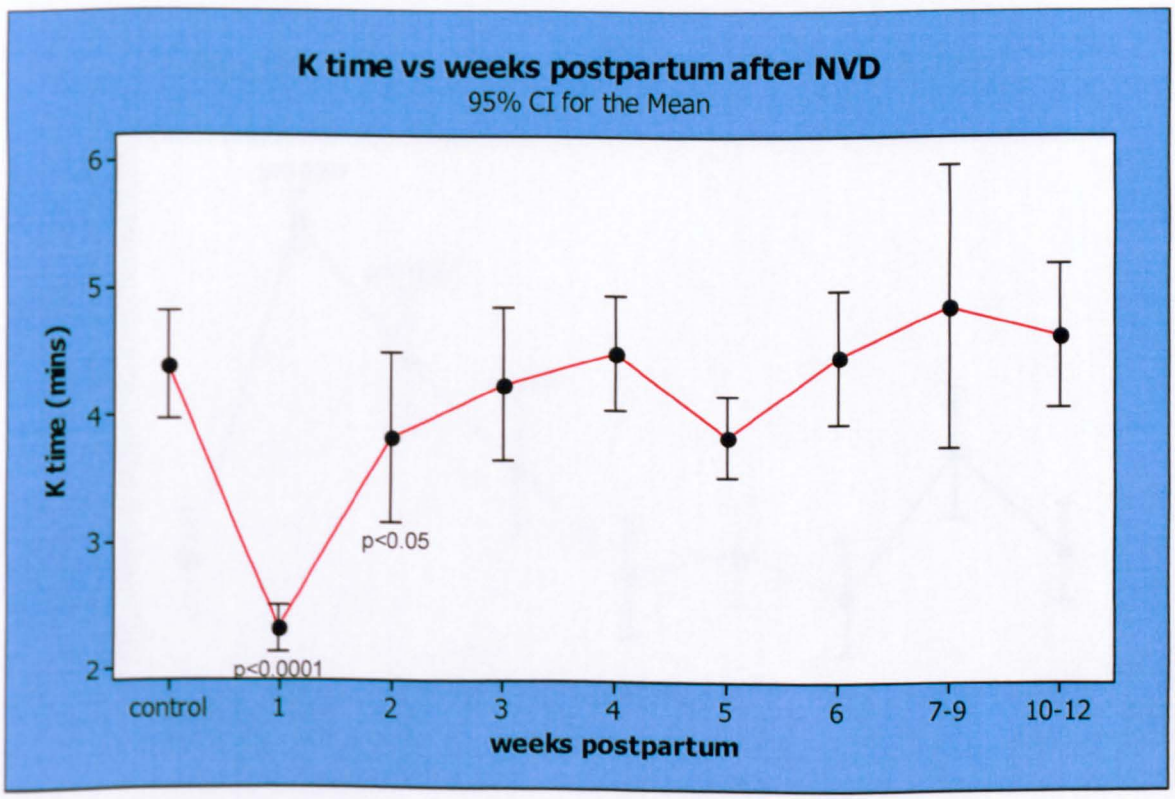

Fig. 8.2. Interval plot of K time vs weeks postpartum after normal delivery

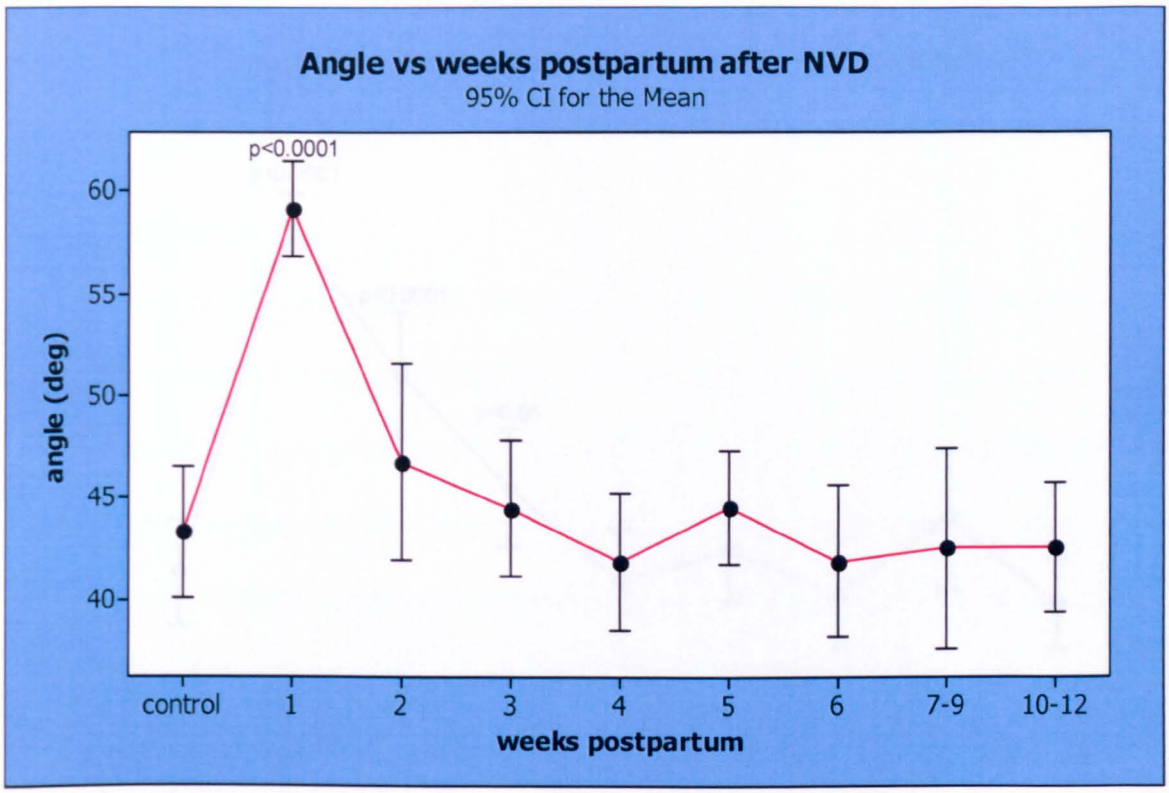

Fig. 8.3. Interval plot of α angle vs weeks postpartum after normal delivery

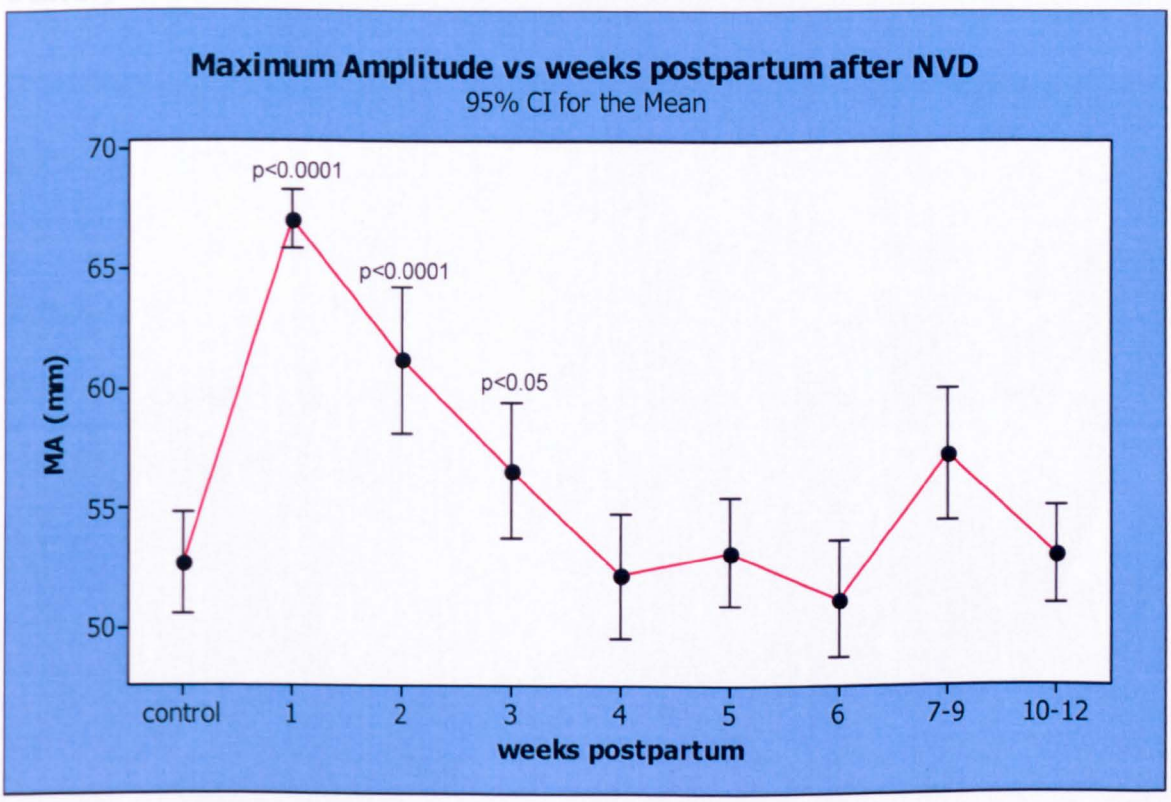

Fig. 8.4. Interval plot of MA vs weeks postpartum after normal delivery

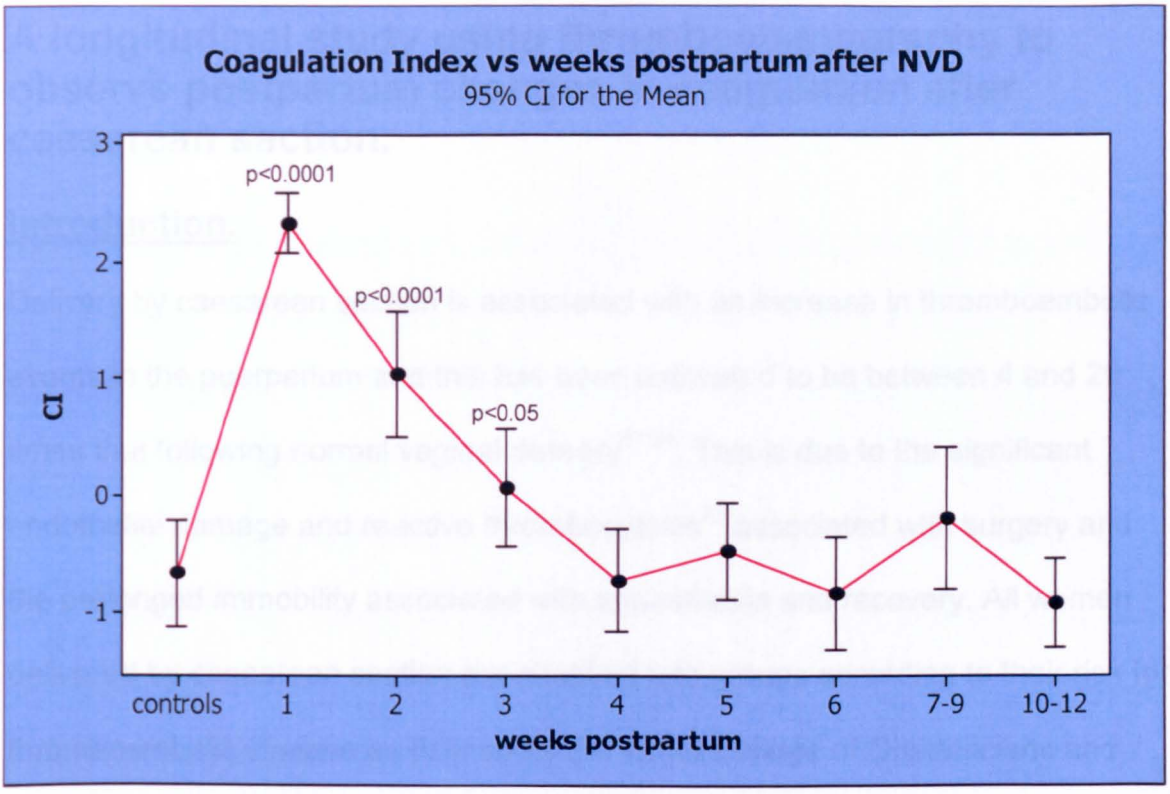

Fig. 8.5. Interval plot of Coagulation Index vs weeks postpartum after normal delivery

A longitudinal study using thromboelastography to observe postpartum changes in coagulation after caesarean section.

Introduction.

Delivery by caesarean section is associated with an increase in thromboembolic events in the puerperium and this has been estimated to be between 4 and 20 times that following normal vaginal delivery[67,64]. This is due to the significant endothelial damage and reactive thrombocytosis[73] associated with surgery and the prolonged immobility associated with anaesthesia and recovery. All women delivered by caesarean section are stratified into groups according to their risk for thromboembolic disease as defined by the Royal College of Obstetricians and Gynaecologists (Appendix 1). The implementation of these guidelines has resulted in a reduction of the number of deaths from thromboembolism after caesarean section as highlighted in the 1997-1999 Confidential Enquiry into Maternal Deaths[75].

Aims.

To determine the time course for global coagulation to return to nonpregnant levels in women delivered by caesarean section.

Methods.

Subjects.

Women who were delivered by caesarean section were approached to take part in the study. Exclusion criteria were multiple pregnancies, delivery of baby weighing less than 2500g, taking regular mediaction and past personal or family history of thromboembolism. Women were sampled weekly for up to 10 weeks post delivery. 32 women were recruited and gave informed consent. All women were stratified into groups of low, moderate or high risk for thromboembolic disease according to RCOG guidelines (appendix 1). The subjects in the low risk group (n=3) received hydration and mobilisation alone. The subjects in the moderate risk group (n=27) received 5,000U unfractionated heparin at the end of the procedure followed by 2,500U low molecular weight heparin (Fragmin ®) for 5 days the first dose being administered 4 hours after removal of epidural catheter. The subjects in the high risk group (n=2) received 5,000U unfractionated heparin at the end of the caesarean section followed by 5,000U low molecular weight heparin (Fragmin ®) for 5 days the first dose being administered 4 hours after removal of epidural catheter.

A control group of 108 nonpregnant women was recruited. These women were healthy volunteers, between 18 and 40 years of age who had no personal or family history of thromboembolism, not taking any regular medication or the combined oral contraceptive pill who donated a single citrated blood sample. This group differs from the control group in the study of postpartum changes in

coagulation after normal delivery because in this study thromboelastography was performed on kaolin activated citrated blood as described below.

Blood Sampling and Analysis.

The blood samples were obtained according to the protocol detailed in chapter 2. Twenty microlitres of 0.2mol/l of calcium chloride were pipetted into a disposable plastic cup, which had been loaded in a prewarmed thromboelastography® machine. The citrated blood sample was inverted 5 times to ensure mixing of the sample and then 1000µl of citrated blood was pipetted into a room temperature vial containing kaolin. This vial was inverted five times and 340µl of kaolin activated citrated blood was added to the cup.

Five thromboelastographic parameters were analysed, R time, k time, α angle, MA and coagulation index.

Statistical Analysis.

Statistical analysis was performed using MINITAB version 15 (Minitab Inc.,PA, USA). To determine the time point at which coagulation in the post-partum women returned to that of the non-pregnant controls non-parametric, 2-independent sample Mann-Whitney U tests were performed on the whole subject group (i.e. low and moderate risk). *P*-values of <0.05 were taken as statistically significant.

Results.

The demographic characteristics of the 32 women recruited and the indication for caesarean section and anaesthetic techniques administered were recorded. The mean age of subjects was 31.7 years (range 22-42yr), mean weight at booking 68.6kg (range 51-76.5kg). Twenty women were delivered by elective caesarean section with a variety of indications: previous caesarean section (n=12), breech presentation (n=4), maternal request to avoid further perineal injury (n=2), pure maternal request (n=1) and fibroid uterus (n=1). All of these women had regional anaesthesia, combined spinal and epidural (n=18) or spinal alone (n=2). The remaining 12 women were delivered by emergency caesarean section with the indications of fetal distress (n=6) and failure to progress (n=6). Eight of these women had epidural anaesthesia for labour which was used for the caesarean section, 2 had combined spinal and epidural anaesthesia and 2 had general anaesthesia. The mean blood loss was 508.6mls (range 300-1500 mls). Fourteen (44%) of the women were primiparous and 18 (56%) multiparous.

Only one sample was obtained from 1 woman due to difficulty in making contact, 1 woman chose to withdraw after the second sample. Of the 30 women remaining 5 had 5 samples taken, 10 had 6 samples taken, 11 had 7 samples taken and 5 had 8 samples taken.

Whole Group Analysis. (low, mod and high)

R Time.

The R time is significantly shorter in the first postpartum week (days 1-4 4.90min vs 6.03min $p<0.00001$, days 6-7 4.72min vs 6.03min $p<0.0001$) and remains shortened in the second week (5.38min vs 6.03min $p<0.005$) (Table 8.2, Fig 8.6). By the third week postpartum there is no significant difference when compared to the nonpregnant controls. The R time is the first parameter to return to nonpregnant values.

K Time.

In the first week postpartum the time taken to reach a defined clot strength (K time) is shortened (days 1-4 1.23min vs 1.93min $p<0.00001$, days 6-7 1.30min vs 1.93min $p<0.0001$) and although this lengthens in the second (1.42min vs 1.93min $p<0.00001$) and third weeks (1.70min vs 1.93min $p<0.05$) it is still significantly shorter than the nonpregnant controls (Table 8.2, Fig.8.7). By the fourth postpartum week the K time does not differ from the nonpregnant control population.

α angle.

The α angle is increased for three weeks after caesarean section, maximally in the first week postpartum (days 1-4 72.65^0 vs 63.56^0 $p<0.00001$). This level of significance remains in the second week postpartum (69.64 vs 63.56 $p<0.00001$).

The α angle reduces in the third week but is still raised compared to the nonpregnant state (66.53^0 vs 63.56^0 $p<0.05$). (Table 8.2, Fig. 8.8).

Maximum Amplitude.

The MA demonstrates a similar pattern to the α angle being maximally increased in the first week postpartum (days 6-7 66.69mm vs 57.25mm $p<0.0001$) (Table 8.2, Fig. 8.9). The mean MA at days 6-7 is higher than at days 1-4 but this difference does not reach significance. In the second week the MA reduces (64.40mm vs 57.25mm $p<0.00001$) and there is a further reduction in the third week (61.01mm vs 57.25mm $p<0.005$) but the mean MA remains significantly elevated compared to the nonpregnant controls. In the fourth postpartum week the MA has reached nonpregnant values.

Coagulation Index.

This parameter is increased during the first 3 weeks after delivery and shows a similar trend to maximum amplitude. The first postpartum week demonstrates the greatest increase (days 6-7 2.27 vs -0.59 $p<0.00001$) (Table 8.2, Fig. 8.10). Although the coagulation index is greater at days 6-7 than days 1-4 this is not a significant increase. In the second postpartum week the coagulation index remains increased but not to the level of the first week (1.37 vs -0.59 $p<0.00001$), in the third postpartum week there is a further reduction in coagulation index but the level remains significantly elevated compared to a nonpregnant population (0.26 vs -0.59 $p<0.05$). In the fourth week postpartum there is no difference

between the coagulation index in the women after caesarean section and the nonpregnant control population.

By the fourth postpartum week none of the parameters differ from the nonpregnant control population that defines baseline blood coagulability in nonpregnant women.

		R min mean	R min 95% CI	K min mean	K min 95% CI	Angle deg mean	Angle deg 95% CI	MA mm mean	MA mm 95% CI	Coag Index mean	Coag Index 95% CI
Controls	N=108	6.03	5.84-6.21	1.93	1.85-2.01	63.56	62.46-64.65	57.25	56.15-58.34	-0.59	-0.89- -0.30
Days											
1-4	N=23	4.90*	4.62-5.18	1.23*	1.15-1.32	72.65*	71.55-73.74	66.36*	64.22-68.50	2.20*	1.80-2.60
6-7	N=9	4.72•	4.36-5.08	1.30•	1.09-1.51	71.90*	69.48-74.32	66.69•	62.78-70.60	2.27*	1.47-3.07
Weeks											
2	N=27	5.38■	5.12-5.64	1.42*	1.28-1.55	69.64*	67.58-71.60	64.40*	61.67-67.73	1.37*	0.87-1.88
3	N=29	5.88	5.53-6.22	1.70▲	1.56-1.84	66.53▲	64.72-68.35	61.01■	58.86-63.18	0.26▲	-0.28-0.80
4	N=29	5.90	5.57-6.24	1.86	1.71-2.00	64.56	63.01-66.11	58.01	55.94-60.09	-0.32	-0.78-0.14
5	N=29	6.02	5.57-6.48	2.04	1.88-2.20	62.88	61.09-64.67	58.12	56.09-60.15	-0.59	-1.21-0.03
6	N=24	6.01	5.59-6.44	1.97	1.79-2.15	63.48	61.47-65.49	57.64	54.87-60.42	-0.50	-1.13-0.12
7-8	N=23	5.73	5.32-6.15	1.87	1.70-2.03	64.51	62.48-66.55	59.83•	57.47-62.20	-0.02	-0.67-0.62
9-10	N=12	5.48	5.01-5.94	1.74	1.61-1.88	66.13	64.60-67.65	57.41	53.85-60.96	0.03	-0.47-0.52

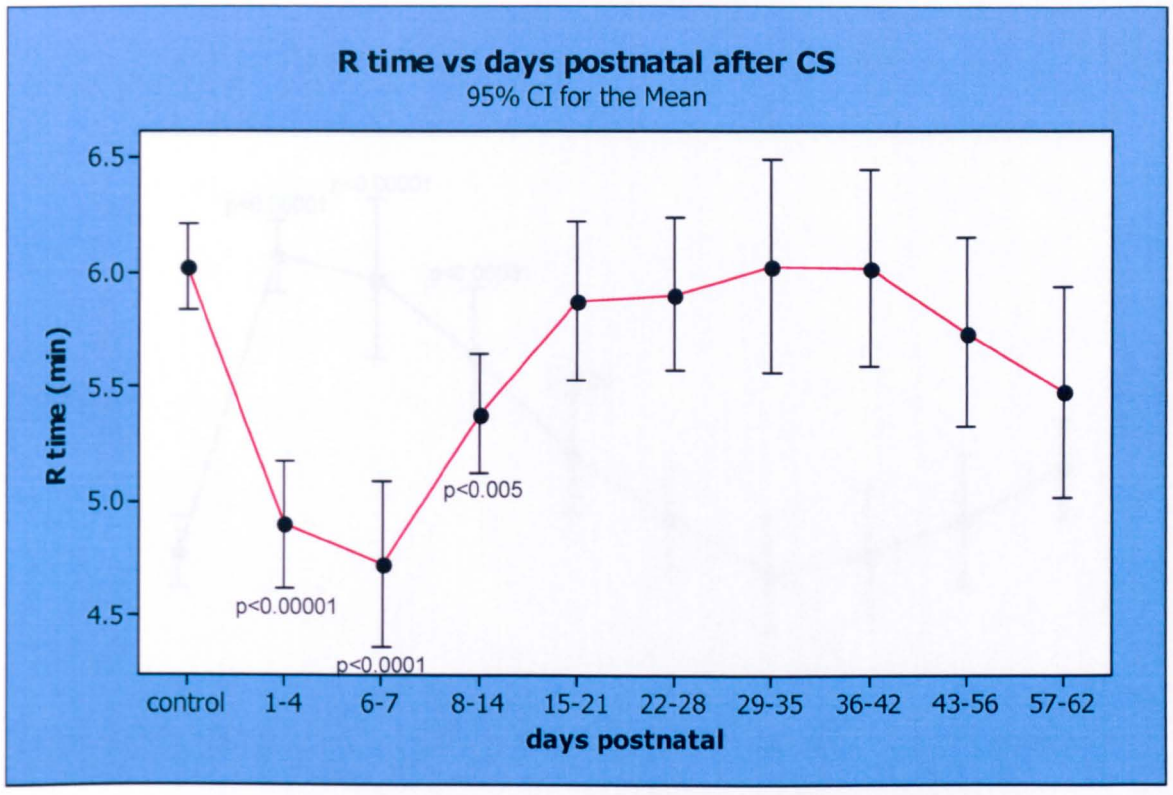

Fig. 8.6. Interval plot of R time vs weeks postpartum after caesarean section

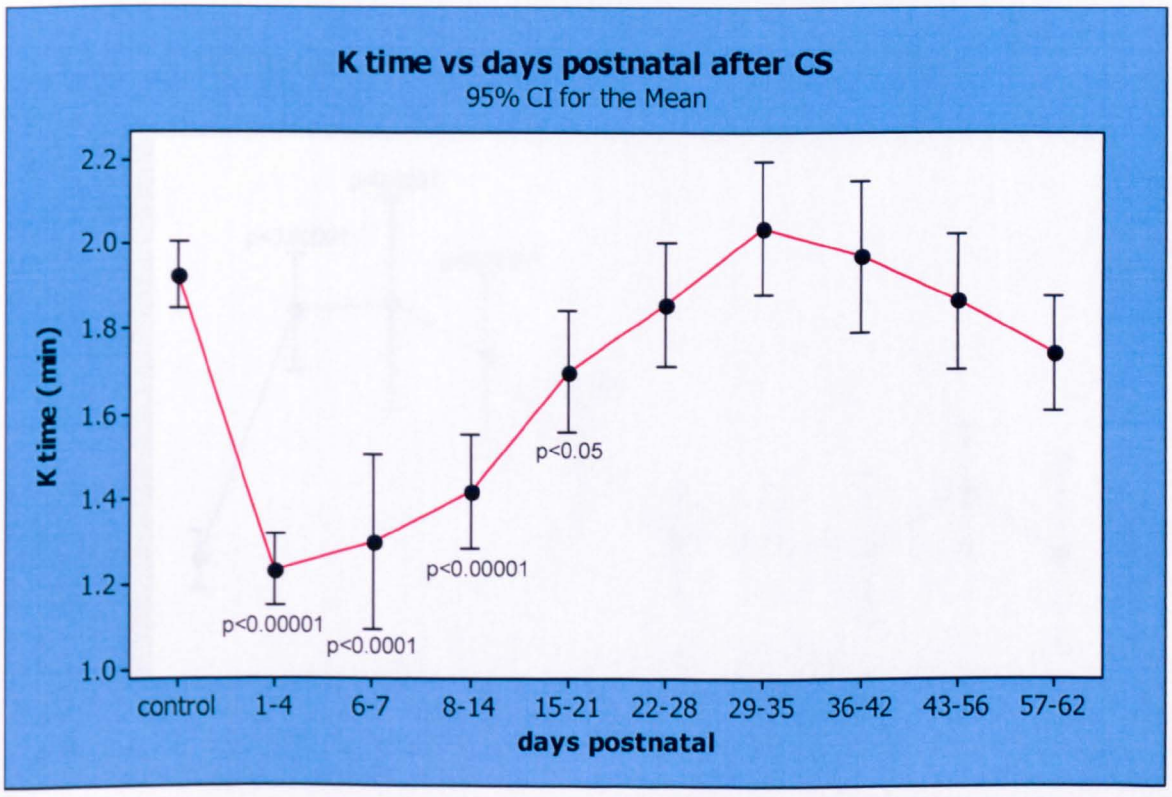

Fig. 8.7. Interval plot of K time vs weeks postpartum after caesarean section

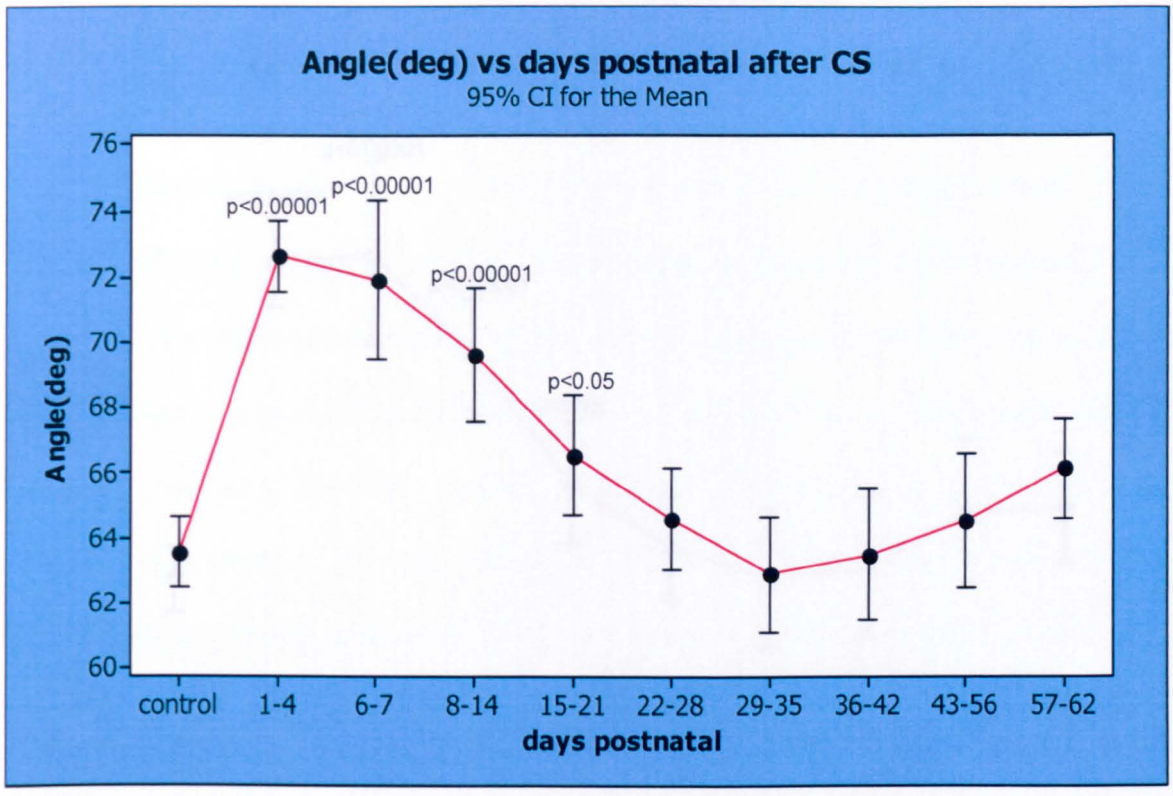

Fig. 8.8. Interval plot of α angle vs weeks postpartum after caesarean section

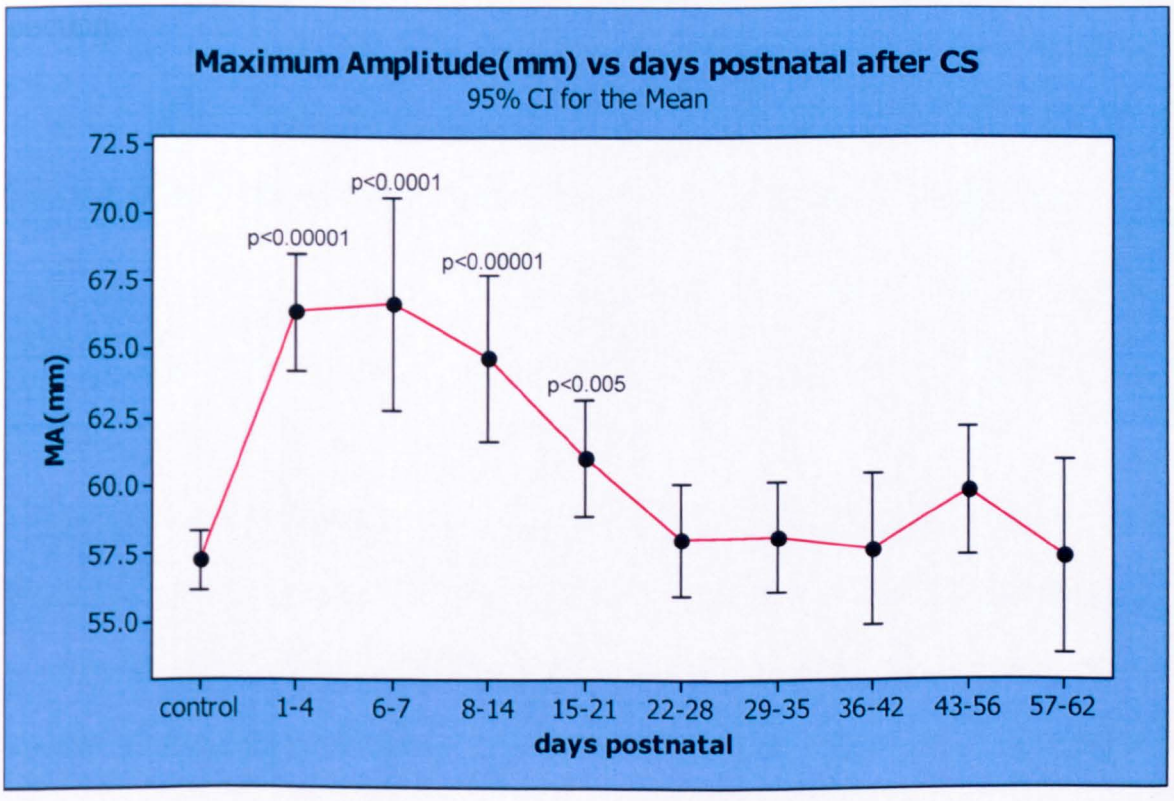

Fig. 8.9. Interval plot of MA vs weeks postpartum after caesarean section

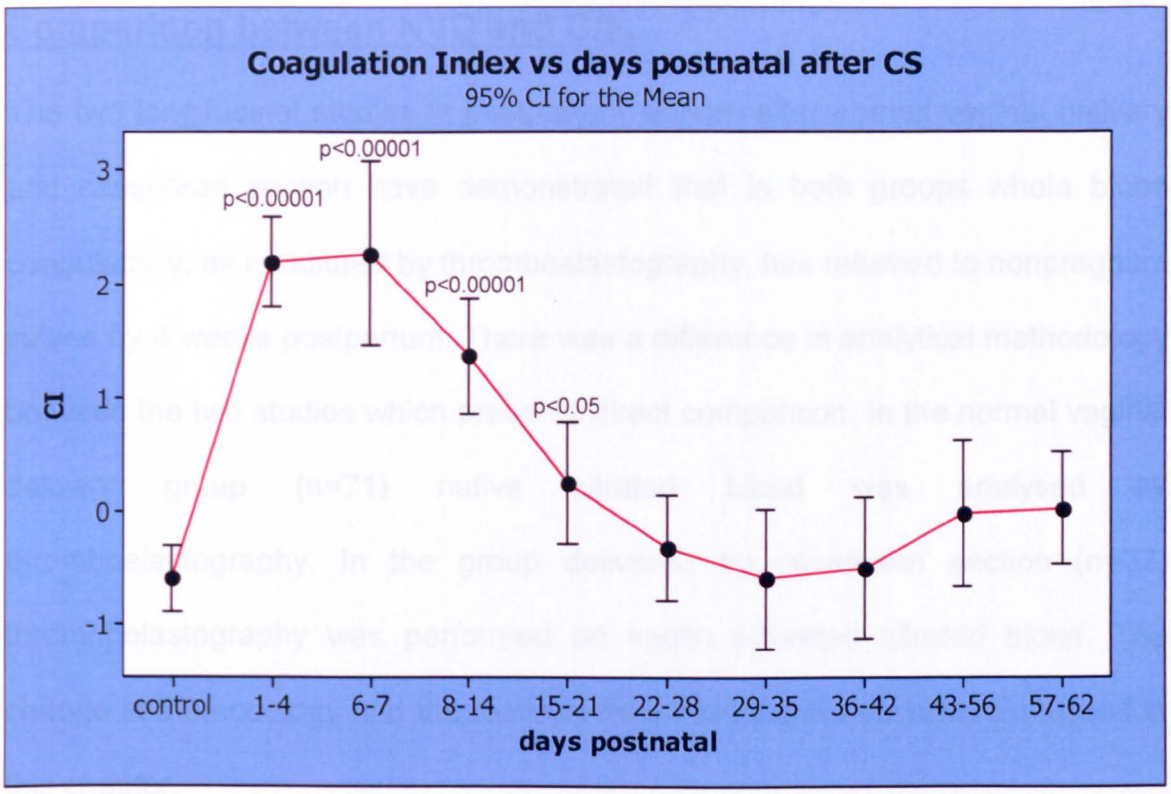

Fig. 8.10. Interval plot of Coagulation Index vs weeks postpartum after caesarean section

Comparison between NVD and C/S.

The two longitudinal studies in postpartum women after normal vaginal delivery and caesarean section have demonstrated that in both groups whole blood coagulability, as measured by thromboelastography, has returned to nonpregnant values by 4 weeks postpartum. There was a difference in analytical methodology between the two studies which prevents direct comparison. In the normal vaginal delivery group (n=71) native citrated blood was analysed by thromboelastography. In the group delivered by caesarean section (n=32) thromboelastography was performed on kaolin activated citrated blood. The change in methodology and the reasons for it have previously been discussed in this chapter.

In order to compare the rate at which coagulability returned to nonpregnant values in women after normal vaginal delivery and caesarean section, ratios have been generated compared to the non-pregnant control groups (table 8.3).

Results.

R time.

In the first week postpartum the women who had a normal vaginal delivery have a comparatively shorter R time than the women who were delivered by caesarean section (Fig. 8.11). The ratios are the same in the second week and the rate of resolution from the 2^{nd} to the 4^{th} postpartum weeks are similar for both groups.

K time.

The k time is shorter in the first week after delivery in the women who have had a normal delivery but by the 2nd week postpartum there has been rapid resolution (Fig. 8.12). The rate of resolution in the women delivered by caesarean section is slower over the first four weeks compared to the women who had normal deliveries.

α angle.

The α angle is very markedly elevated in the women who had a normal delivery in the first postpartum week. The ratios are the same in the 2nd postpartum week as is the rate of resolution in both groups from the 2nd to the 4th postpartum weeks (Fig. 8.13).

Maximum Amplitude.

The maximum amplitude is higher in the women who had a normal delivery in the first postpartum week and remains so into the second postpartum week. In the 3rd and 4th postpartum weeks the ratios of the two groups are similar as is the rate of resolution (Fig. 8.14).

This comparative study demonstrates that the time taken for resolution of blood coagulability to the nonpregnant state is unaffected by mode of delivery. The relative hypercoagulability of the women who had a normal delivery compared to

those who had a caesarean section can be explained by the thromboprophylaxis which was administered to 29 of the 32 subjects delivered by caesarean section.

	R Time (min)		K Time (min)		α angle (deg)		MA (mm)	
Week	NVD	C/S	NVD	C/S	NVD	C/S	NVD	C/S
1	0.69	0.80	0.52	0.65	1.36	1.14	1.27	1.16
2	0.88	0.90	0.86	0.74	1.08	1.09	1.16	1.12
3	0.96	0.98	0.97	0.88	1.03	1.05	1.07	1.07
4	1.00	1.00	1.02	0.96	0.97	1.01	0.99	1.01

NVD, normal vaginal delivery; C/S, caesarean section.

Table 8.3. Ratios of TEG parameters compared to non-pregnant controls in women for 4 weeks after normal delivery and after caesarean section.

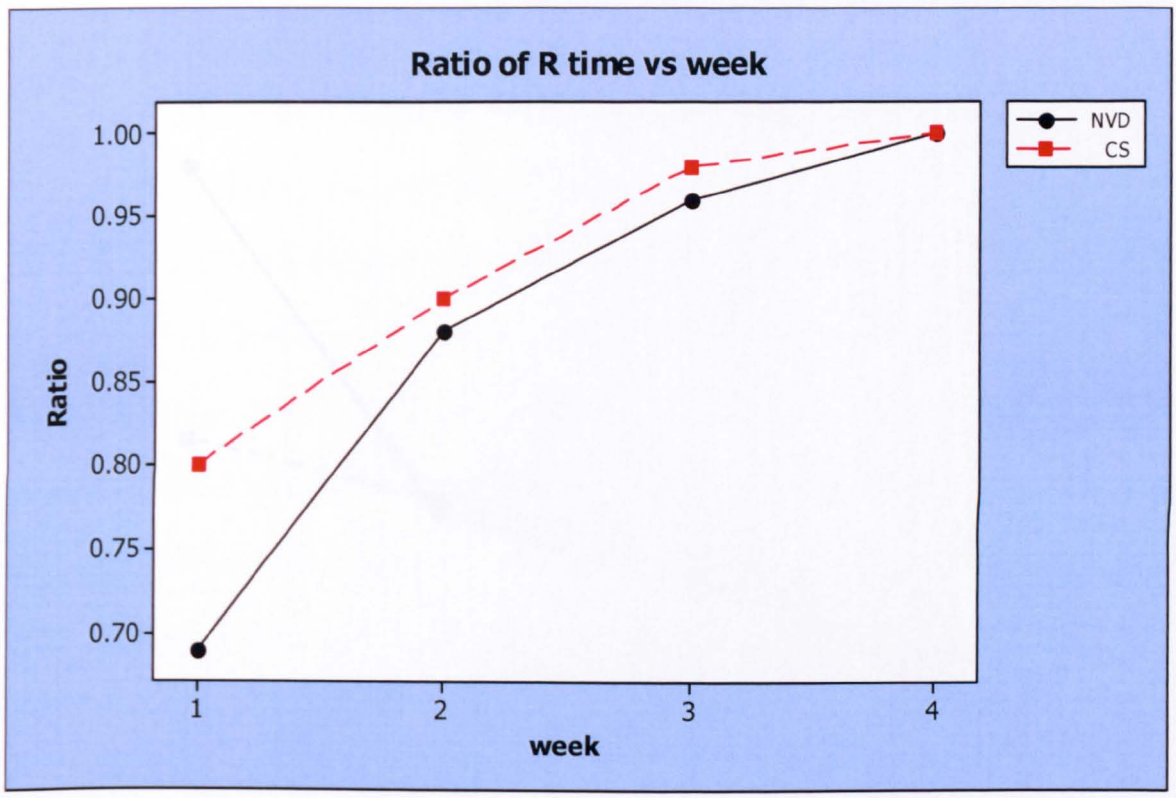

Fig. 8.11. Ratio of R time vs week postpartum for NVD and CS

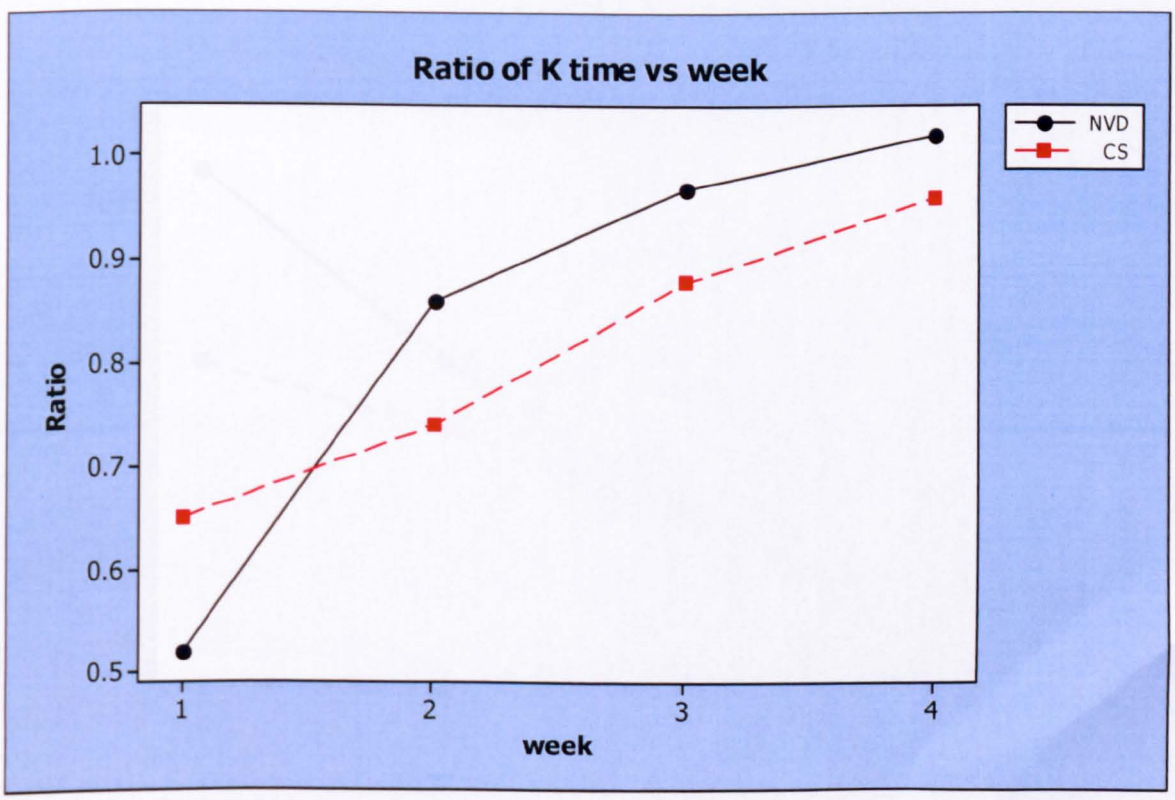

Fig. 8.12. Ratio of K time vs week postpartum for NVD and CS

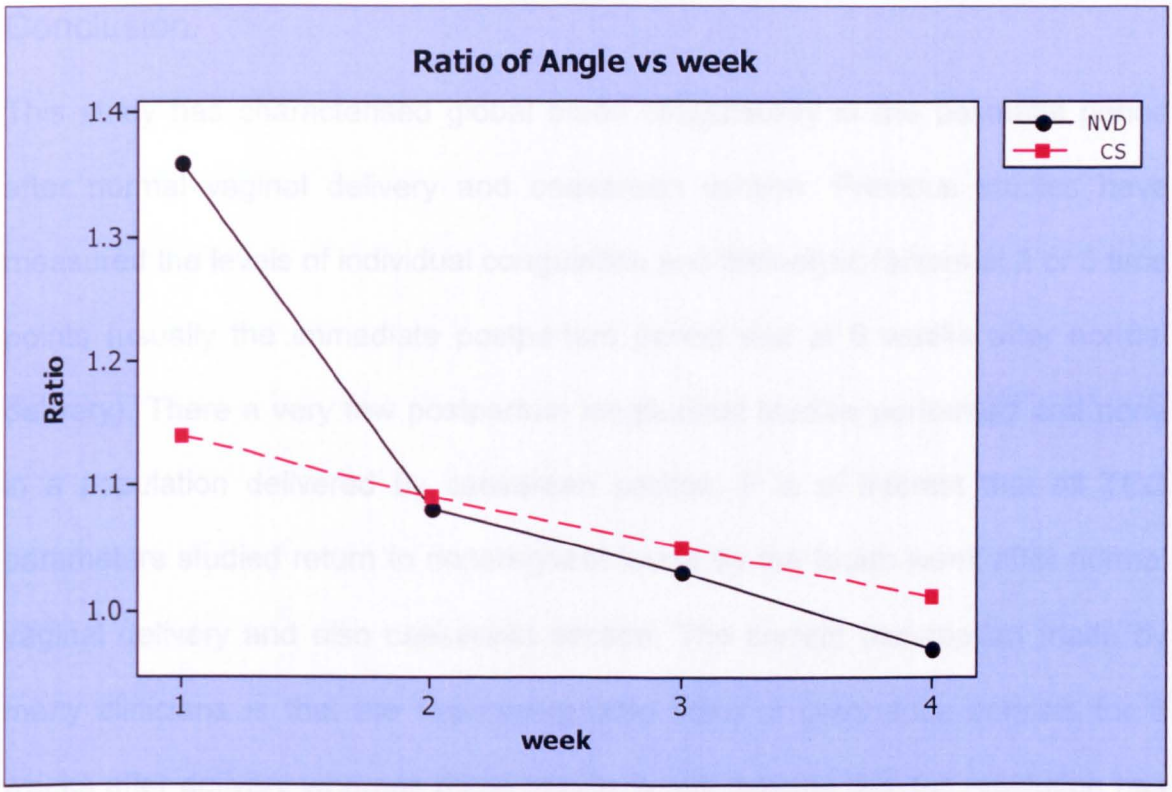

Fig. 8.13. Ratio of α angle vs week postpartum for NVD and CS

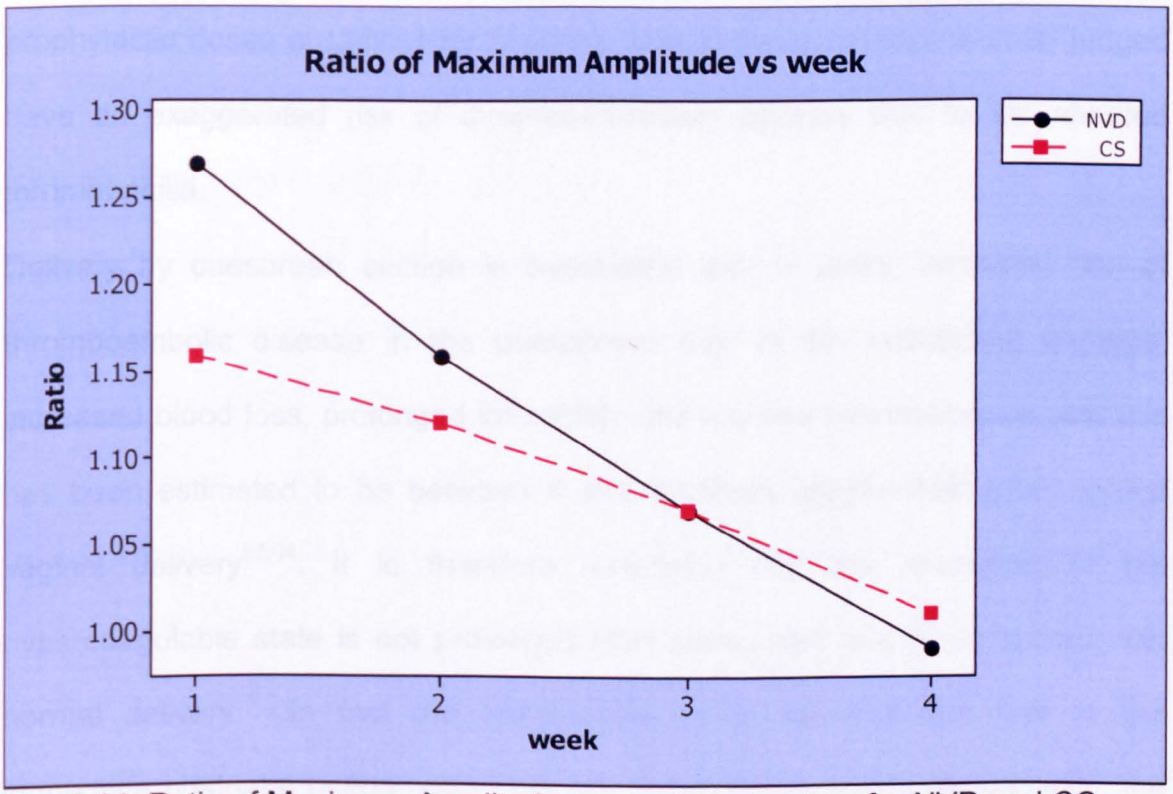

Fig. 8.14. Ratio of Maximum Amplitude vs week postpartum for NVD and CS

Conclusion.

This study has characterised global blood coagulability in the postnatal period after normal vaginal delivery and caesarean section. Previous studies have measured the levels of individual coagulation and fibrinolytic factors at 2 or 3 time points (usually the immediate postpartum period and at 6 weeks after normal delivery). There a very few postpartum longitudinal studies performed and none in a population delivered by caesarean section. It is of interest that all TEG parameters studied return to nonpregnant levels by the fourth week after normal vaginal delivery and also caesarean section. The current assumption made by many clinicians is that the hypercoagulable state of pregnancy persists for 6 weeks after delivery whereas these results would indicate that full resolution has occurred 2 weeks earlier. This may influence the practice of administration of prophylactic doses of LMWH for 6 weeks after delivery to those women judged have an exaggerated risk of thromboembolism possibly due to an inherited thrombophilia.

Delivery by caesarean section is associated with a vastly increased risk of thromboembolic disease in the puerperium due to the endothelial damage, increased blood loss, prolonged immobility and reactive thrombocytosis and this has been estimated to be between 4 and 20 times greater than after normal vaginal delivery[67,64]. It is therefore surprising that the resolution of the hypercoagulable state is not prolonged after caesarean section compared with normal delivery. In fact the comparative study demonstrates that in the immediate postnatal period, when the risk of thromboembolism is highest[60], the

women who had an uncomplicated vaginal delivery were relatively more hypercoagulable than those delivered by caesarean section. As stated previously this may be explained by the administration of prophylactic LMWH for 5 days to 29 of the 32 women studied after caesarean section. These findings also reflect the reduction in maternal deaths due to thromboembolic disease in recent years due to the widespread introduction of formal thromboembolic risk assessment in all women undergoing caesarean section and the administration of prophylactic measures including LMWH[73]. Over the same time period there has not been a reduction in the number of women dying after vaginal delivery. In 2001 the confidential inquiry into maternal deaths 1997-1999 reported that of the 10 women dying after vaginal delivery only one was after instrumental delivery, the other women were either over 35 years of age and/or overweight[74]. This prompted the RCOG to advise that all women undergo assessment for the risk of venous thromboembolic disease during pregnancy, labour and after delivery regardless of mode of delivery[75].

The results of this study demonstrate very significant differences in all the TEG parameters in the postpartum period after vaginal delivery which are maximal in the first 7 days after delivery. Heit et al have estimated that the risk for pulmonary embolism in first postpartum week is 25 times that in the third trimester[58]. As a group, the demographics the vaginal delivery group place them at low risk for thromboembolism, yet they are relatively hypercoagulable compared to the women after caesarean section. This would suggest that women who have additional risk factors for venous thromboembolism such as age over 35 years,

instrumental delivery or postpartum haemorrhage would be even more hypercoagulable and therefore be at greater risk of thromboembolism.

CHAPTER 9.

CONCLUSIONS.

Thromboelastography is a technique that enables the global assessment of haemostatic function from a single blood sample with rapidly available results. To date its use in obstetric populations has been limited. A wide variety of methodologies have been employed in studying small groups of women with a variety of pathologies and disease processes.

The description of a reference range of TEG parameters in uncomplicated pregnancy is essential before this technique can be employed in obstetric practice. This thesis defines a reference range in a tightly defined low-risk group with robust and reproducible methodology. This study, together with the longitudinal study of haemostatic function in pregnancy demonstrates that hypercoagulable changes are detectable very early in pregnancy, at 6-9 weeks gestation, and the hypercoagulable state progresses with advancing gestation.

This thesis has also examined the resolution of the hypercoagulable state after normal delivery and caesarean section and demonstrated that the resolution has occurred by the 4^{th} postpartum week and is not affected by mode of delivery. In the immediate postpartum women who delivered by caesarean section were relatively less hypercoagulable than those who had normal deliveries and it is likely that administration of low molecular weight heparin to the majority of women who delivered by caesarean section accounts for this.

The intensive study of the peripartum coagulation profiles in women delivered by caesarean section demonstrates that pre-operative starvation results in significant hypercoagulability and we would suggest that early administration of fluids should be considered. This study also demonstrates that women who, after

formal risk assessment, are judged to be at moderate and/or high risk of thromboembolism are hypercoagulable when compared to the women at low risk of thromboembolism. This suggests that the demographic characteristics used for risk assessment stratify women into the correct groups for risk of thromboembolic disease.

Further research is required to explore the use of thromboelastography in groups who experience adverse pregnancy outcomes including pre-eclampsia and intrauterine growth restriction.

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