The Effect of Endurance Exercise and its Intensity in Middle-aged Runners; Are they Thrombogenic?

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2024

Abstract

Introduction

Despite the well documented benefits of regular exercise, acute exercise induces a transient hypercoagulable state with increasing risk of thrombotic disease with age and intensity. While prior studies have used various conventional coagulation tests in studying the influence of exercise on coagulation, limited attention has been given to clot microstructure and contraction profile in well-trained individuals of middle to older age. Our aim was to identify effects of exercise on these variables using hemorheological biomarkers.

Materials and methods

Twenty-eight male and female runners aged over 40 years completed a 10 km run at moderate intensity. Of these runners,14 were reinvited to complete a 3 km run to exhaustion. Blood samples were drawn at three time-points, baseline, immediately after exercise and after 1 hour of recovery. Structural biomarker d_f and measurements of mature clot mechanical properties (Maximum Contractile Force and G'_{Max}) were analysed alongside conventional coagulation markers.

Results

While d_f remained stable following long moderate intensity exercise, higher intensity exercise caused an increase in d_f indicating a hypercoagulable phase. Following an hour of rest, d_f returned to baseline. These results indicate that the effect of acute exercise on hypercoagulability is intensity dependent and transient. Maximum Contractile Force (CF_{Max}) was reduced by exercise, irrespective of intensity. This effect was lower after an hour of rest,

suggesting that some unknown initial compensatory mechanisms are outlasted by a longer period of reduced contractile force.

Conclusion

d_f and CF_{Max} detected the hypercoagulable phase that occurred in trained older individuals as a result of exercise. Investigating these effects in more sentient populations could allow risk stratification of exercise rehabilitation programmes and their intensity.

Keywords: Clot microstructure, clot mass, clot contraction, exercise, physical activity, middle-aged adults, endurance-trained, runners

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This work has not previously been accepted in substance for any degree and is not being concurrently submitted in candidature for any degree.

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Table of Contents

Acknowledgement

I am entirely grateful to my supervisor and mentor Professor Adrian Evans for his undying support and trust, he had given on this project. His expertise and wisdom have become my inspiration and guidance in every step of the research process. My sincere thanks to you Professor Adrian. I am also grateful to my second supervisor Professor Karl Hawkins for his expertise in rheology which allow me to understand and apply scientific details to this research project and helped me appreciate its direct application to coagulation studies.

I would like to acknowledge the team in Welsh Centre for Emergency Medicine Research (WCEMR) namely Dr. Suresh Pillai, Dr. Oliver Watson, Dr. James Gregoire and Mrs. Janet Whitley for all the support they had willingly offered during the entire research activity. Surely, this will not be all possible without your help. I would like to also acknowledge Professor Ylva Hellsten for imparting her expertise in human physiology and exercise.

My sincere thanks also go to all staff and students in Rheology Laboratory, Centre for NanoHealth, Swansea University for their technical expertise and their patience while I am in their laboratory. I am also grateful to all staff in Laboratory Medicine, Morriston Hospital especially to Rachel, Jenna and Non for providing their technical expertise in analysing blood tests in this study. My gratitude also extends to the Emergency Department Morriston Hospital for the support you have given to me during my stay in WCEMR.

I would like to also acknowledge my family Dominador, Lovilia, Jonas, Jeric, Junel and Angel for the love and support you have given me all throughout this journey. Thank you

for helping me realise that nothing is impossible if you put your hearts and minds on it. I will always strive to do good things wherever my career takes me.

I would like to express my sincere appreciation to my confidante and lovingly wife, Mariette. Thank you for your patience, love and being my number one supporter, allowing me to pursue my dream and passion. Your support has been vital in this study.

Lastly, to almighty God! Thank you for your providence. I am forever humbled by your works and creation. This is for your greater glory!

List of Figures

List of Tables

List of Abbreviations

ADP	Adenosine diphosphate
APC	Activated Protein C
APTT	Activated partial thromboplastin time
APHMR	Age-predicted maximal heart rate
AT	Antithrombin
ATIII	Anti-thrombin III
ATP	Adenosine Triphosphate
β2	Beta 2
CF_{max}	Maximum Contractile Force
CI	Confidence Interval
Cm.	Centimetre
D	Dimension
d_f	Fractal Dimension
EPCR	Endothelial cell protein C receptor
EDTA	Ethylenediaminetetraacetic acid
ETP	Endogenous thrombin potential
FDP	Fibrin degradation products
FVII	Factor VII
FVIII	Factor VIII

FXII	Factor XII
FXIIa	activated Factor XII
G'_{max}	G Prime Max
GP	Gel point
GPIb/IX/V	Glycoprotein Ib/IX/V
HEX	Healthy Exercise
HMWK	High molecular weight kininogen
ILS	Institute of Life Science
INR	International Normalized ratio
IU	International units
IQR	Interquartile Range
Km.	Kilometre
L	Liter
LT	Lactate Threshold
LMI	Long moderate intensity
MI	Myocardial Infarction
Mmol	millimole
NO	Nitric Oxide
PAI-1	Plasminogen activator Inhibitor
PAF	Platelet-activating factor

PDGF	Platelet-derived growth factor
PE	Phosphatidyl Ethanolamine
PEH	post-exercise hypotension
PG	Gel point
PGE2	Prostaglandin E2
PGI2	Prostacyclin I2
PK	Prekallikrein
PRP	Platelet-rich plasma
PS	Phospholipids phosphatidyl serine
PT	Prothrombin time
RM-ANOVA	Repeated Measures Analysis of Variance
RBC	Red blood cells
SAOS	Small amplitude oscillatory shear
SD	Standard Deviation
SEM	Scanning electron microscope
SHI	Short high intensity
TAFI	Thrombin activatable fibrinolysis inhibitor
TF	Tissue Factor
TFPI	Tissue Factor Pathway inhibitor
TT	Thrombin time

TGP	Time to Gel Point
t-PA	Tissue Plasminogen Activator
t-Pa	Tissue Plasminogen activator
UK	The United Kingdom of Great Britain and Northern Ireland
u-PA	Urokinase-plasminogen activator
VEL	Viscoelastic liquid
VES	Viscoelastic solid
vWf	Von Willebrand Factor
WBC	White blood cells
WCEMR	Welsh Centre for Emergency Medicine Research

Chapter 1: The Interplay between Exercise and Cardiovascular Dynamics

1.1 Introduction and Contextual Framework

1.1.1 Exercise, Aging and Cardiovascular Vulnerabilities

Exercise is an engaging activity that helps improves one's well-being psychologically and physically. It has many well-known benefits such as improving mood [1], weight management [2], increase quality of sleep and sustained energy levels. It is well established that exercise increases longevity [3-5] and reduces the risks of acquiring major illnesses such as coronary heart disease, stroke, type 2 diabetes and cancer [6]. The importance of this has been highlighted in the UK's government proposal that adults should try to remain physically active for 150 minutes in a week to maintain an optimum level of body balance, muscle and bone functionality [7]. While these benefits are recognised to health improvement, evidence suggests that the risk of severe cardiac problems from effort-related adverse events is high especially to people with undiagnosed vascular conditions [8-10] and age-related atherosclerotic disease [11]

Sudden cardiac deaths in running or marathon events are uncommon however incidences are well documented [12-19]. Further investigation revealed that deaths were mainly caused by undiagnosed cardiac condition and life-threatening arrhythmias in both young [20, 21] and older population [15, 19] with men belonging to the highest-risk group [22]. Some of these deaths also occur in non-elite competitive events or recreational sports [12]. In Wales alone, over 400,000 adults run regularly [23] with vast majority who participates for social or recreational activity. Although the Wales national survey did not specifically mention the number of late-age adults engaged in running, it was estimated that

664,000 individuals aged more than 40 years old chose at least one type of sport or physical activity [24]. Middle-aged adults belong to a unique age category where there is an increased risk of acquiring cardiovascular diseases and its prevalence further increases with age [25, 26].

One of the contributing factors to this extensive risk is aging. It is well recognised that the vascular system undergoes structural change as people grow older [27]. Although the vascular wall is designed to withstand lifelong stress and hormonal changes, these properties can change through exposure to known modifiable and non-modifiable cardiovascular risk factors. The reduction of its overall compliance may eventually lead to a much higher systemic blood pressure [28-30], altered flow [31] and plaque formation [32-34] vulnerable to rupture, erosion or the development of necrotic core [35]. Another important component of the vessel wall is the endothelial lining. With age, increase production of endothelin, a potent vasoconstrictor and procoagulant becomes more notable. By contrast, nitric oxide, a potent vasodilator which has haemostatic properties, decreases in the vascular circulation [27]. All in all, these conditions when combined favours a highly prothrombotic environment and if such occurrence are found in middle-aged adults with acquired lifestyle diseases, the risk of myocardial ischemia or sudden cardiac death from thromboembolic events increases considerably.

1.1.2 Blood flow and thrombogenicity in health and disease

It is well recognised that exercise in healthy individuals produces an increase laminar blood flow and plasma volume change within the circulatory system. Physiologically, alterations in laminar flow among healthy individuals can be expressed by an elevated heart rate and may vary depending on the level of physical activity. Heart rate zones and lactate

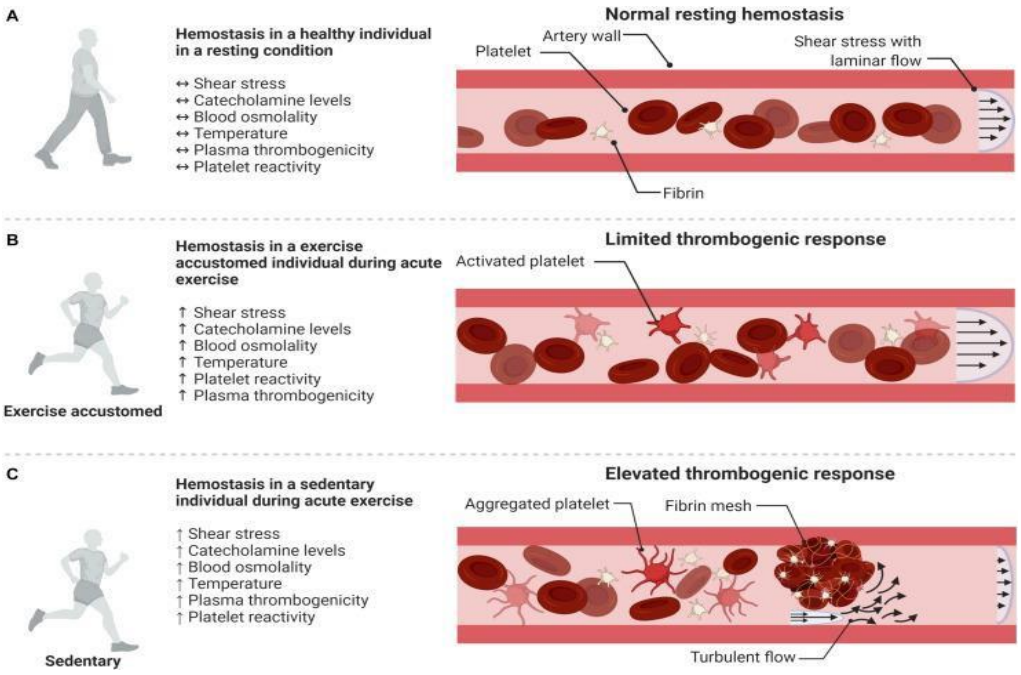
levels are prominent markers of exercise intensity commonly used by recreational runners. Consequently, a marked effect on the clotting system is known to occur that may lead to a temporary prothrombotic states [36-38] even to individuals with no underlying vascular pathology. However, this prothrombotic states subsides after 1 to 2 hours with no subsequent systemic effect [39-44], a prime example of our evolutionary adaptation to physical movement. Conversely, notable risks may persist to individuals who have early and undiagnosed atherosclerotic formation. The reason for that is because exercise can increase blood flow or shear stress around the atherosclerotic plaque which may induced a potential catastrophic activation of the coagulation system.

1.1.3 Exercise adaptation and the need for research

Exercise related risk can be mitigated by chronic adaptations [45].Regular training improves endothelial function [46], reduces platelets hyperactivity [47] and lowers systolic blood pressure when compared to sedentary controls [48]. Figure 1.1 provides a schematic illustration between healthy, well-adapted and sedentary individuals. Despite recent studies supporting the role of exercise-related adaptations, it remains uncertain how different levels of physical exertion influence clot microstructure. In addition to this, the paucity of evidence related to exercise intensity and its effect on clot microstructure underscores the need for further research and investigation. If topic is explored, we can assess how exercise and its relationship to thrombosis could lead to an aberrant clot structure and condition.

Figure 1.1

Schematic illustration of acute exercise and habitually active lifestyle on haemostasis



Note:Effect of acute exercise on (A) healthy individuals, (B) well-trained or accustomed individuals, (C) sedentary individuals. Image reproduced from "Does Exercise Influence the Susceptibility to Arterial Thrombosis? An Integrative Perspective" by Olsen et al. licensed under CC BY 4.0 [49].

In summary, exercise is known to instil beneficial effects to the human body. However, there are exercise mortality related incidences which are closely link to in vivo thrombotic changes. Risk of sudden cardiac death due to vigorous activity is high with middle and late-age adults. Fortunately, known adaptations through exercise seems to mitigate such risk. Henceforth, it is important to understand the effects of exercise and its direct impact on thrombogenicity and how exercise intensity relates to the mechanistic

development and properties of the clot itself. This could be facilitated by using a new functional biomarker of clot microstructure, namely the fractal dimension (d_f).

In the next section, a brief historical perspective on exercise themed coagulation studies will be discussed.

1.1.4 Brief historical perspective on exercise themed coagulation studies

The interesting relationship between coagulation and exercise was first known during the late 18th century by John Hunter where he carefully observed that animals who ran to death exhibited impaired clotting mechanism. However, years passed, and a much newer study was published showing a different finding [50]. It is only in the mid-20th century that research have been extensively carried out to understand the complex coagulation processes involve during exercise. Subsequent experiments observed that blood sampled post-exercise tend to clot faster when compared to blood collected during rest [51-53] supporting a previous conclusion by John Hunter and were found to be consistent between varied exercise routines [54] and intensity [55-58]. Blood clotting factors, fibrinolysis and platelet function received the most attention in many research activities during this time [59].

Over the years, a wide range of biomarkers exploring the relationship between physical activity and cardiovascular diseases have been extensively studied [60, 61]. For example, hypoxanthine is recommended to identify cardiac ischemia and exercise training status, Natriuretic peptides can be used as an indicator for cardiac muscle stress while cardiac troponin can detect cardiac tissue damage following exercise. This resulted in a much better understanding of the role of physical activity and its physiological impact particularly to the early detection of the harmful effects of vigorous activity that might be unsuitable to one's level of fitness.

However, sophisticated tests may not be readily available for urgent decision-making especially in acute clinical settings. Longer processing times and high cost are one of its many disadvantages. Alternatively, there are point of care tests developed to give clinicians better understanding on an individual's coagulation status. For example, D-dimer test can reflect the fibrinolysis (clot breakdown) during and after a physical activity. Prothrombin time (PT) and International normalized ratio (INR) are used to evaluate the extrinsic pathway. Similarly, PT and INR, activated partial thromboplastin time (APTT) are used to monitor changes in coagulation through the intrinsic pathway. Fibrinogen, platelet count, haemoglobin and haematocrit have been used to provide understanding on the interplay of red blood cells and plasma components in the coagulation pathway during exercise.

Despite notable scientific advancements, the complex effects of exercise-induced coagulation with its microstructural components remains insufficiently explored. This pertains particularly to variation across different intensities in between physical activity performed by a selected age group. Hence, there is a compelling demand for a global biomarker that can readily assess changes of coagulation with sound scientific principles. Since 2010, Fractal dimension *(d_f)* has been used and extensively studied in a wide variety of pathological conditions and has been successful in structurally identifying blood clots in healthy individuals [62]. As a biomarker, the fractal dimension represents the structural complexity of an incipient clot which can detect tighter and denser clots without the need for a labour-intensive test such as scanning electron microscope (SEM) and confocal microscopy. These tightly woven and denser clots specifically present during prothrombotic conditions are known to have properties resistant to clot lysis [63, 64].

In summary, the amount of research time poured into the field of exercise and coagulation has proven to be beneficial in our understanding of blood stasis and has opened ways to explore its role in physiology, sports science, and medicine, in general. However, more research is needed to accurately pinpoint the global changes of coagulation status while engaging in exercise.

Chapter 2: Sequential Activation of Coagulation in Exercise: Insights and Impact from the Literature

By following a sequential approach in the activation of the coagulation system, a more in-depth study investigating the impact of exercise will be discussed in this literature review.

2.1. Overview of the Coagulation system

The primary role of the haemostatic system is to serve as a protective mechanism to prevent any further blood loss by limiting further seepage of blood in the injured sites. The whole process is controlled to restrict unregulated coagulation which could progress within the vasculature and limit blood flow to the adjacent systems. The dynamic mechanism involved can be further broken down into three different phases such as primary haemostasis which corresponds to endothelial injury and formation of the platelet plug, secondary haemostasis entails the propagation of the clotting process by the coagulation cascade and tertiary haemostasis which includes the termination of clotting and the removal of clot through fibrinolysis [65]. Haemostasis depicts the equilibrium between procoagulant response and direct inhibitory pathways, all aimed at averting excessive coagulation beyond the site of injury. Disruptions in this balance leads to serious pathological conditions.

2.2. The endothelium and haemostasis

The endothelium is considered a dynamic organ that encompasses the regulation of many physiological processes in secretory, synthetic, metabolic, and immunologic functions.

It is far from what had been described before as an inert, anatomical barrier between blood and vessel wall [66]. Arteries and veins compose the largest blood vessels in the human body which have a thick, tough wall connective tissue and successive layers of smooth muscle cells. The wall is lined by a thin film of endothelial cells coated by the basal lamina which separates the surrounding outer layers. The amount of smooth muscle and connective tissue in each blood vessel may differ depending on the vessel's diameter and function. Additionally, endothelial lining is always present and can be found even in the finest branches of the vascular tree. For example, capillaries and sinusoids' walls are made up only of endothelial cells and basal lamina along with a few, sporadic amount of functionally important pericytes. Therefore, endothelial cells are evenly present in the vascular system from the heart to the smallest capillaries with the capacity to effectively transport materials such as white blood cells and active compounds of coagulation into and out of the bloodstream [67].

With its wide range, healthy endothelial cells play an active role in keeping the overall balance of the coagulation system. It serves as the binding site for anticoagulant and procoagulant factors on the cell surface which maintain blood fluidity [68]. The endothelium secretes natural anticoagulants such as nitric oxide, prostacyclin I_2 (PGI_2), prostaglandin E_2 (PGE_2), heparin sulphate and thrombomodulin. Endothelium-derived growth factor which is structurally known as nitric oxide (NO) not only plays a role in the relaxation and dilation of vascular smooth muscle through subsequent increases of cyclic guanylate monophosphate in the smooth muscle, but it also inhibits adhesion and aggregation of platelets [69] and has been shown to mediate the effect of Tissue Factor (TF) gene expression [70]. The simultaneous vascular dilation and relaxation of smooth muscles through the release of NO contributes to the reduction of vessel-obstructive related thrombosis which limits the extent of tissue ischaemia [71].

The interaction between endothelial cells and platelets or further production of thrombin enables the release of PGI_2 and PGE_2 which are known platelet antagonists [72]. Furthermore, the presence of Heparin sulphate in the endothelial glycocalyx which has high affinity to a potent thrombin inhibitor antithrombin (AT) deactivates thrombin, factor IXa and Xa in the blood circulation which prevents further activation of the coagulation system [73] [74]. Thrombomodulin which is highly expressed in endothelium combined with endothelial cell protein C receptor (EPCR) stimulates the release of activated Protein C (APC), a serine protease with potent anticoagulant activity irreversibly inactivating factors Va and VIIIa [75, 76]. All these compounds found in the vessel wall help maintain haemostasis in vivo.

However, during physical exercise, vascular laminar shear stress increases. Directed by two principal vectors, one perpendicular and the other parallel to the wall, they both exert frictional force on the surface of the endothelium [77-79] and stimulates the release of NO in the vascular system which promotes vasodilation, upregulation of eNOS activity, and superoxide production [80, 81]. Chronic exposure to such compounds leads to improvement of endothelial function accommodating long term benefits such as reduction of atherosclerotic complications. The endothelial lining produce tissue plasminogen activator (t-PA), its release is critical to clot dissolution in the system. However, unlike sedentary individuals, t-PA release is maintained in older adult men who regularly exercise [82].

With sustained dedication to exercise, endothelial sensitivity and vascular tone improves which negates acute thrombosis [83]. This aligns with notable bioavailability of NO which counteracts platelets adhesion and aggregation [84] reducing intravascular shear stress and platelet-vessel wall contact [85]. It is crucial to emphasise that persistent changes resulting from exercise were notably more pronounced in the endothelial lining compared to other coagulation indicators such as aPTT, PT, TT, FVII and FVIII [86-88]. Undoubtedly,

endothelial lining plays a crucial role in maintaining haemostatic balance both at rest and during heightened physical activity, a phenomenon highly observable to people who have undergone extensive adaptation to exercise.

2.3 Turbulent and laminar blood flow in the vasculature

There are two types of flow occurring in the vasculature namely laminar and turbulent blood flow [89] as shown in Figure 2.1 and 2.2. The location of the maximum velocity acts as the main difference between each flow. Laminar flow occurs as a linear flow. Its maximum velocity is mainly found in the middle of the vessel. Thus, follow its usual pattern. While turbulent flow as the name suggest comes from the turbulence or the disruption of the laminar flow. It is mainly found in areas where there are bifurcation [90] or plaques in the vessel wall [91, 92]. However, there are variables which affect the outcome of blood flow in the vasculature. Reynold's number is used to predict the blood flow by considering variables such as blood's density, velocity, and diameter of the concerned vessel wall. An increase of such number indicates higher blood flow or turbulence while the complete opposite happens in laminar flow. Exercise is known to increase shear stress and highly dependent to intensity and type of exercise [93]. Higher intensity produces more turbulence in blood flow [93]. Hence, the likelihood of thrombosis is increased.

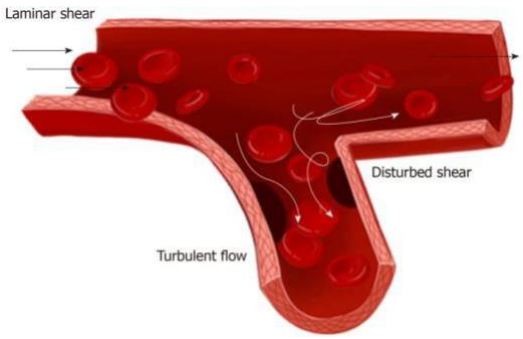
Figure 2.1

Laminar and Turbulent flow

Note: (Left) Laminar flow, (right) Turbulent flow. The image was sourced from Athel Cornish-Bowden, Laminar and turbulent flow, CC BY-SA 4.0.

Figure 2.2

Different patterns of blood flow in the vasculature



Note: Blood flow adapting to bifurcation, branching and curvature of the vessel wall. Atherosclerosis may easily develop following turbulent flow and increase shear stress in the branch points. The image was sourced from "Mechanoresponse of stem cells for vascular repair" by Tian et al. It is licensed under CC BY-NC 4.0 [94].

2.4 The Coagulation Dynamics and Exercise-Induced Modulation in Haemostasis

2.4.1 Primary Haemostasis

The first phase of haemostasis starts from the activation of platelets on the site of vascular injury. It forms the foundation of a platelet plug often referred to as the initial haemostatic response to prevent further bleeding. Vascular injury may occur in different circumstances such as atherosclerosis [95] brought about by increased response to oxidised LDL [96] or shear stress. The exposure of the subendothelial matrix and collagen creates ways to promote the recruitment of platelets, other cell types and procoagulant factors. The most important section to take part in this phase is the exposure of vWF to platelets, especially in high shear rate environments such as during exercise. It acts as a bridge between tissue and collagen at the site of injury through the GPIb/IX/V glycoprotein receptor and together with lipid mediator platelet-activating factor (PAF) synthesized by endothelial cells supports the activation and adhesion of platelets in the endothelial lining. Platelets procoagulant response are directly proportional to endothelial shear stress and exercise intensity [97-99].

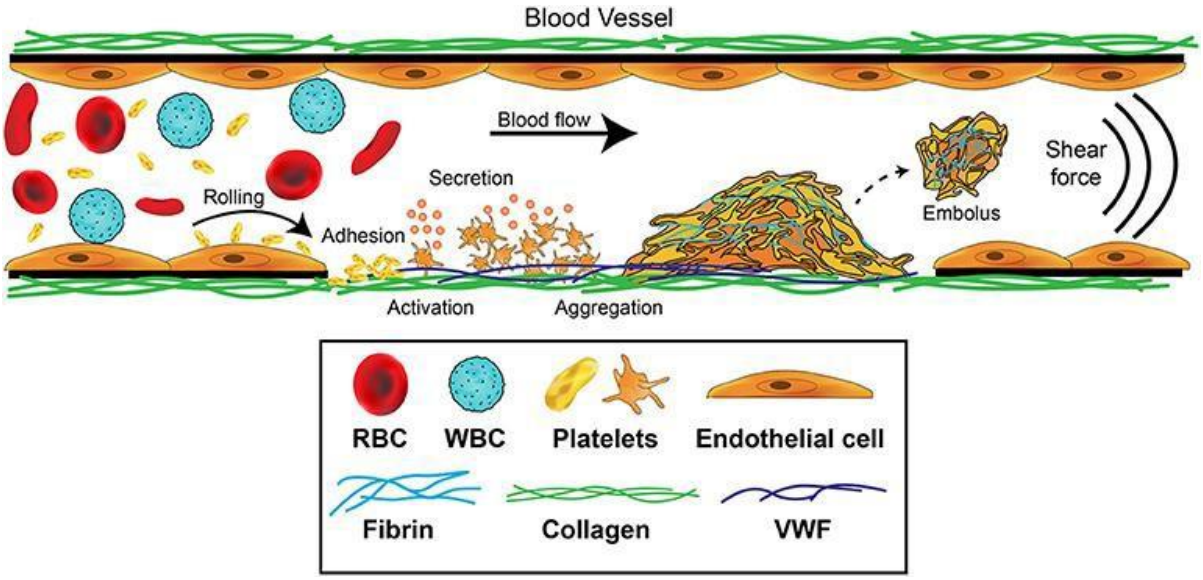
2.4.1.1 Platelet adhesion

There are four different processes at play during platelet activation. These are adhesion, aggregation, secretion and successive procoagulant activity. Platelet adhesion happens when a platelet encounters a break in the integrity of the vascular lining. It immediately interacts with collagen fibrils which subsequently allow platelets to change their morphology losing their discoid shape and projects multiple filopodial as it undergoes cytoskeleton

rearrangements. This transformation makes them extremely adhesive. Platelets further secrete thromboxane A2 (TxA2) and adenosine diphosphate (ADP) in the blood circulation. The release of these compounds enables more platelets to collectively adhere to the injured site and allows smooth muscle contraction necessary for the build-up of the haemostatic plug. It occurs due to the release of endothelin by the injured endothelium [100] and through vascular myogenic response [101]. The inability of the damaged endothelium's vessel wall to produce innate compounds such as NO, heparin sulphate and thrombomodulin further enhances the coagulation process. Figure 2.3 briefly illustrates this process.

Figure 2.3

Platelet's role in the vasculature during thrombus formation



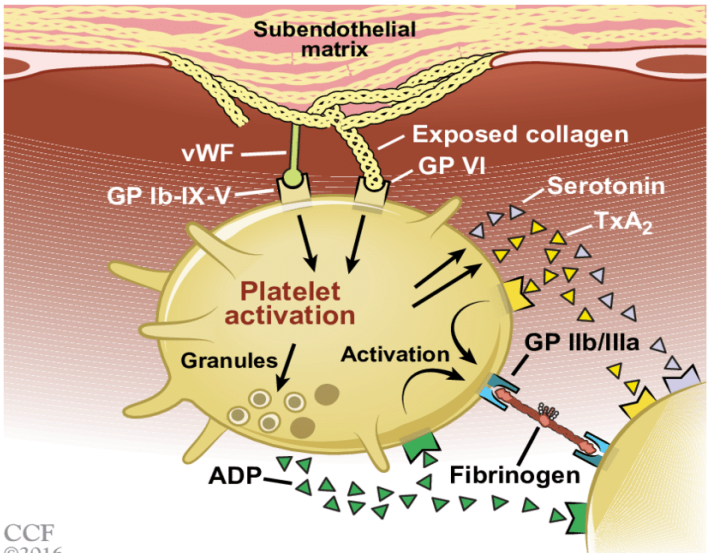
Note: Reaction of blood components including platelets to subendothelial injury and how shear force enacted on the vessel wall contributes to embolus formation. The image was sourced from "Imaging Platelet Processes and Function—Current and Emerging Approaches for Imaging in vitro and in vivo" by Montague et al., licensed under CC BY 4.0.

Current exercise-related studies by Wang et.al claimed that overall, there is an increase platelet adhesion following acute exercise [102-105]. This is evident by a stronger platelet-surface interaction which authors viewed as an exhaustive response to physical activity. However, it cannot be denied that there are studies who disprove such claim. One study proved a decrease of platelet adhesion to fibrinogen as determined by shear-stress using rotational viscometer [106] while other studies using different experimental techniques showed no changes after acute bouts of activity [107, 108].

2.4.1.2 Platelet aggregation

Figure 2.4

The platelet aggregation cascade



Note: The contact of platelet to subendothelial matrix triggers adhesion to vessel wall, activation, and aggregation. This image was sourced from "Dual antiplatelet therapy for acute coronary syndromes: How long to continue?" by Halkar et al. Image taken with permission for

Platelets also bind to circulating fibrinogen through an abundant platelet integrin aIIb3 formerly known as glycoprotein IIb/IIIa [109, 110] as shown in Figure 2.4. A large clump of platelets can easily accumulate in the injured site. Each platelet contains 40,000 to 80,000 copies of aIIb3 on its surface. Following platelet activation integrin aIIb3 transforms from a low affinity to a high affinity fibrinogen receptor leading to an "inside-out" signalling mechanism. Since fibrinogen has a divalent symmetrical molecule, it allows the binding of two activated aIIb3 complexes on two activated platelets thus enabling the cross-linking process. In addition to this, aIIb3 also binds to inactive VWF in the subendothelial matrix through its cytosolic components and easily binds to a platelet cytoskeleton resulting in platelet spreading and clot contraction also known as "outside-in" integrin signalling. The cytosolic interactions by the activated aIIb3 complex enable receptor-ligand interactions on the external surface of the membrane in two directions [110, 111]. Hence, the resultant platelet plug structure becomes more stable as platelet aggregation happens over time.

Platelets are known to be activated after exercise and its numbers increased momentarily. This change is a combination of haemoconcentration and rapid release of platelets by spleen, liver and kidneys and may be triggered by elevated epinephrine and collagen in the system [112] and were also found out to be dependent on exercise intensity [113-115]. Notably, a study by Ebrahimi et.al, showed that circuit training produces higher increases of platelet activation than resistance training [116]. Additionally, in a study by Ersöz et.al [112], platelet aggregation was induced by the release of collagen during submaximal exercise of upper extremity with no effects in thromboxane B2 and ADP. In

contrast, incremental exercise activates collagen, thromboxane B2 and ADP. Therefore, an intensity dependent physical activity induces platelet aggregation through collagen-led activation.

2.4.1.3 Platelet Secretion

Platelet secretes two types of granules, alpha and dense. Alpha granules contain proteins such as fibrinogen, vWF, thrombospondin, platelet-derived growth factor (PDGF), platelet factor 4 (PF4), and P-selectin while dense granules owing to their dense appearance on the electron microscope have Adenosine Diphosphate (ADP), Adenosine Triphosphate (ATP), ionized calcium, histamine and serotonin. Dense granules are far more specific and less than its counterpart alpha granules [117].

Research investigating the role of biochemicals secreted by platelets following exercise have shown conflicting results [37]. The majority of these studies supported increases of alpha granules PF4 [102, 118-120], vWF [86, 121, 122], fibrinogen [39, 123, 124], while P-selectin showed varied results interpretation [37], and no studies were found in relation to thrombospondin and PDGF responses. Dense granules, however, showed a similar picture with notable increase in ATP [125] 21, ADP [125-127]15, 80, 21 and calcium [104, 105, 128, 129] following exercise.

2.3.1.4 Platelets procoagulant activity

The procoagulant activity as determined by the interplay of platelets with other clotting components is an important aspect of platelet plug formation needed to support

complex adhesive and platelet-activation-dependent procoagulant mechanisms and the activation of the clotting cascade or “cell-based” model of coagulation [130]. The process involves linear polymers inorganic polyphosphate (polyP) from the dense granules leads to the activation of factors V, XI and XII, deactivation of tissue factor pathway inhibitor (TFPI), enhancement of thrombin activatable fibrinolysis inhibitor (TAFI) and downregulation of fibrinolysis. Secondly, for thrombin generation to be initiated and propagated, a partially activated factor V is released from the platelet’s alpha granules which supports the activation of the extrinsic pathway. Lastly, once the platelet is activated, its inner membrane releases negatively charged phospholipids phosphatidyl serine (PS) and phosphatidyl ethanolamine (PE) which supports the assembly of tenase and prothrombin complex on platelet outer leaflet membrane [131, 132].

2.4.2 Secondary Haemostasis

This section aims to present the two models of coagulation namely the cascade and cell-based model of coagulation. It is crucial to emphasize that cell-based model of coagulation is the most updated body of knowledge focusing on the roles of specific cell surfaces in controlling the process of coagulation. However, to provide much deeper context, organised thought delivery, basic information with regards to enzymes activation and synchronicity with past exercise-themed studies, the cascade model will be presented accordingly.

2.4.2.1 The Cascade model of Coagulation

In the coagulation system, the activation of enzymes leads to a cascade effect of serine proteases being activated altogether via limited proteolysis. The final goal is the polymerization of fibrin and the activation of platelets to form a stable plug in the site of injury. The coagulation cascade can initially be subdivided into two parts, the intrinsic and extrinsic pathway, which later merges into a common pathway to form a blood clot as illustrated in Figure 2.5.

2.4.2.1.1 Intrinsic Pathway

The intrinsic pathway starts from the activation of FXII, a zymogen latent serine protease, which converts to Factor XIIa when exposed to endothelial collagen after an endothelial injury. This is facilitated by high molecular weight kininogen (HMWK) and prekallikrein. The activation acts as a catalyst for factor XI to be activated to FXIa which then subsequently, leads to the activation of factor IX to factor IXa. Factor IXa with its cofactor (factor VIII) form a tenase complex on a phospholipid surface to activate factor X into its active form Factor Xa [133, 134]. This step-by-step process was considered by David, Ratnoff and Macfarlane as a “cascade” or “waterfall” effect as the activation of proenzymes leads to the downstream actuation of more enzymes [135]. However, it is important to point out that when a particular step in the cascade is completed, the concentration of the subsequent factors increases. For example, once factor XI is activated, the concentration of factor IX in the blood will be much higher. Furthermore, once the intrinsic or extrinsic pathway activates factor II, it allows reinforcement of the intrinsic pathway through positive

feedback by factors V, VII, VIII, XI, XIII making factor XII, a less critical component of this mechanism [136].

The intrinsic pathway is clinically measured through a conventional coagulation marker activated partial thromboplastin time (aPTT) and has been widely acknowledged that during exercise the primary activator of coagulation is the contact or intrinsic pathway through subsequent expression of factor VIII in the system [40, 42, 43, 53, 86, 87, 98, 122, 137-141] and is highly related to exercise intensity and individuals training status [40]. The activation of FVIII during exercise may have been the result from the activation of previously inactivated FVIII [142, 143] and the protective effect from activated protein C proteolysis by the highly expressed formation of vWF-FVIII complex in the vasculature [144-146]. As a result, Factor VIII may increase 200-400% during exercise [147-149].

2.4.2.1.2 Extrinsic pathway

Once a rupture in a blood vessel occurs, the plasma is exposed to tissue factor (TF) expressing cells in the vasculature or in the extracellular matrix such as fibroblasts. The plasma circulating FVII becomes activated and easily binds to TF forming FVIIa-TF complex. This newly formed complex activates FIX and FX. The activated FXa triggers more FVII conversion into its activated form, FVIIa. Hence, accelerating the whole coagulation process through a positive feedback loop mechanism. Factor Xa alone, in the absence of cofactor FVa can produce small amounts of thrombin from its interaction with prothrombin which can lead to the activation of FV and FVIII, cleave FIX to its activated form FIXa, and initiate platelet activation through PAR-1 and PAR-4 [150, 151]. The activation of factor Xa leads to the common pathway which unifies the intrinsic and extrinsic pathways into the

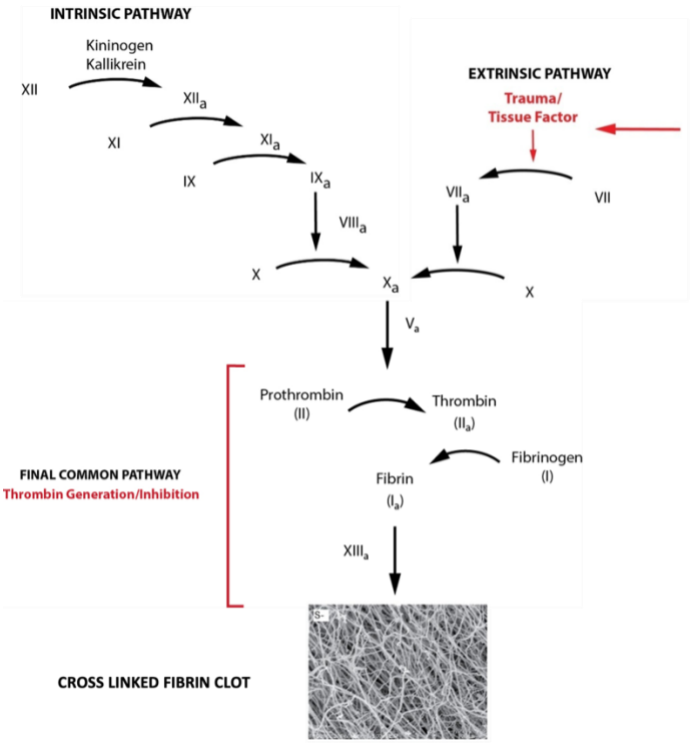
common pathway. The extrinsic pathway takes a shorter time than its intrinsic counterpart and is clinically measured as the prothrombin time (PT).

2.4.2.1.3 The common pathway

When factor X is activated to factor Xa, factor II (prothrombin) gets actuated into factor IIa (thrombin), which allows the activation of fibrinogen into fibrin. The presence of thrombin permits other factors of the intrinsic pathway such as factor XI and cofactors V, VIII and factor XIII to be activated. Fibrin subunits congregate to form fibrin strands while factor XIII acts on it to create a fibrin mesh. The formed meshed structure stabilised the platelet plug.

Figure 2.5

The traditional based model of coagulation cascade



Note: The step-by-step activation of enzymes from two pathways namely intrinsic and extrinsic which leads to the formation of a cross-linked fibrin clot. The image was sourced from Prof. Phillip Adrian Evans with permission to use and reproduce in this dissertation.

2.4.2.2 Cell based model of coagulation.

The cell based model of coagulation is divided into three overlapping phases namely the initiation, amplification and propagation [152].

2.4.2.2.1 Initiation

It has been widely accepted that Tissue Factor (TF) is the main physiologic initiator of coagulation. TF can be activated by plasma contact through the TF-bearing extravascular cells. Circulating factor VII in the plasma can quickly binds to TF and subsequently cause the start of coagulation. Once FVIIa/TF complex is formed, it can activate Factor X and factor IX. Factor Xa activates plasma factor V. On the one hand, Factor X can be inhibited by TFPI or ATIII if it goes beyond the protected environment of the cell surface. On the other hand, Factor Xa which remained in the cell surface can combine with Factor V which in turns generate a small amount of thrombin [153]. This vital process of thrombin generation within the cell surface activates platelets and FVII and plays a major role in the next phase.

2.4.2.2.2 Amplification

To understand this phase, it is crucial to remember the damage incurred earlier in the vasculature. This break in the system allows platelets and plasma components to get in contact with extravascular tissues. As platelets quickly adhere to subendothelial matrix, it undergoes further activation and adhesion secreting further procoagulant particles. This gets amplified by the thrombin already generated during the initiation phase, fully activating surrounding platelets, factors V, VIII and XI [153]. Now that all cofactors are activated, a large influx of thrombin starts to flood the system and leads to the next phase.

2.4.2.2.3 Propagation

It is important to highlight that during this phase, several “tenase” and “prothombinase” complexes are formed on the platelet surface. Platelets are known to have

high affinity binding sites for factors IX, X and XI.

To begin with, unlike factor X, factor IXa can easily reach the platelet surface since it is not rapidly inhibited by ATIII. It assembles a tenase complex known as factor VIIIa/IXa. In addition to prior reaction, circulating thrombin generated from the amplification phase allows factor XI activation and binds to platelets. This opens the synthesis of additional factor IXa in the platelet surface. The overexpressed factors VIIIa/IXa complex activates factor X and subsequently forms a complex with its cofactor V. The combined Factor Xa/Va allow generation of more thrombin paving the way for the formation of haemostatic fibrin clot.

2.4.2.2.4 Uses of cell-based model of coagulation

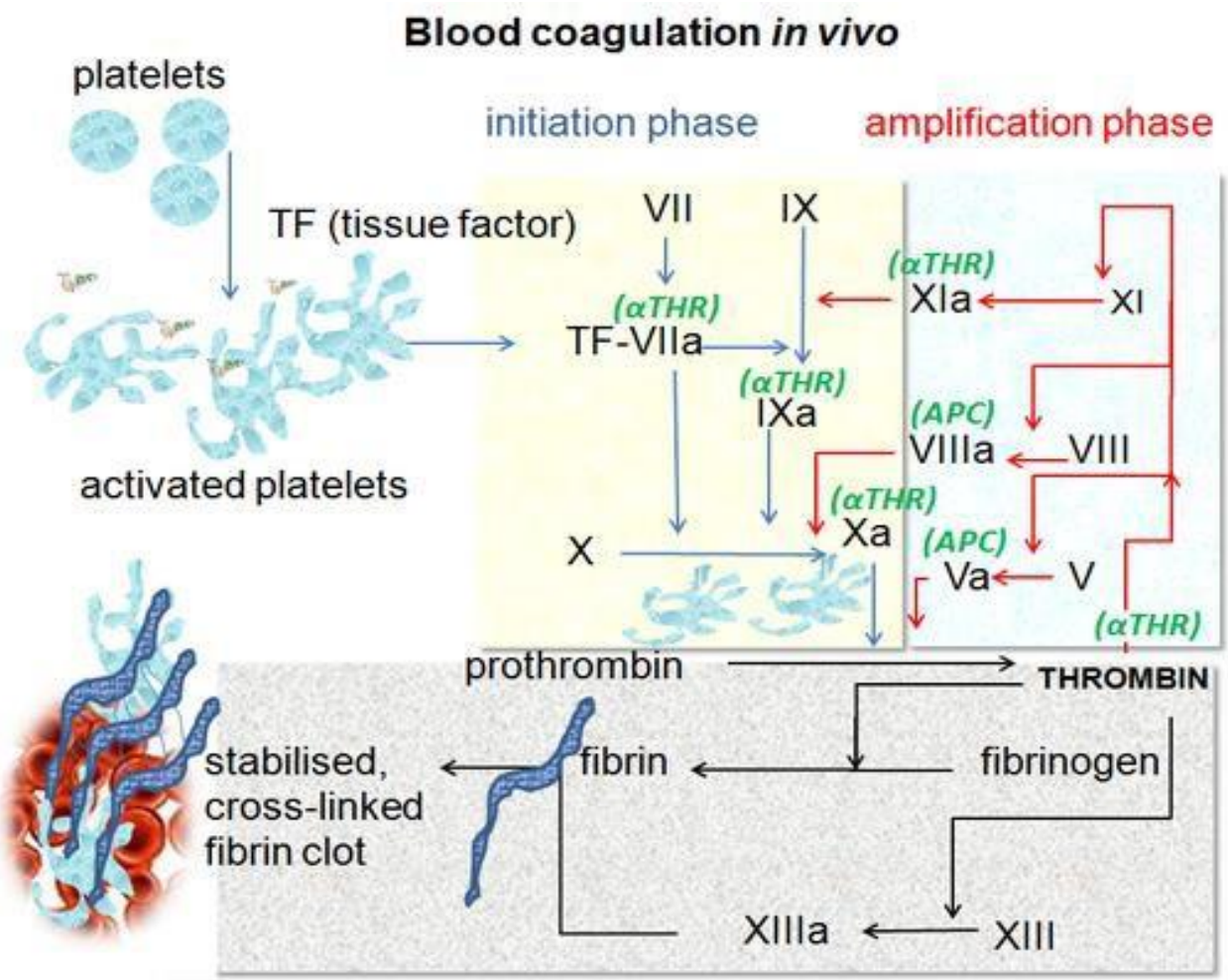
The cell-based model of coagulation as illustrated in Figure 2.6 was able to explain concepts behind bleeding diathesis previously unsupported by the cascade model. It was able to establish the pathophysiology of clotting factors deficiencies. For example, in many biological systems deficiencies in the initial components of intrinsic pathway (FXII, HMWK or PK) allow a marked prolongation of aPTT with no associated bleeding tendencies. Furthermore, some mammalian species such as whales and dolphins maintain blood stasis despite a lack of FXII in their system. Additionally, a condition involving FXI deficiency could led to variable haemostatic effects with others presenting a severe form of haemorrhage. Moreover, people with FVIII and IX deficiency i.e., haemophilia A and B can experience haemorrhagic episodes despite an intact extrinsic pathway. Similarly, even though people with FVII deficiency have an intact intrinsic pathway, they can still present with bleeding episodes.

Clearly, cell-based model of coagulation explains the dependence of each clotting factors reacting in parallel pathways to maintain haemostasis while the cascade model utilised non-

physiologic representation of clotting process in vivo. However, since cascade model was formed in a structure that can easily be understood, it can be a tool to help form our basic understanding of clotting factors' subsequent activation and reaction in the coagulation system.

Figure 2.6

The cell-based model of coagulation



Note: The cell-based model of coagulation and how different enzymes interlinked to activate and stabilised a cross-linked fibrin clot. The Image was sourced from Dr Graham Beards, Coagulation in vivo, CC BY-SA 3.0.

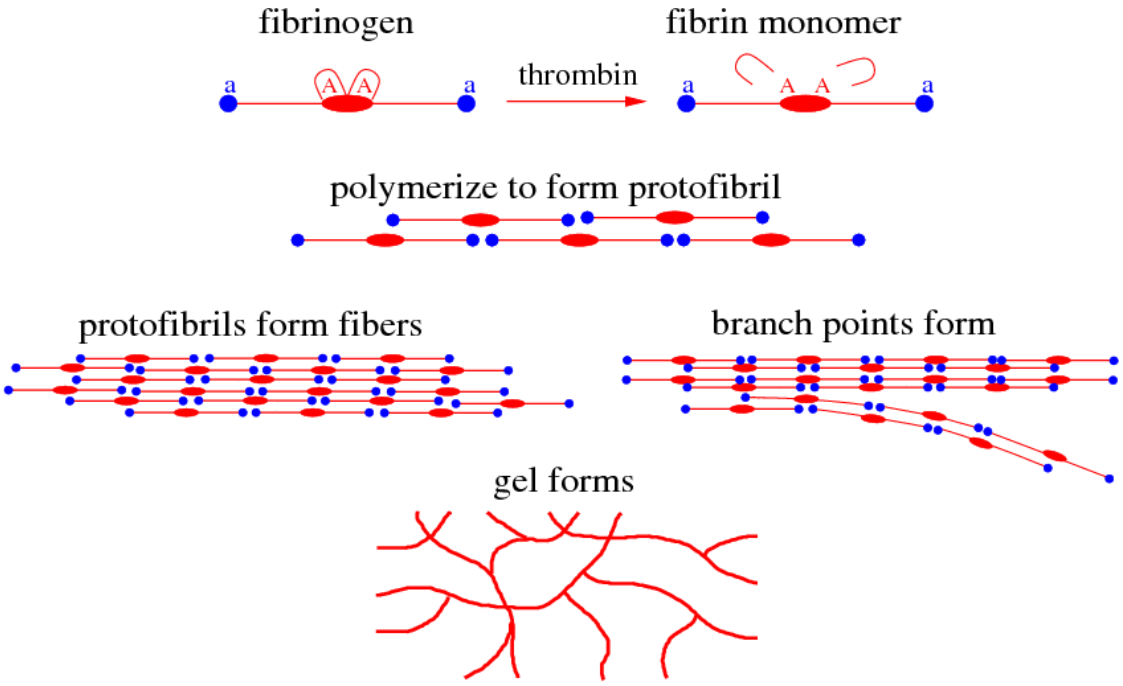
2.4.2.3 Formation of Fibrin Strands

Fibrin clot is the end-product of many processes and interactions of several clotting factors with fibrinogen as schematically illustrated in Figure 2.7. However, its importance not only lies at the end stages of the coagulation cascade but also plays a role in the initial linkage of platelets during its activation and aggregation stages [154]. A steady stream of plasma fibrinogen comes from the hepatocytes of the liver- which can synthesize 1.5 to 7 grams per day. The source can also come from body's own intracellular reserve [155]. Its production is regulated by transcriptional and translational mechanisms. After such, they assemble into Aa- and Bb-y precursors, which in turn becomes Aa/Bb/y molecules and are converted to hexameric complexes known as $(Aa/Bb/y)_2$ before being released to the circulation.

Fibrinogen remains dormant until thrombin cleaves N- terminal fibrinopeptides from the Aa and Bb chains. The newly exposed a and b knobs insert into a- and b- holes in the gC and bC regions of the D nodule of another fibrin monomer. This formation allows half-staggered association of developed fibrin monomer into protofibrils. Following this, protofibrils aggregate into fibres which allows build-up of crosslink fibrin mesh that helps to stabilise the blood clot [156-160].

Figure 2.7

Schematic of fibrin formation



Note: Schematic representation of formation of fibrin highlighting the role of thrombin fibrin polymerization. This image was reproduced from University of Utah (pending permission).

There are conflicting results pertaining to the effects of exercise on fibrinogen [161] [141]. Other studies support the idea that fibrinogen concentration increases following maximal exercise and can quickly return to baseline level after 30 minutes [124] [39], however there were also research that completely disprove this finding [161] and could be due to different methods used. Additionally, training could have a large impact on the improvements of fibrinogen levels to specific individuals. Adaptations to exercise are well reflected to people who previously do not exercise or just relatively healthy. In a study made by Aloulou et.al. and Kilic-Toprak et.al., they support the notion that sedentary individuals and healthy young males produced less fibrinogen after a series of prescriptive regimens [162, 163]. Similarly, such findings are found with people who had ischemic events [164].

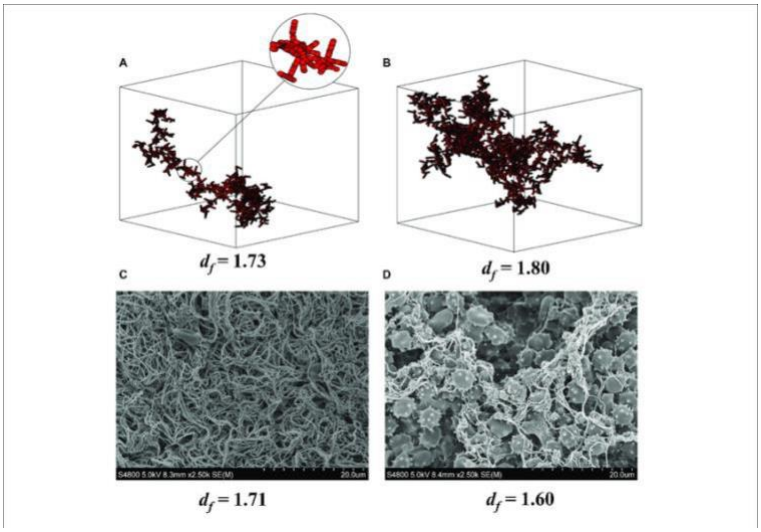
On the other hand, endurance runners and trained athletes showed no change of fibrinogen levels even after an intense activity or exercise [122, 138] suggestive of the body's adaptability or ability to accommodate increases of prothrombotic episodes following exercise. It is also important to highlight that fibrinogen concentration has been shown to have been associated with age [122, 138]. Researchers linked such association to the pro-inflammatory condition of older individuals.

2.4.2.4 Clot microstructure

The importance of clot microstructure and its contribution in identifying the structure functionality of the formed clot is only just beginning to be fully understood. A newly formed clot produced from the interplay of different factors during the early phase of coagulation ends up with a fibrin network that is entangled and disordered. A much denser tightly packed fibrin network composed of thinner fibres is far less susceptible to lysis due to its impermeable characteristics. By contrast, loose, open, and permeable fibrin network enables lysis enzymes to degrade blood clots easily. By understanding this effect, a more broaden structural perspective of the coagulation system has opened. Figure 2.8 depicts a clear comparison between two different fractal networks of a blood clot.

Figure 2.8

Comparison of two different fractal networks



Note: (Above) Computer modelling of (A) normal fibrin fractal network, (B) highly dense fibrin fractal network. (Below) SEM of (C) normal fibrin network (D) less dense fibrin network. Image courtesy of Prof. Phillip Adrian Evans.

Currently, we are beginning to explore its role in identifying haemostasis in healthy population [62, 165] and thrombogenicity on highly prevalent pathological vascular conditions [166-173]. Its full potential may lie on identifying the structural component of the clot and its deterministic way of predicting its own future morphology [174-176]. This can be done by utilising the latest and most sophisticated rheological techniques exploring ex vivo clots viscoelastic properties. This technique produces a single quantitative value rather than the subjective identification of the clot microstructure (e.g. more, less, tightly, dense) [177].

Rheology draws upon principles of physics and theory of polymerisation that allows a numerical description of the fibrin network.

Furthermore, it has been well validated that in acute exercise, clot microstructure showed a similar thrombogenic characteristic consistent with other exercise-themed studies [178, 179]. However, the difference of the population group may pose variation in its clot microstructure profile as previous studies focus was different, volunteers were mainly untrained yet young individuals. Hence, undeniably more research is still needed to fully grasp its mechanistic implications in the coagulation system especially in older population

2.4.2.5 Clot contraction

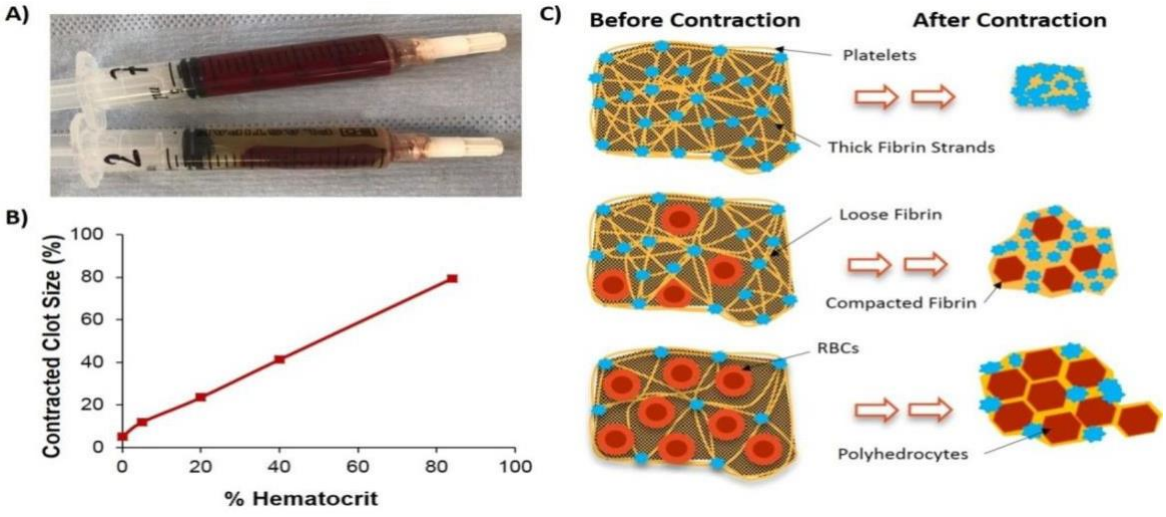
Clot contraction refers to the shrinkage of the blood clot over time by a contractile protein followed by a subsequent expulsion of blood plasma [180]. It results to the tightening of the clot network. The whole mechanism is well known to play an integral part in the overall physiologic process and has been shown to be impaired in many known disease conditions. An illustration showing this effect can be seen in Figure 2.9.

One of the main functions of clot contraction in vivo is to enhance haemostasis by stabilising the clot and assist in building up a strong and impermeable seal at the site of injury. It allows recanalization making sure that there is enough blood flow supplying the structure distal to the injured site. It starts when non-muscle myosin IIA located in the cytoplasm of activated platelets interacts and pulls on actin filaments which in turn generate traction forces along the fibrin network through mechano-transduction of adhesive molecules [181]. This action allows clot mechanical compression with subsequent squeezing of the plasma serum [182].

As previously discussed in section 2.2.2, the integrin aIIb3 serve as the main mechanical and structural bridge between extracellular fibrin matrix and intracellular actin. The complex is bound to integrin through talin [183]. The aIIb3-fibrinogen binding initiates outside-in signalling that enhances platelet contractility [184]. When viewed microscopically, platelet filopodia latched to fibrin fibres can make a kink on individual fibres and in the process creates a pulling motion toward the platelet body [185] as seen in Figure 2.10. As platelet aggregates over time, it causes compaction of the surrounding network and induce dramatic remodelling which decreases clot volume accompanied by increased density and stiffness as well as reduced porosity and permeability of the build-up clot [180].

Figure 2.9

Blood clot contraction



Note: Schematic of blood clot contraction. The image was sourced from "Mechanical behaviour of in vitro blood clots and the implications for acute ischemic stroke treatment" by Johnson et al. with permission to reuse from BMJ journal.

Unfortunately, there are limited accounts employing the most up to date techniques measuring clot contraction in exercise. Most of these studies remained focus on pathological

diseases. On the other hand, there were known exercise-themed studies which had previously focused on aIIb3 activity and platelet aggregation as well as the response of thrombin and fibrinogen components during exercise. As they play a major role in clot contraction, it is necessary to review to such topics that may partly support an association to the mechanism of clot contraction during physical activity.

2.4.2.6 *aIIb3 and platelet activity potential role in clot contraction*

Following platelet activation, aIIb3 present in the platelet's outer surface acts as link between extra and intracellular components of platelets and fibrin. However, previous studies showed conflicting results in determining the multitude effects of physical activity on aIIb3. Some demonstrated increases [186, 187] while others showed no changes [47, 106, 125, 188-190]. Similarly, studies associated with platelet aggregation defined inconsistent findings, with a majority showing an increase of platelet aggregates after exercise [112, 126, 128, 186, 191-198], some showing no such effects [107, 199-201], or even direct inhibition of platelet aggregation by exercise [118, 140, 202, 203]. Furthermore, the associated platelet response to clot contraction may differ with different participant profiles. For example, evidence of increase platelet aggregation to trained individuals [204] were noted while exhaustion of platelet response has been observed after repeated and routine exercise [86] which in longer terms could lead to reduced platelet aggregation [47]. Therefore, there is no definite conclusion that can be made in terms of aIIb3 activity and subsequent platelet aggregation in trained individuals.

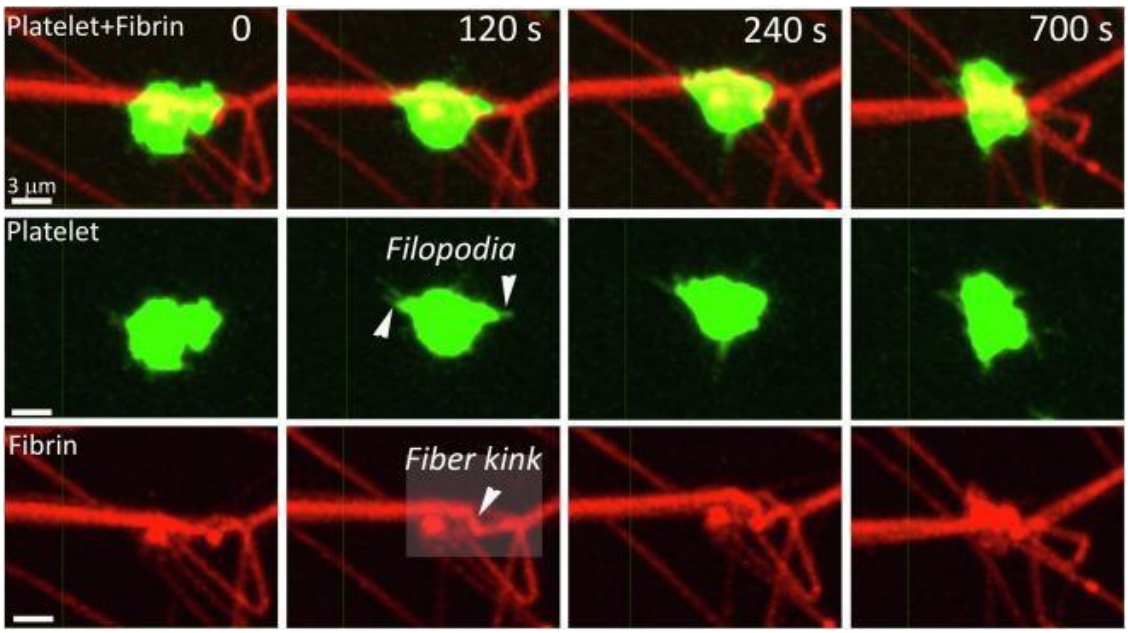
2.4.2.7 Thrombin and potential role in clot contraction

In a study made by Li et al, direct thrombin inhibitors such as Argatroban and Enoxaparin were used to study thrombin's response following exercise. Authors found that platelet activation occurred despite the absence of thrombin activity [205]. Notably, a parallel study was carried out studying the effect of unenhanced endogenous thrombin potential (ETP) immediately after exercise with athletes. Results showed no increases of ETP after exercise despite a moderate 5% increase of ETP seen after 2 hours [138]. Consistent results were observed in middle aged runners following a marathon race [122] and could be suggestive of an individual's adaptation to thrombin activation [206]. On the other hand, Rock et al. argued a 42% to 152% increase of thrombin activity on distance runners [119]. These contrasting views could be the result of different methods used, or conditions previously set to test the research hypothesis and might have been greatly influenced by confounding variables.

Furthermore, it is important to emphasize that up-to-date literature pertaining to clot contraction imaging used in this study utilised a thrombin reactant in a fixed concentration to initiate clot response in-vitro [207]. A more detailed approach on this test will be discussed in a later chapter. Nonetheless, clot contraction thrombin-led response following exercise remains inconclusive.

Figure 2.10

Clot contraction and fibrin components.



Note: Platelets reaction to fibrin contact, releasing its filopodia to initiate fibrin to fibrin kink, a precursor to clot contraction. Image was sourced from "Blood clot contraction: Mechanisms, pathophysiology, and disease" by Litvinov et al., licensed under CC BY-NC-ND 4.0 DEED.

Following an in-depth discussion of fibrin in section 2.3.3, it is important to reintegrate its use in clot contraction. Since platelet's filopodia latched itself and enable hand-over-hand action to proximal fibrin fibres, its presence allows the squeezing of plasma serum by activated platelets. Its absence or presence in the structural framework can contribute to clot's contractility. As trained middle-aged individuals were known to exhibit a maintained level of fibrinogen after exercise [122], sedentary untrained individuals with its higher fibrinogen levels may produce a much tighter fibrin network [178]. Therefore, a distinction can be made between these two separate groups that may lead to a different clot contraction profile.

In totality, owing to many components involve enabling clot contraction such as interaction with fibrin networks, thrombin components and reactivity to platelet [208], it remains unclear as to how exercise or increase level of physical activity affect clot contraction in vivo

2.4.3 Tertiary Haemostasis

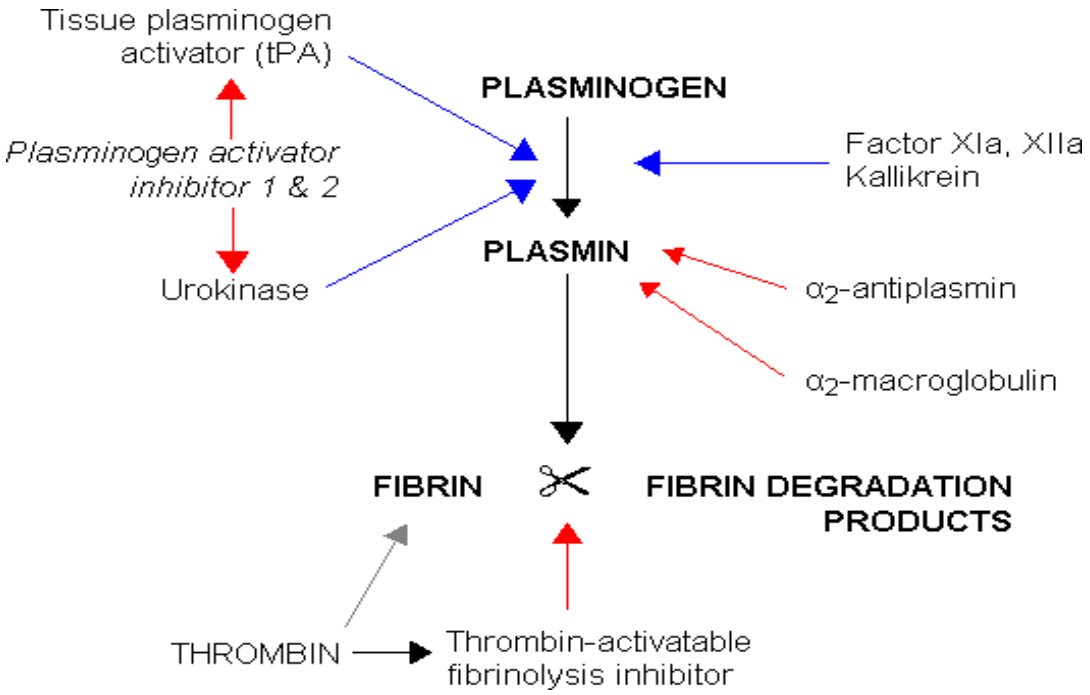
2.4.3.1 Fibrinolysis

The third part of haemostasis refers to the parallel fibrinolytic system that acts to limit the size of the formed blood clot and enables restoration of the blood flow in the vascular system. This mechanism as seen in Figure 2.11 avoids overexpressed thrombosis, vascular inflammation, tissue injury and ischemia. The primary catalyst bringing the breakdown of fibrin is the release of t- PA or urokinase-plasminogen activator (u-PA) from the vascular endothelium. Its activation can be triggered by tissue occlusion or presence of thrombin, epinephrine, vasopressin, and strenuous exercise [209].

It starts when the naturally occurring t-PA in the vascular endothelium reacts to the plasminogen circulating in the bloodstream. The plasminogen can be converted by t-PA into plasmin. The newly converted plasmin digest fibrin strands initially formed after activation of the coagulation. During this process, fibrin degradation products (FDP) commonly known as d-dimer are released. In clinical settings, d-dimer are used to assess the fibrinolytic response of the body.

Figure 2.11

Fibrinolysis



Note: The role of Plasminogen and plasmin in breaking down blood clots. Imaged sourced from Jfdwolff, Fibrinolysis, CC BY-SA 3.0

Several studies have supported the role of fibrinolysis during exercise. It has been widely acknowledged that frequent exercise enhances the fibrinolytic response of the body [210]. Exercise effectively lessens renal blood flow in the system which in turn drops PAI-1 levels and reduced clearance of t-PA in the liver. Subsequently leading to increase responses of fibrinolytic marker t-PA and decreased attenuation of PAI-1 in the system [211]. Primarily, there is a demand for the body to mitigate the possible adverse effect of fibrin deposition in the vascular system [41] and supports the balance between coagulation and fibrinolytic potential. It was also extensively reported that the level of exercise intensity is highly associated to fibrinolytic response. Short duration of high intensity leads to a much increase response of fibrinolysis than of moderate intensity and longer duration [86, 211-213].

This type of reaction was known to last between 60 minutes to 24 hours following maximum exertion [214].

2.4.4 Summary and research gap

Haemostasis plays an important role in the overall function of the body. This physiologic process involves multi-lateral and complex activation of enzymes and different blood components in the vasculature. Without such, biologic lifeforms may succumb to bleeding or develop uncontrolled thrombosis. The mechanism in place to maintain such delicate balance is the coagulation system. It involves the endothelium, platelets, clotting factors, and fibrin components and upon activation can undergo subsequent and overlapping phases leading to primary, secondary, and tertiary haemostasis. Interestingly, one physiological condition known to influence this intricate biological response is exercise.

Physical activity or vigorous exercise are known to activate blood coagulation through the intrinsic pathway. Increase shear stress from a turbulent or laminar blood flow exposes subendothelial matrix of the vascular system which in turn activates vWF, collagen, platelets, and clotting factors. However, anticoagulatory effects from NO, tPA and other endothelial vascular mechanisms restore haemostasis after exercise, preventing unwanted effects from severe thrombotic response. While sedentary individuals and those with vascular diseases may be at heightened risk of developing cardiovascular events due to an overexpressed coagulation, trained athletes were known to exhibit adaptability to transient thrombosis brought about by an enhanced fibrinolysis and a healthy endothelial vasculature.

Additionally, age also play a significant factor influencing the complete sequence of coagulation, with older individuals known to have a much higher risk of prothrombotic

activity. Nonetheless, the extent of mechanistic and adaptive change to exercise with age remains elusive and poorly understood. Clearly, individual biological profiles may lead to varied responses to coagulation. This highlights the need to further investigate variables affecting transient thrombosis in vivo such as chronic exercise adaptability and intensity for us to gain more comprehensive and global view in understanding the haemostatic imbalances which an increase physical activity may initiates.

2.3.5 Research Focus and Objectives

This postgraduate project delineates a set of primary research objectives and focal points. First is to expand our understanding of clot microstructure and blood coagulation profiles among a cohort of middle-aged adults, a domain not completely characterised.

Secondly, to understand the impact of physical activity on coagulation markers and observe its trend before, immediately following exercise and one hour post recovery.

Third, as there are known risk involve in recruiting sedentary individuals to perform intense physical activity, the sample group will be mainly centred on middle-aged yet trained adults. This project endeavours to understand their haemostatic capabilities and adaptation. It aims to determine if a new biomarker d_f and other hemorheological markers reflect the changes in coagulation during short-term and prolonged exercise regimen.

Lastly, by doing this research, a thorough assessment of the exercise intensity could be linked to specific coagulation markers to validate and differentiate physiological in between different exercise regimens. This can serve as basis for pre-clinical modelling with great emphasis on clinically related intervention such as but not limited to cardiac and stroke

rehabilitation regimens. Identifying the extent and limit of an exercise intensity can help clinicians understand the acceptable range of exercise modalities.

Chapter 3: Research Methods

3.1 Overview

In the previous section, we discussed that physical activity activates coagulation. Coagulation balance is maintained by a subsequent anticoagulatory response from the vascular system supported by an efficient fibrinolytic activity. This response may vary depending on the individual's level of fitness, age, and the intensity of exercise. Many studies have investigated such responses by measuring coagulation pathways with different biomarkers. While there are important findings that have given us insights in understanding coagulation in vivo, further research is still needed to investigate the impact of exercise to its microstructural components with great emphasis on its role to thrombogenicity.

In this chapter, discussion will be made pertaining to the methods used to recruit participants, blood testing involving coagulation and rheological markers as well as physiological scoring used in determining the effect of exercise intensity in coagulation. To give more context and readability, selected sections were structured by stating its research context and the approach used to measure it methodologically. In this way, the reader can fully evaluate and follow the step-by-step approach used by the researcher if needed, to ensure reproducibility of the study.

3.2 Participants, study design, inclusion, and exclusion criteria

Participants were recruited through advertisements sent to local running clubs and running events. Inclusions are as follows, adults aged over 40 who regularly engaged in

aerobic exercise, upwards of 3-4 activities in a usual week is suggested. Participants were screened for any chronic medical conditions, particularly those relating to cardiovascular health such as ischaemic heart disease, hypertension, stroke, and diabetes. Gender, social background, and drug history were recorded. Those taking regular medications were not invited to participate, except hormone replacement therapy (HRT) to treat menopausal symptoms or medications for depression and other mental health conditions. Further demographics were recorded including smoking status, occupation and weekly alcohol intake, height, weight and BMI, and exercise background. No controlling variables were implemented.

All participants were asked to complete a healthy volunteer questionnaire prior to the study to ascertain their inclusion. Researchers received a signed consent form prior to the start of trial. Study took place all week from 9 am to 12pm. Researchers made sure that schedule was tailored fit to participant's availability.

3.3 Study Site

3.3.1 Laboratory

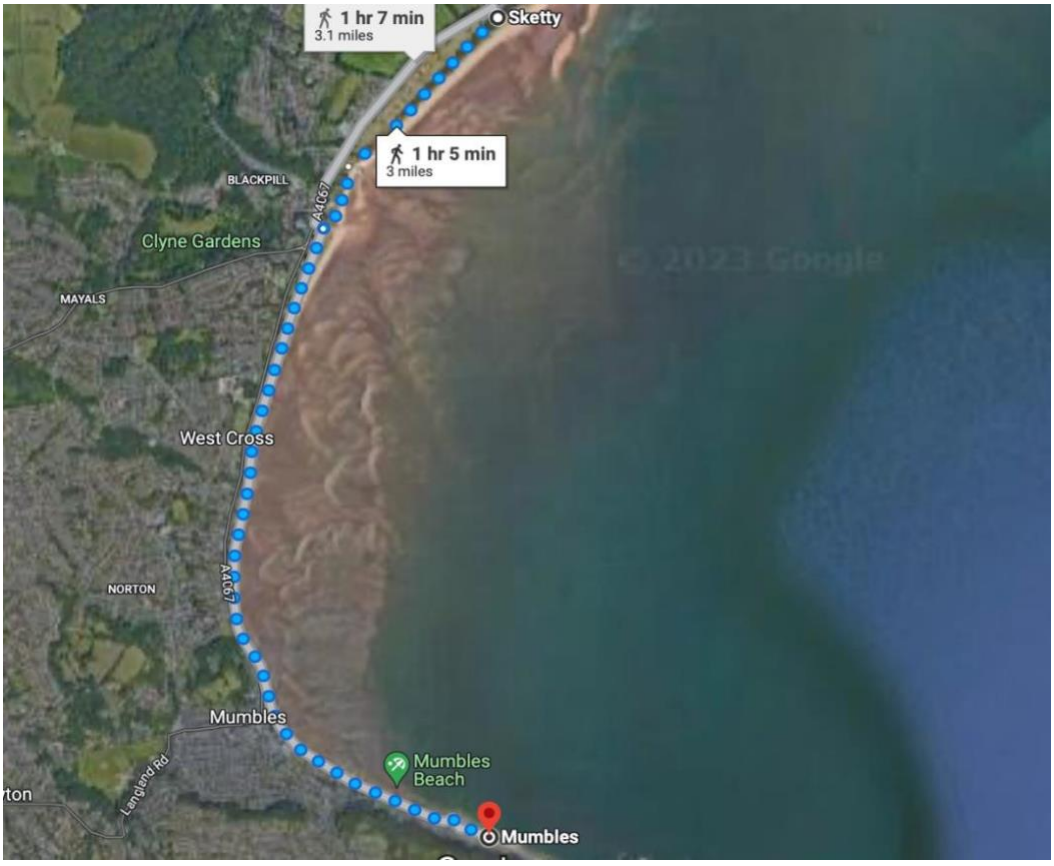
All sampling points were completed in Institute of Life Science (ILS) Building 2 Rheology laboratory, lower ground floor. Its proximity to running tracks and athletics sports park make it an ideal place to conduct the study. Also, ILS 2 is equipped with cutting edge laboratory equipment designed to process samples efficiently and with ease. All collection of blood samples were done in a clean, well-lit room where privacy is always observed, please see picture below.

3.3.2 Running Routes

The following illustrations (Figure 3.1 and 3.2) are the running routes used for long moderate intensity and short high intensity studies. The Swansea Bay running track leading to Mumbles area was used by runners to run the distance of 10 kilometres, self-paced while Swansea Bay Sports Park athletics track was used to conduct the 3 kilometres short high intensity. Participants were asked to run at their maximal pace and complete 7 laps in the track. They were all instructed to come back immediately once the desired distance was completed. Both running routes are illustrated below. All routes are mostly flat.

Figure 3.1

The 10 km. route of low moderate intensity run.



Note: Image sourced from google.com.

Figure 3.2

The 3 km. route for Short high intensity exercise

Note: Image sourced from google.com.

3.4 Sampling points

Sampling points were chosen to ascertain baseline levels and follow the haemostatic response of participants, purposefully making sure that known interaction between exercise and coagulation were well covered. In Fig. 3.3, the process flow of this study is illustrated.

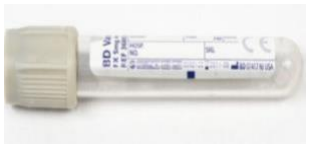
Researchers trained in phlebotomy collected blood samples aseptically in a well-lit, quiet, and controlled environment using BD Vacutainer Precision Guide multiple sample needle, either gauge 21 or 23 depending on participant's vein size. Brachial arm was the preferred site for blood extraction with alternating turns for each sampling points. To account for the presence of tissue factor, initial 2 to 3 ml. of blood were discarded. Following

successful blood backflow, the sequence of sample bottles introduced in the collection system were listed in Table 3.1.

Table 3.1

List of blood bottles used for this study.

Sequence	Name of blood bottle	Amount of blood needed	Number of bottles needed	Test performed
First	None- Discard 3 ml. of blood as per protocol	3 ml.	None	None
 Second	Greiner Bio One VACUETTE® TUBE 9 ml - No Additive, White Cap, Black Ring, Non-ridged - 16 x 100mm	9 ml.	1 pc.	d_f, GP, CF_{max} and G'_{max}
Third 	BD Vacutainer® Blue Citrate Blood Collection Tubes	2.7 ml.	3 pcs.	PT, APTT, FVIII, Fibrinogen, D-dimer

Fourth 	BD Vacutainer 3 ml. EDTA Purple blood collection tubes	3 ml.	1 pc.	Full blood count
Fifth 	BD Vacutainer tube Fluoride / Oxalate 4 ml. Grey Blood Collection Tubes	4 ml.	1 pc.	Lactate

3.4.1 Long moderate intensity Studies

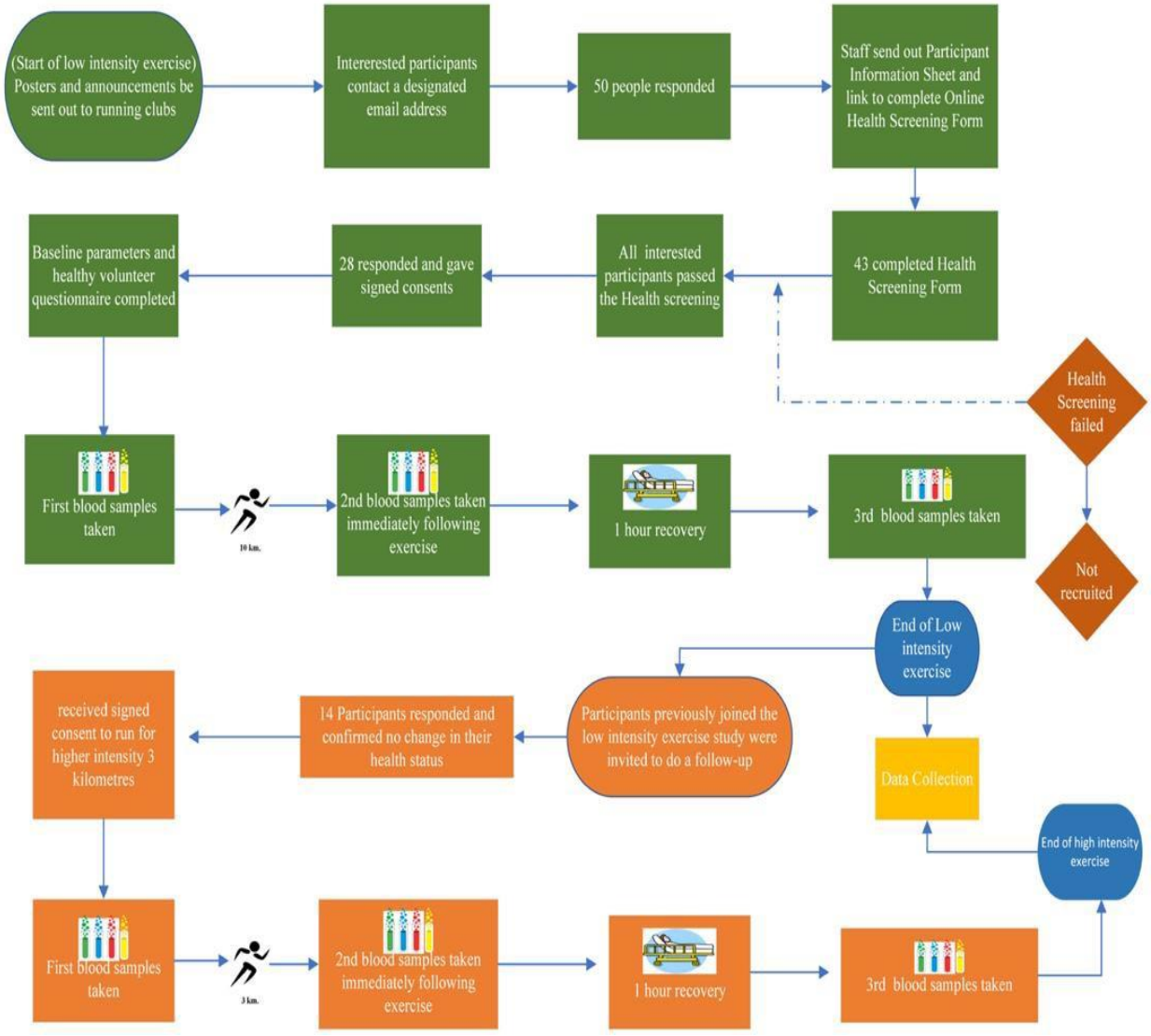
For the long moderate intensity studies (LMI), participants were requested to run at a steady self-selected pace along a defined flat 10km route. The steady pace was advised to be a pace at which they could maintain a conversation. Blood samples were collected before exercise, immediately after and 1 hour post exercise. These time points were chosen to depict the overall response of blood at baseline, during exercise and recovery.

3.4.2 Short High intensity Studies

For the short high-intensity experiments (SHI), recruited individuals who initially joined the long moderate intensity run were re-invited to run 3-km distance in the athletics track on maximal capacity. Participants were given the option of a self-selected warm-up routine before this effort. Blood samples were collected before exercise, immediately after and 1 hour post exercise.

Figure 3.3

Flow process of Exercise study



3.5 Physiological parameters of exercise intensity

Research Context

Nowadays, the availability of portable gadgets such as smartwatches enabled sports enthusiasts and recreational endurance runners to easily monitor their own physiological parameters. It has been proven to be an inexpensive way of analysing exercise performance and exertion. Conveniently, the American College of Sports Medicine has released a guideline on how to differentiate moderate and vigorous exercise intensity based on using age-predicted maximal heart rate (APHMR) [215].

For moderate exercise intensity, the target heart rate is between 64% and 76% of an individual's maximum heart rate. The maximal age-related heart rate can be estimated by subtracting 220 with current age. For example, a 40-year-old's maximal heart rate is 220-40= 180 beats per minute (bpm). With this, the 64%-74% of a 180 maximum heart rate is 115 and 133 bpm, respectively. One would have achieved a moderate intensity if heart rate raised up to 133bpm during physical activity. The same principle applies with vigorous exercise intensity. The only difference is that a much higher threshold of 77% and 93% is required to achieve maximum effort. For example, the same 40-year-old would have achieved 139 to 167 bpm maximal heart rate when a vigorous exercise is performed. However, caution should be advised as maximal heart rate may not be used to estimate the overall energy expenditure during exercise. This incidence may be explained by a lag or dissociation of HR against speed and V02max of runners [216]. Furthermore, Heart rate levels on this study will be used to differentiate baseline levels against a series of time points. An increase of such would give us a better estimate of the cardiovascular effort of an individual.

Another physiological marker which is used to monitor cardiovascular response to incremental exercise is blood pressure. It is measured by determining preload and afterload of arterial pressure between each heartbeat. As an immediate response to physical activity, blood flow to vital organs increases. Smooth and skeletal muscles demand more oxygen needed to adapt to the increase metabolic requirement of the vascular system which in turn increases the overall blood pressure. However, it has been documented that trained athletes, unlike people who are hypertensives [217], showed a different response to acute effects of blood pressure and to some extent can lead to post-exercise hypotension (PEH) [218]. Indeed, because of these conflicting results, there is a need to validate such effect to middle-age endurance runners.

Methodological approach

For this study, initial baseline BP and HR were taken before the exercise, immediately after exercise and 1 hour after recovery. Automatic upper arm blood pressure monitor (Omron M2) manufactured by Omron Healthcare was used to detect blood pressure and Heart rate of the participants. The device's cuff was wrapped in the upper arm 2 to 3 cm. above the brachial artery. The procedure was carried out in a quiet, well-lit, and ambient room condition. The participant was lying down in a reclined bed during BP measurement. Heart rate during the run was recorded by using the participant's smart watches. Data generated from this activity was extracted and recorded accordingly.

3.6 Metabolic markers of exercise intensity

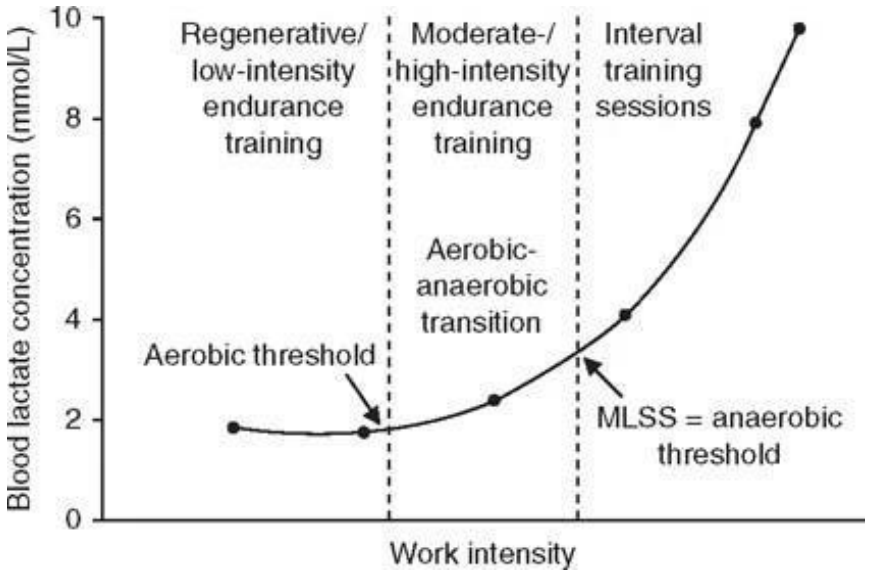
Research Context

Lactate levels is one of the metabolic markers that can be used to assess exercise intensity [219-222]. It is a biproduct of glycolysis which is constantly produced by the body to keep up with the energy demand [223]. The pyruvate molecule which is an intermediate product from the breakdown of glucose to ATP can be used to generate energy anaerobically. Likewise, pyruvate utilised by mitochondria in the cells can produce energy aerobically. These two energy source systems are physiologically utilised during increase demand such as physical exercise.

With constant lactate production because of metabolism, an imbalance may occur if clearance is not sufficiently met. The boundary of metabolic limit is the lactate or anaerobic threshold as depicted in Figure 3.4, where the inflection points corresponds to the level of exercise intensity and the transition from aerobic to anaerobic energy production. Predominantly, it is used by many endurance runners and sports enthusiast to differentiate between aerobic and anaerobic threshold and could be used to distinguish the impact of physical activity to their body systems [224]. Hence in this study, we will use the lactate threshold (LT) as a reasonable estimation of exercise intensity.

Figure 3.4

Lactate threshold workload plot.



Note: A lactate workload plot demonstrating increasing lactate levels in response to work intensity. Image was sourced from "Lactate threshold concepts" by Faude et al., with permission from Springer to use in this dissertation.

Methodological approach

Lactate levels were taken using a butterfly needle G.21 in the antecubital vein. Collection tube BD Vacutainer REF 368921 (Gray) was used to collect blood. Analysis was performed using Roche assay in Cobas 8000 for measurement of venous plasma lactate concentration on each time points.

3.7 Haematological markers during exercise

Research Context

It has been widely acknowledged that blood components are physiologically altered during exercise. Leucocytes or White Blood Cells (WBC) partly activates the coagulation cascade through release of cytokines which promotes expression of procoagulant and adhesive molecules on vascular endothelial cells [225]. WBC are known to increase transiently immediately after exercise [226] and may persists for the next 4 hours [227]. The increase of WBC especially during short high intensity exercise routine may lessen blood's fluidity in the vessel wall [228]. Measuring WBC in this study will give us an idea on the role it plays during an exercise routine.

Red blood cells (RBC) or erythrocytes have a significant impact in haemostasis and thrombosis in vivo. Previously, having played a passive role in the coagulation system, it is now receiving attention [229] because of its expanding influence on clot structure heterogeneity [230], modulation of platelets reactivity through release of ATP and ADP during low pO2 and pH environment as well as its response to mechanical deformation [231, 232]. Physiologically in high shear rate environment, mature RBC do not adhere to vascular wall as it axially migrates and deforms, however, under low shear conditions, along with fibrinogen, it can create a rouleaux formation [233]. Thrombosis may occur if RBC

aggregates with higher numbers of fibrinogen. RBC also play a significant role on the overall ability of the clot to contract [207]. Hence, they possess unique characteristics that can contribute to the overall quality of the blood clot.

During exercise, RBCs deformed its shape and squeeze to the smallest region of capillaries enabling transport of oxygen to the muscles. This is made possible by a protein named haemoglobin which carry oxygen ready for uptake and usage by the contracting muscle. Several studies have concluded that during increase physical activity, more RBC aggregate [163, 226, 227, 234-236] and plasma viscosity [226, 236, 237] increases. However, it is important to emphasize that there are distinct differences between profiles of sedentary and trained individuals engaged in exercise. Trained individuals are known to present, which most researchers called “sports anaemia”, where Haematocrit levels- the proportion of RBC compared to the overall plasma volume, are lowered when compared to the sedentary group [237-239]. This can be attributed to the increase of red blood cell mass, perhaps through constant training, and may have been acquired by adaptation. The total mechanism as to how this phenomenon may have occurred remains debatable [239]. While trained athletes showed increase blood fluidity [240], sedentary individuals have higher blood viscosity and RBC aggregation [238]. The differences in these profiles make endurance runners’ RBC, haemoglobin, and haematocrit as well as its mechanistic effect on clot structure and contraction all worth exploring.

Methodological approach

In this study**,** 4mL of blood was collected in a plastic dipotassium EDTA vacuette (Becton Dickinson, Plymouth, UK Ref 367839) for Full Blood Count (FBC) test using a Sysmex XN reagents and was analysed in Sysmex XN9000. Results yielded WBC, RBC, HGB,

HCT of each participant.

3.8 Coagulation, fibrin, and fibrinolysis markers Research Context

Different aspects of the clotting system can be measured by commonly used clinical tests. These tests are time-based assay of the coagulation system. PT measures the extrinsic pathway while APTT measures the intrinsic pathway. Both tests help evaluate the time it takes the clot to form. They are both commonly employed in many exercise-themed studies to assess the impact of exercise in the coagulation system.

Fibrinogen test can be used to assess the fibrinogen protein in the blood coagulation system. A lower fibrinogen levels may lead to bleeding while higher levels of fibrinogen support thrombosis in vivo. D-dimer test was also used to assess the breakdown of clots in each blood sampling points.

3.8.1 FVIII test

As what previously discussed in the related literature section, FVIII is one of the primary components being activated during exercise and is highly related to the level of intensity one individual performs. In this study, it is our endeavour to analyse the factor VIII activity during exercise in relation to both aforementioned and subsequent measurements.

Methodological approach

Two 2.7mL samples were collected into PET 0.109M 3.2% citrated vacutainers (Becton Dickinson, Plymouth, UK Ref: 363095). Routine coagulation studies were performed on the first citrated vacutainer including PT, PTT, Clauss fibrinogen and were measured using a Sysmex CS5100 analyser with reagents for PT-Siemens Innovin, APTT- Siemens Actin FS and Fibrinogen- Siemens Thrombin. D-dimer, a marker of fibrinolysis, analysis was carried out using a reagent Siemens Innovance D-dimer and was analysed in Sysmex CS5100 (Instrumentation Laboratory, Warrington, UK).

The second citrated sample were placed in a centrifuge using Eppendorf (model) at 2000G for 10 minutes to obtain platelet-rich plasma (PRP). For platelet-poor plasma (PPP), remaining plasma volume from PRP samples were re-spun for another 10 minutes at 2000G. Plasma samples were used for FVIII testing.

3.9 Rheological markers *Gel-point and df Research Context*

Viscoelastic tests using rheological measurements has been used to quantify gel network microstructures [241]. The development of an incipient gel structure or the first appearance of a spanning network can serve as a predictor of a mature gel structure [242]. This gelation phenomena can be quantified by employing a technique known as small amplitude oscillatory shear (SAOS), which measures evolving rheological properties of a material undergoing gelation and is capable of detection of a gel point [243].

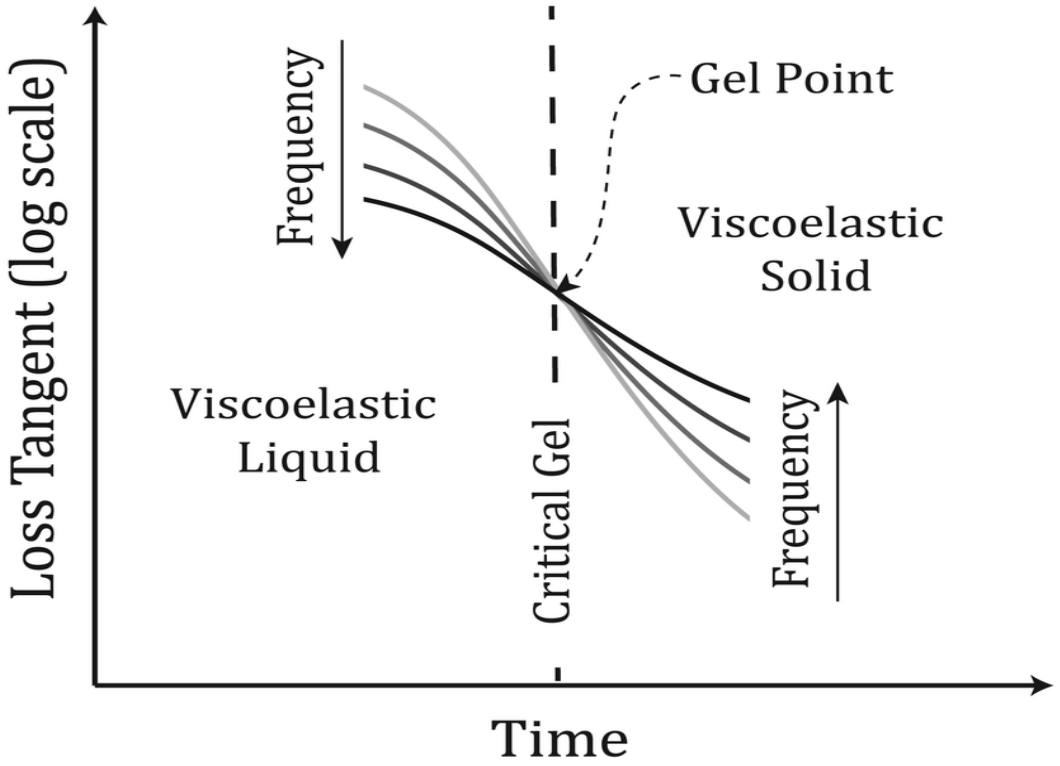
The resulting deformation of the material to a sinusoidal stress is recorded and analysed to establish the complex modulus $G^*(\omega)$ ($=G' + iG''$). It can further be broken down to its real

and imaginary parts, G′ and G″, and are commonly referred to as the storage and loss moduli, respectively [243, 244]. Storage modulus pertains to the extent of energy storage while loss modulus identifies the energy dissipation of the material on each completed cycle. With this, the loss tangent, tan δ, can be accurately determined through the following calculation, $\delta = G''/G$. Subsequently, the ratio of elastic energy storage and viscous energy dissipation can be identified on each entire cycle, with $\delta = 90°$ (i.e. $\tan \delta = \infty$) and $\delta = 0°$ (i.e. $\tan \delta = 0$) denoting purely viscous and elastic behaviour, respectively.

Furthermore, the phase angle of materials describes a combination of viscous and elastic properties and can then be assigned with any value within the above limits. However, to accurately define whether a material exhibits viscoelastic liquid (VEL) or viscoelastic solid (VES) properties; G* and/or tan δ must be determined over a range of frequency [245]. Through utilising SAOS, gelation can be monitored as tan δ decreases and transitions from a VEL phase to a VES according to its relationship with the frequency of measurement. The gel point is defined as the critical point to which frequency independence of tan δ is observed [245] and indicates the establishment of the incipient gel network [242, 246] as shown in the illustration below.

Figure 3.5

Profile of rheometric patterns during gelation



Note: Fig. 16 depicts the linear viscoelastic response of a material undergoing gelation. Prior to the establishment of GP, the material behaves as a VEL while post GP the material behaves as a VES. Image was sourced from "Control of collagen gel mechanical properties through manipulation of gelation conditions near the sol–gel transition" by Holder et al., licensed under CC BY 3.0.

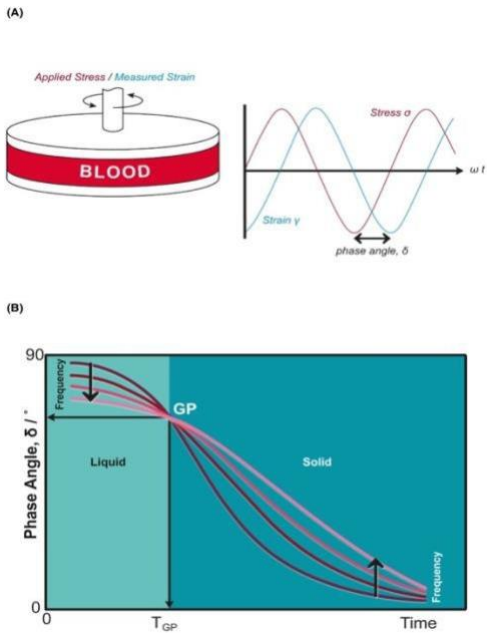
By using the gel point to precisely determine the first instance of the "sample-spanning" network, the structural complexity of the material can be measured using a mathematical relationship that predicts a value of fractal dimension. The term fractal dimension was coined by a mathematician Benoit Mandelbrot in 1975 to explain the complex shapes of nature [247] and how it can accurately be quantified. Furthermore, its existence as a

fundamental mathematical framework can be traced back in the 1600s [247, 248]. Its main function is to describe quantitatively the space filling characteristic of the fractal using a non-integer value. In addition to this, fractals are known for its self-similarity feature- wherein the incipient network self-replicates until it reaches its final form [247, 249].

Blood is known to exhibit viscoelastic properties. It can transform from a highly fluid state to a solid material and before reaching the solid phase, with pure observation, can obtain a gel-like characteristic. Using SAOS, we can identify the 3-dimensional network cluster formed at the GP from the analysis of viscoelastic data using a well-defined relationship [250] d_f = (D+2) (2a-D)/2(a-D), where D is the space dimension (D = 3 herein), see Figure 3.6 which demonstrates this relationship. The greater the value of d_f, the more compact and complex is the network structure, whereas low values of d_f equate to a more open/permeable networks.

Figure 3.6

Gel point (GP) analysis.



Note: (A) Demonstrates the blood within the geometry's surface. Small amplitude oscillatory measurements are performed, and the resultant strain waveform is recorded. The differences between stress and strain are accurately calculated to depict the phase angle. (B) Represents a typical gel point experiment with four independent frequencies measuring the oscillation according to time. The cross over in between frequencies determines the Gel point and represents the transition of the material from being in a liquid to solid. Image was sourced from "The effects of apixaban on clot characteristics in atrial fibrillation: A novel pharmacodynamic biomarker" by Lawrence et al., licensed by CC BY-NC-ND 4.0 DEED.

Methodological approach

A 6.8 ml. aliquot of whole blood was immediately loaded into a TA instruments AR-G2 controlled-stress rheometer (Please see Fig. 3.7 TA Instruments, New Castle, DE, USA) at 37°C (±0.1°C) with a double concentric cylinder geometry for visco-elastic testing. This

approach has been fully described previously in the literature and has been validated as a reliable technique for measuring the visco-elastic properties of blood and as a marker of clot initiation, the templating effect and mechanical properties of the mature clot [62]. Briefly, visco-elastic measurement was performed using SAOS measurements at varying frequencies. The incipient clot was detected at the point when the blood transitions from behaving as a visco-elastic liquid to visco-elastic solid. This is defined as the gel point and the time to reach this point is recorded as Time to Gel Point (T_{GP}). At this point, the phase angle was recorded as the difference in phase between the stress and strain waveforms recorded in the oscillations. Fractal Dimension ($d_{f\,f}$) was calculated as stated in the earlier section. And Phase Angle (δ) relates to α by the equation $\delta = \alpha\pi/2$.

Figure 3.7

AR G2 Rheometer

Note: An AR-G2 capable of measuring d_f and CF_{max}. Image sourced from Prof. Phillip Adrian Evans, with permission to re-use in this dissertation.

G Prime Max Research Context

The storage modulus G' (G prime, in Pa) depicts the elastic behaviour of the sample and quasi represents its solid state. When the sample is measured, rheological analysis identifies the maximal elasticity achieved by the sample within the observed time-period and in this case the maximal elastic deformed state of blood after reaching its solid phase.

Cf_{max}

Research Context

Recent studies have highlighted the importance that clot contraction plays in the final phase of normal clot formation. Altered contractile forces in clot development have been noted to be an important factor in abnormal clot formation in vascular disease [207, 251, 252]. Thrombotic states such as stroke, are known to be associated with a reduction in the process of clot contraction, however the effect of exercise on clot contraction has not previously been described. We assessed the influence of exercise on Maximum Contractile Force during both the hypercoagulable phase and the recovery period. CF_{max} is calculated from the absolute difference between maximum and minimum recorded measurements of Normal Force and represent a measurement of the contractile forces generated by blood during clotting [180, 251-253]. Measurements of the Normal Force generated during coagulation were carried out on the same samples using a second rheometer. As clotting progresses, the matrix scaffold interlinked by the fibrin network, platelets and other components generate and apply forces to the surrounding microstructure. Normal Force measurements were conducted using a Parallel Plate geometry (60mm diameter) of an AR-

G2 Rheometer, with the lower plate controlled at a temperature of 37°C. Approximately 0.84 ml of sample of blood was loaded onto the lower plate of the rheometer and the upper plate was gradually lowered to confine the sample between the plates at a fixed gap of 300 microns. Standard 10 mPa.s silicon oil (Brookfield) was placed around the edge of the geometry to prevent evaporation of the sample. The measurements involved the application of Small Amplitude Oscillatory Shear at a single frequency of 1 Hz, to provide values of viscoelastic parameters (including G'). The axial force generated between the two plates was recorded by the normal force transducer fitted to the lower plate. The contractile force generated by the fibrin clot was defined as the difference between evolving Normal Force and the registered Normal Force corresponding to the Gel Point i.e. at the point which indicates the establishment of the first sample spanning fibrin network. This ensured that the measured contractile force was attributable to the forces exerted on the fibrin network. The test ran for 75 minutes in order to record the maximum value of G', representative of the elasticity of the fully formed clot, and the CF_{Max} generated by the clot.

Methodological approach

A 0.84 ml. aliquot of whole blood was loaded onto the lower plate of the rheometer, which is held at 37C. The upper 60mm diameter parallel plate was gradually lowered to confine the sample between the plates at a fixed gap of 300 microns. Standard silicon oil was placed in the periphery of the geometry to prevent evaporation of the material. SAOS measurements at a fixed frequency of 1 Hz provided a measurement of G' as clotting evolved, up to a maximum value as defined as G'_{max}. Furthermore, the normal force generated between the two plates was monitored by the normal force transducer fitted to the lower plate. The contractile force generated by the clot was defined as the difference between normal force measured and the

registered normal force at time equal to zero. The maximum Normal Force difference generated will be used in reference to the Gel point parameters initially stated above.

3.10 Calculation of sample size and data collection

Each group's sample size is estimated using IBM SPSS software. Alpha level was set to 0.5 using a power level of 0.8 based on a previous report measuring fractal dimension after acute exercise, drug interventions and clinical conditions. The sample size needed for long moderate intensity activity is 15 while for short high intensity is 9 to detect a meaningful clinical significance.

Personal information was collected using the healthy volunteer questionnaire and was kept in a secured location. Once consent was given, participants were assigned a unique identifier using acronyms HEX which stands for Healthy Exercise with number assigned and a letter to determine different time points. "A" for before exercise, "B" immediately after exercise and "C" for after 1 hour of recovery. For example, HEX 1A for Healthy Exercise participant number 1-before exercise. The same codes were used all throughout the study to identify the participant's blood samples and to provide anonymity in data processing.

For d_f, Gel point, G-prime max and Cf_{max}, parameters were collected from the files generated by TRIOS software version 5.1.1. Data was saved in accordance with University Data Storage policy. Computers are password protected. Rheological markers were sent to an independent reviewer for thorough analysis independent of the person doing the test. The purpose of this is to remove observational bias from involved researchers. Transmittal of data was made using University One Drive secured database hosted by Microsoft Systems.

For blood assayed tests, assigned identifier was labelled on each bottle. Labels include identifier, birthday, gender, date, and time of sample collection. The same data was written in the blood forms approved by Laboratory Medicine in Morriston Hospital Samples was transported in accordance to Transfer agreement approved by Human Tissue Act Officer of the Swansea Bay University Health Board and Swansea University. Test results was accessed through Welsh Clinical Portal. Participants were completely anonymised in this computer system.

All data generated was saved in Excel spreadsheet using One Drive Cloud file storage and accessible only to primary researchers. The account is completely Swansea University owned. No personal identifier except Date of Birth and Date of sample collection was encoded in this spreadsheet.

3.11 Statistical analysis

Each groups sample size was estimated using IBM SPSS software. Alpha level was set to 0.05 using a power level of 0.85 based on previous reports measuring fractal dimension [62, 171, 254-256]. The sample size needed was 10 for each additional time point. For attrition, we recruited 28 endurance runners aged over 40 for the LMI group, 14 were invited to return for the SHI arm of the study.

IBM SPSS Statistics 29.0.1.0 (171) were utilised to perform statistical analysis. Normality of the data was confirmed using Shapiro-Wilk Test, non-normality was detected with p-value <0.05. Statistical analysis was performed on all data points. Outliers were detected using box-plot analysis in IBM SPSS; however, none were excluded as there was no justification from either a clinical or technical standpoint. Statistical significance was

accepted at $p \leq 0.05$. Normally distributed data were reported as a mean (*M*) and Standard Deviation (*SD*) and displayed in figures as a bar chart with uncertainty bars while non-normally distributed data were shown as median (*Mdn*), interquartile range (*IQR*) (25^{th} and 75^{th} percentile) presented as box and whisker plot.

All statistical analysis were performed using Repeated- Measures Analysis of Variance (RM-ANOVA) with a Bonferroni post hoc test for normally distributed data set involving three time points and Friedman Test for non-normally distributed data followed by non-parametric Wilcoxon matched-pairs signed rank test with a Bonferroni adjustment level set at $p<0.016$ which helps correct type I error for any variables showing significance value of $p<0.05$. For RM-ANOVA, if the assumption of sphericity was violated, Greenhouse-Geisser correction p-value was accepted for interpretation.

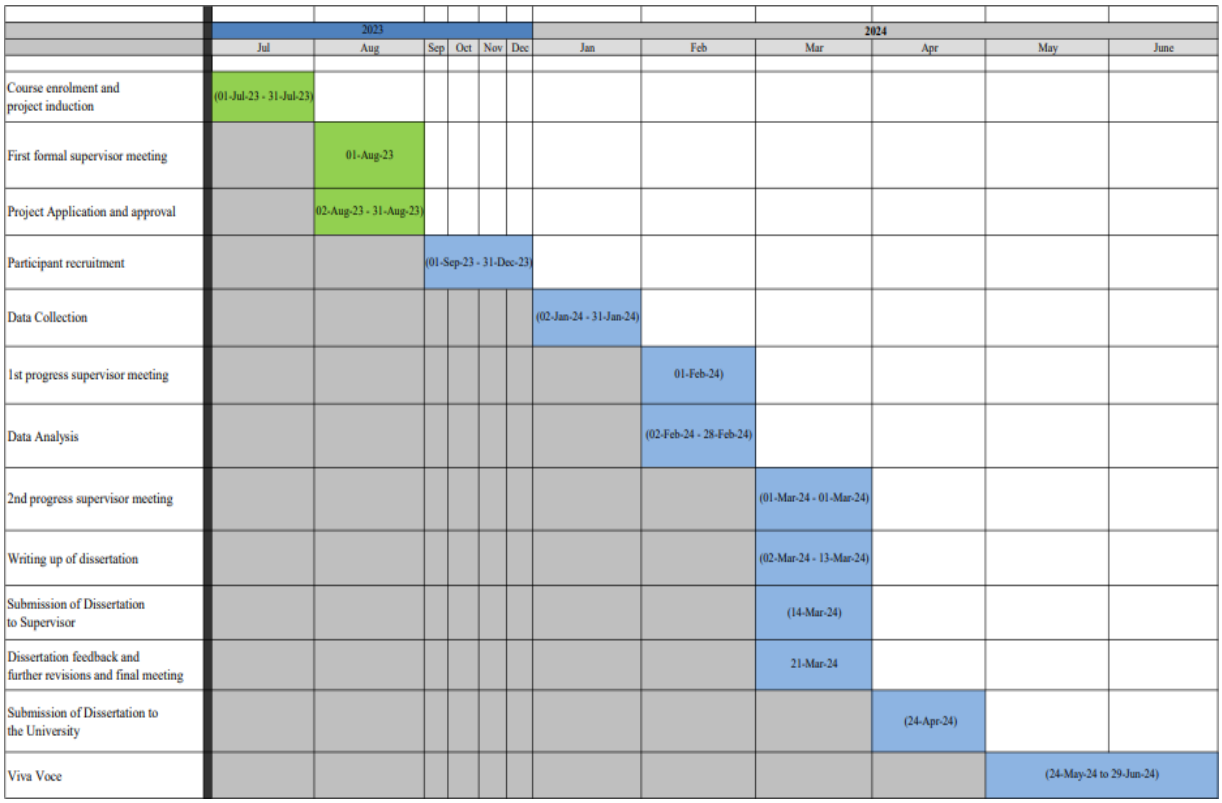
For comparing individual variables between groups, paired t-tests were carried out for normally distributed data and Wilcoxon matched-pairs signed rank test as its non-parametric counterpart. The difference scores were assessed for distributional assumption. If unmet, appropriate data transformations or sign test was utilised. Figures were created using GraphPad Prism (GraphPad Software, version 9.3.1).

3.12. Ethical Approval

Ethical application was completed and submitted to Swansea University Internal Ethics Review Committee. Approval was given for the start of recruitment in long moderate intensity exercise study reference number 1 2023 6945 576. A subsequent amendment was submitted for short high intensity exercise study and was given a favourable ethical opinion by the ethics board.

3.13 Project Timescale

Table 3.2



	2023						2024					
	Jul	Aug	Sep	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	June
Course enrolment and project induction	(01-Jul-23 - 31-Jul-23)											
First formal supervisor meeting		01-Aug-23										
Project Application and approval		(02-Aug-23 - 31-Aug-23)										
Participant recruitment			(01-Sep-23 - 31-Dec-23)									
Data Collection							(02-Jan-24 - 31-Jan-24)					
1st progress supervisor meeting								01-Feb-24)				
Data Analysis								(02-Feb-24 - 28-Feb-24)				
2nd progress supervisor meeting									(01-Mar-24 - 01-Mar-24)			
Writing up of dissertation									(02-Mar-24 - 13-Mar-24)			
Submission of Dissertation to Supervisor									(14-Mar-24)			
Dissertation feedback and further revisions and final meeting									21-Mar-24			
Submission of Dissertation to the University										(24-Apr-24)		
Viva Voce											(24-May-24 to 29-Jun-24)	

Table 3.2 illustrates the project timeline of this study, which clearly describes the step-by-step approach taken by the researcher to organise each project activity. It has been arranged to comply to the requirements set out for this one-year full-time postgraduate study. This arrangement was mutually agreed upon by the supervisors and student. Notably, all agreed dates were adhered and successfully met in a timely manner by the researcher.

3.14 Limitations and hurdles

One of the challenging feats that this study had to overcome was the number of people needed to gather sufficient data. It involves widespread dissemination and contact with other

colleagues who offered help and distribute flyers to running clubs. Many people found it interesting but due to conflict of schedule, they cannot find a good time to participate. Weather was also a big factor as sometimes cancellation happened on the day owing to a sudden change of weather. People found it also hard to join as it involves three blood tests in a day. The logistics involved in collecting bloods, transporting them to Morriston Hospital Laboratory and measuring, blood pressure and heart rate simultaneously during blood collection time points to ascertain body's response on specific exercise intensity posed significant additional challenges to this study. Furthermore, due to variety of smartwatches used by individuals during exercise, accurate readings of their heart rates cannot be validated. Hence, exclusion of their results was deemed appropriate. Additionally, measurements involving nitrous oxide and inflammatory markers were excluded due to budgetary restrictions associated with this project.

Chapter 4

Results

4.1 Demographics

Figure 4.1

Graphical demographic representation

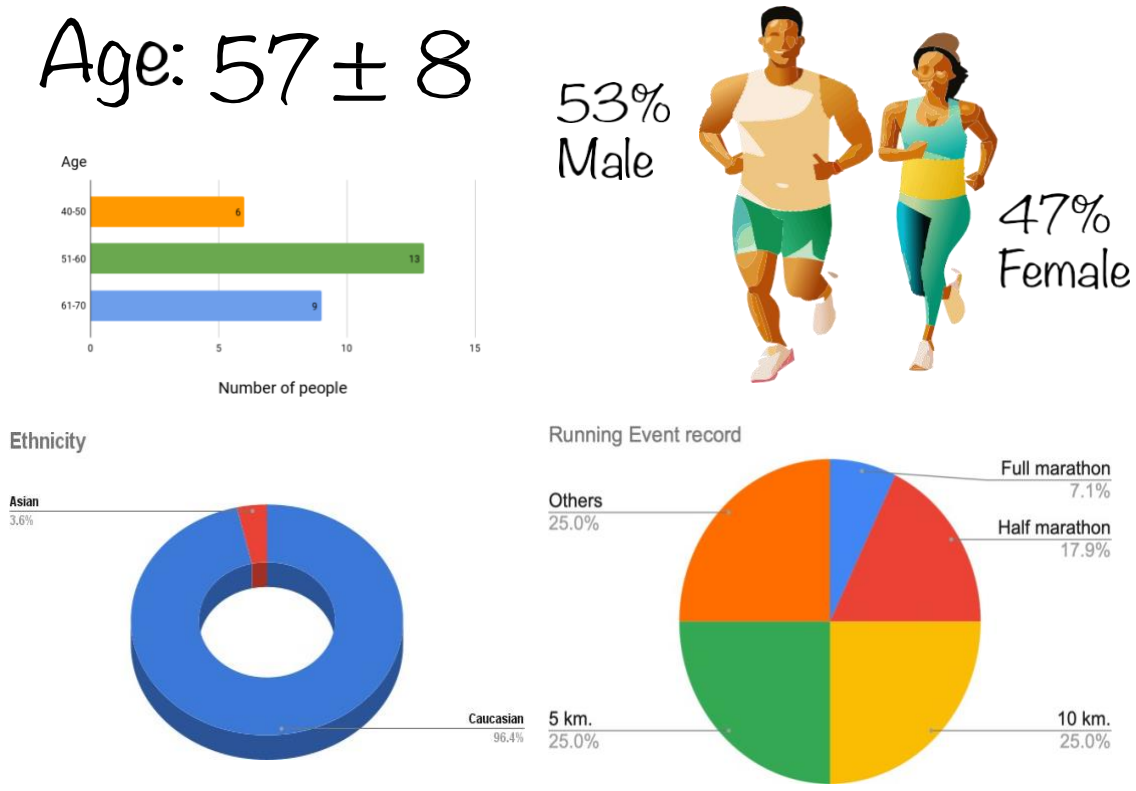


Figure 4.1 illustrates the demographic characteristics and participation records of the study population involved in a running event. The gender distribution indicates almost equal participation in both males (53%) and females (47%) individuals. Age distribution is represented in a bar chart, expressing the largest group of participants within the 51–60 age range (13 participants), followed by the 61–70 age range (9 participants), and lastly the 40–50 age range (6 participants).

Ethnicity data is displayed in a donut chart which indicates that most participants are Caucasian (96.4%), with a small proportion identifying as Asian (3.6%). The running event records are shown in a pie chart, which highlights the diversity of running events usually participated by these group of runners; 25% of participants each completed 5 km and 10 km events, while 17.9% participated in half marathons, 7.1% in full marathons, and 25% in other types of events. This figure proves that these selected individuals are continuously engaged in physical activity before participating on this study.

A total of 28 healthy volunteers more than 40 years old participated in the lower exercise intensity study. Participants with no changes in health status were invited to do a follow-up higher exercise intensity, 14 of them kindly accepted the invitation. Table 4.1 demonstrated further demographic details.

Table 4.1

Further demographic details

Demographic	LMI intensity (10 km.) *n*=28	SHI (3 km.) *n*=14
Height (cm.)	168 ± 9.5 cm.	167 ± 12.8 cm.
Weight (kg.)	68 ± 13.5 kg.	67 ± 12.9 kg.
BMI (kg/m^2)	23.5 ± 3 (kg/m^2)	23.7 ± 2.2 (kg/m^2)
Minute/km. (mm: ss)	5:25 ± 49 sec	4:57 ± 45 sec.

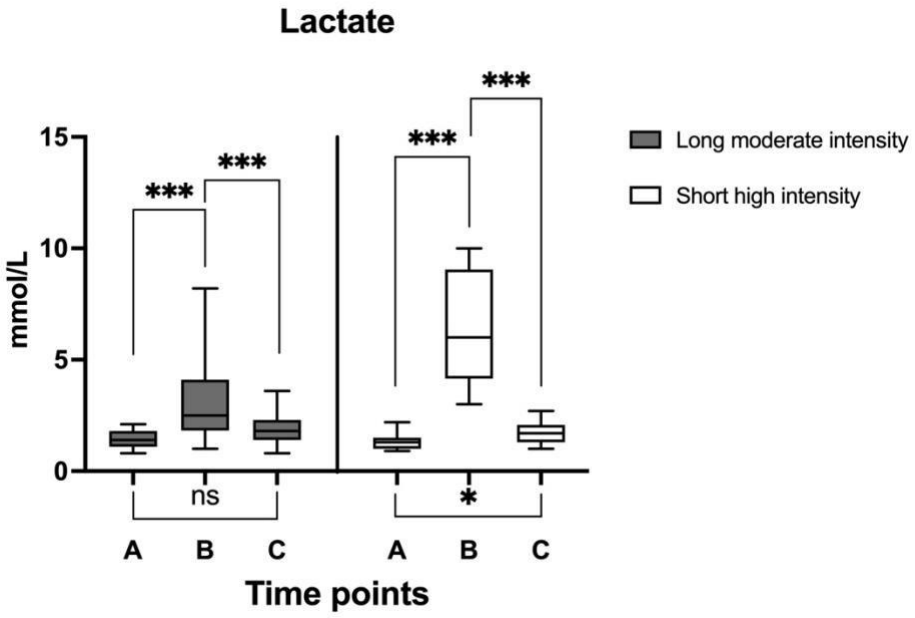
Training History	3 to 6 times weekly
Medical History	1-asthma 1 Raynaud's 26 no acute/chronic medical condition
Family History	
Blood clot in the leg/lung	3 out of 28 participants
Stroke or heart disease	10 out of 28 participants
Cancer	10 out of 28 participants
Bleeding disorder	1 out of 28 participants
Drug History	
Anticoagulant/antiplatelet therapy	None
Aspirin taken within the last 7 days	None
Current medications	Levothyroxine, Hormone Replacement Therapy patch, Omeprazole

4.2 Understanding the trends of biological markers within lower and higher exercise intensity groups across three time points.

4.2.1 Lactate Levels

Figure 4.2

Lactate levels across three time-points.



*Note: Lactate levels of long moderate and Short high intensity group in between three time points. Time point A (before exercise), Time point B (immediately after exercise), Time point C (1 hour after recovery).. Error bars represent IQRs for median values. *p <0.05), **<0.01, *** p <0.001 denotes statistical level, ns not significant.*

A Friedman test was run to determine if there were differences in lactate concentration in long moderate intensity group. However, as the distribution follows the assumption of non-normality. Pairwise comparisons were performed [257] with a Bonferroni correction for multiple comparisons. Lactate levels in long moderate intensity group was statistically significantly different at each time points during the exercise activity, $\chi 2(2) = 23.82$, $p < .001$.

Post hoc analysis revealed statistically significant change in lactate concentration from pre- (*Median*= 1.40, with an IQR of 0.70 mmol/L [1.10-1.8 mmol/L]) to immediate post- exercise (*Median* = 2.5, IQR of 2.65 mmol/L [1.83-4.48 mmol/L]) ($p < .001$) and after 1 hour of recovery (*Median*= 1.80, IQR of 0.85 mmol/L [1.4-2.25 mmol/L]) ($p =$ <0.001). Pre-exercise and after 1 hour of recovery failed to meet statistically difference based on the Bonferroni adjustment previously set at p <0.016 (*median difference*= 0.45 mmol/L, p= 0.04).

For short high intensity group RM-ANOVA was utilised to detect changes across three time points, the assumption of sphericity was violated, $\chi2(2) = 21.83$, $p =$ <0.001. Therefore, a Greenhouse-Geisser correction was applied ($\varepsilon= 0.53$). The exercise intervention elicited statistically significant changes in lactate concentration over time, F (1.1, 11.66) = 45.36 $p <$ .001, with lactate levels increasing from baseline value of (*Mean* = 1.34, *SD* ± 0.38 mmol/L) to immediately post-exercise (*Mean* = 6.3, *SD* ± 2.51 mmol/L) before plummeting to (*Mean*= 1.76, *SD* ± 0.51 mmol/L). Post hoc analysis with Bonferroni adjustment results as follow, pre-exercise to immediately post-exercise (*Mean difference* = 4.97 mmol/L, 95% CI [2.9, 6.9], $p < .001$), and immediately post exercise to after 1 hour of recovery (*Mean difference* = 4.5 mg/L, 95% CI [2.5, 6.5], $p =$ <0.001). Before exercise and after 1 hour of recovery (*M*ean difference= 0.43 mmol/L, 95% CI [0.32, 0.82], $p = .03$).

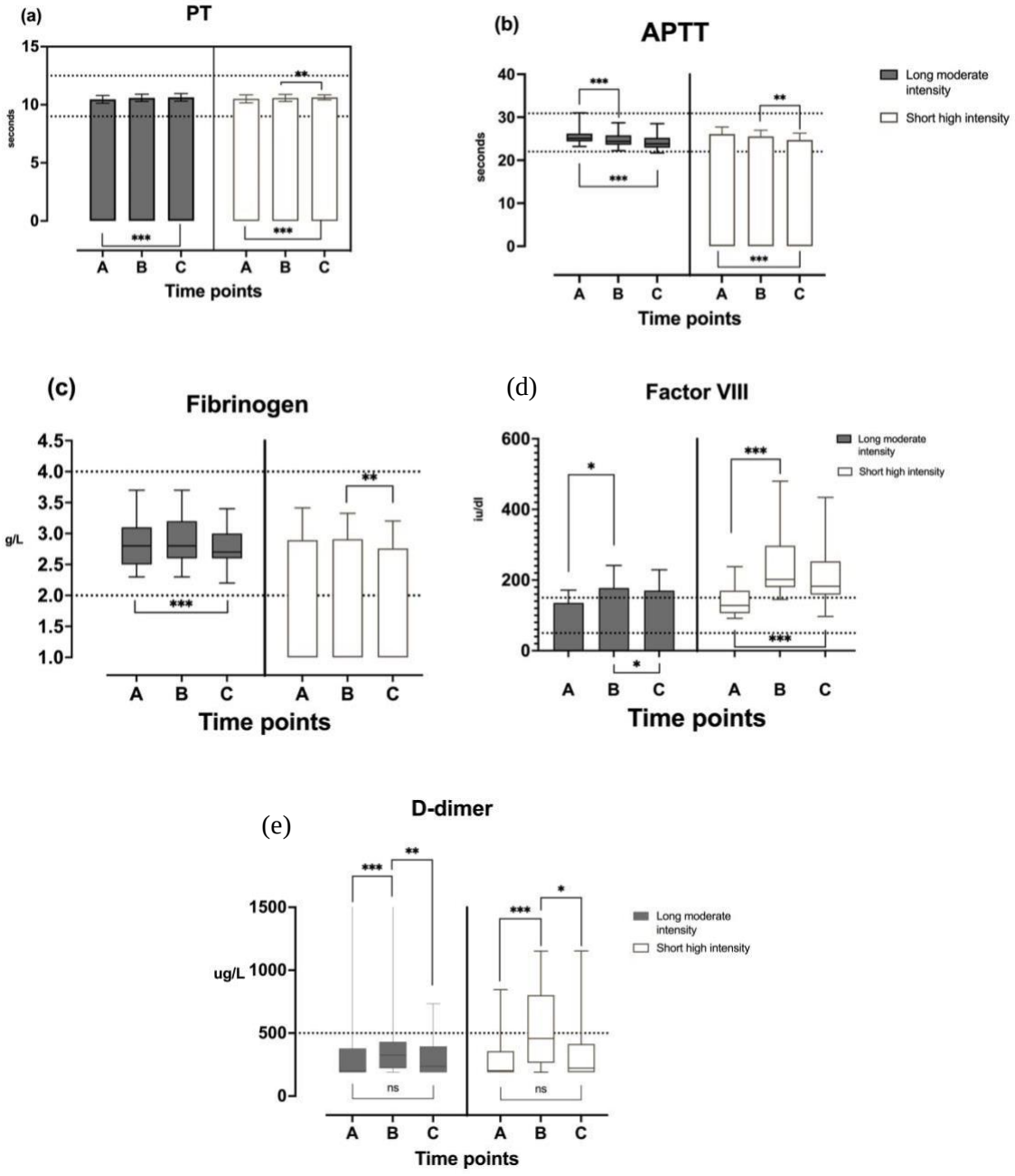
Overall, there was a significant increase of lactate levels in both exercise groups depicted in Figure 4.2, a determinant of physical exertion [219, 258, 259]. Short High intensity group significantly exceeded their anaerobic threshold more than the long moderate intensity group.

Nevertheless, both groups' lactate levels in time point B reached the upper bounds of the pre-established range, a clinically meaningful increase appropriate for the design of this study.

4.2.2 Coagulation markers

Figure 4.3

Coagulation markers across three time-points



*Note: Coagulation markers of LMI and SHI group in between three time points. Time point A (before exercise), Time point B (immediately after exercise), Time point C (1 hour after recovery). Dashed line in the y-axis depicts upper and lower ranges. Error bars represent SDs for mean and IQRs for median values. *p <0.05), **<0.01, *** p <0.001 denotes statistical level, ns not significant.*

There were statistically significant changes in all coagulation markers in both low and high intensity groups as illustrated in Figure 4.3. The assumption of sphericity was violated in long moderate intensity group FVIII data, as assessed by Mauchly's test of sphericity, $\chi2(2) = 7.05$, $p = .029$. Therefore, Greenhouse-Geisser correction was applied ($\varepsilon = 0.68$) specifically on that data point only. Nonetheless, the exercise intervention elicited statistically significant changes in **(A)** PT (LMI) long moderate intensity group F (1, 0.202) = 37.85, p <0.001, PT (SHI) short high intensity group $F(2, 20) = 25.2$, <0.001 **(B)** APTT (LMI) $\chi2(2) = 22.72$, p <0.001, APTT (SHI) $F(2, 20) = 17.31$, p <0.001. **(C)** Fibrinogen (LMI) $\chi2(2) = 16.02$, p <0.001, Fibrinogen (SHI) F (2, 20) = 5.95, p 0.009 **(D)** FVIII (LMI) $F(1.3, 16.3) = 11.3$, p 0.002, FVIII (SHI) $\chi2(2) = 18.17$, p <0.001 **(E)** D-dimer (LMI) $\chi2(2) = 21.7$, p <0.001, D-dimer (SHI) $\chi2(2) = 9.89$, p 0.007.

Although there are statistically significant changes in all coagulation markers, only FVIII in both exercise groups and D-dimer in short high intensity group exceeded their pre-established clinical normal range. Following post-hoc analysis, short high intensity group's d-dimer was observed to have increased from its pre-exercise to immediately post exercise levels at (*median*= 202, IQR of 176 μ/L [190-366 μg/L]) to (*median*= 560, IQR of 581 μg/L [275-856 μg/L]) (*median difference*= 221.5 μg /L, z= 2.6, p 0.01) before decreasing back to

baseline levels at (*median*= 231, IQR of 227 μg /L[190-417 μg/L]) (*median difference*= 29 μg /L, z= 1.5, p 0.13). Following data transformations as Time point B and C failed to meet distributional assumption, a statistically significant difference between two time points was observed at (*median*= Ln 6.22 μg/L, IQR of 1.16 [5.5-6.7 μg/L]) and (*median*= Ln 5.4 μg/L, IQR of 0.78 [5.24-6 μg/L]) (*median difference*= Ln 0.22 μg/L, z=-2.5, p 0.013), respectively.

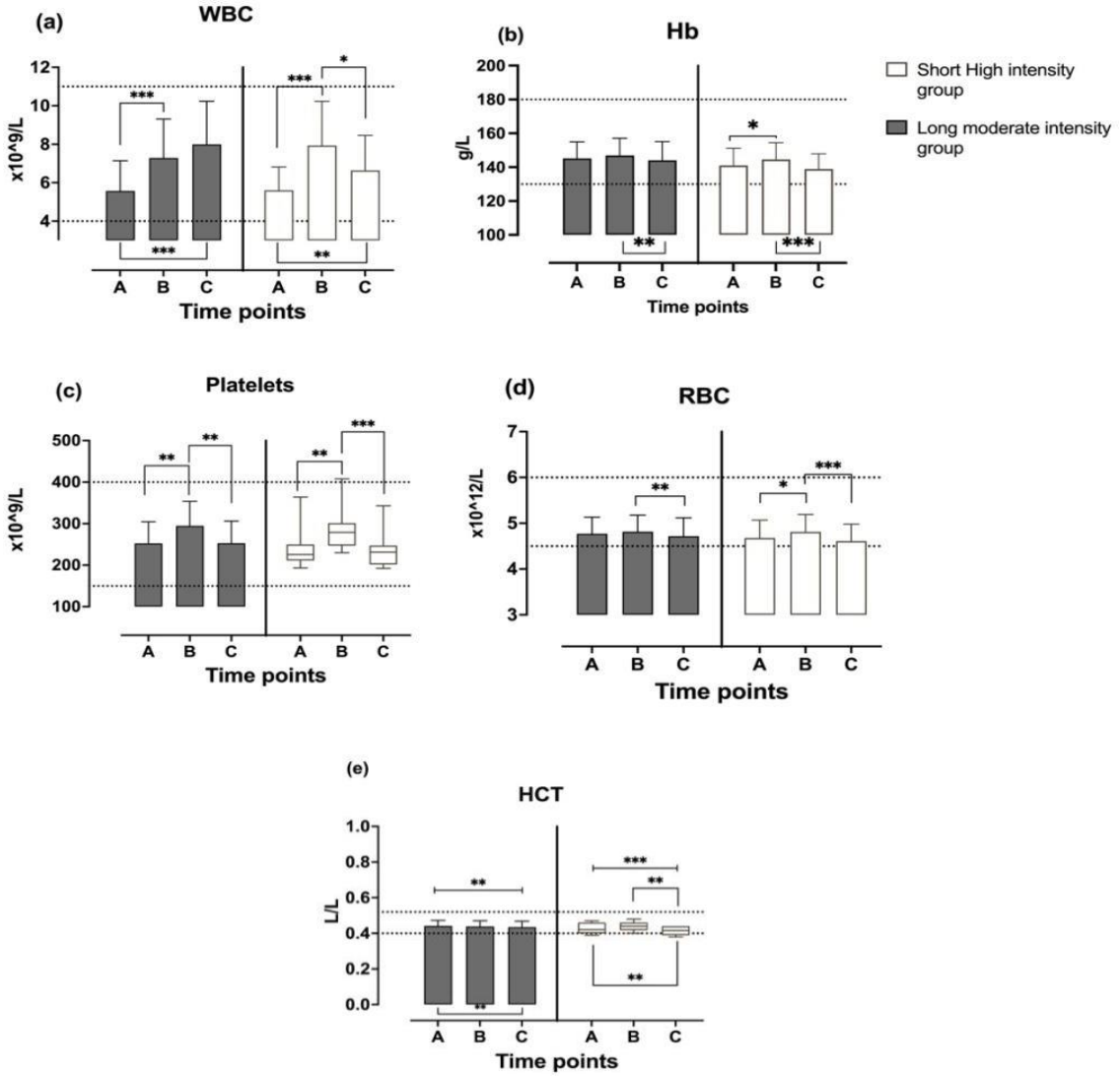
Factor VIII followed a similar pattern. Post hoc analysis revealed that the LHI group was statistically significantly increased from their baseline values to immediate post-exercise measurement (*Mean difference* = 42.3 iu/dl, 95% CI [9.1, 75.3], *p* = 0.012), while SHI group's FVIII increased significantly from (*median*= 128, IQR of 57 iu/dl [112.6-169.9 iu/dl]) to (*median*= 202, IQR of 117 iu/dl [180.2-297.1 iu/dl]) (*median* difference= 76.9 iu/dl, p <0.001). Notably, when compared to their initial readings, FVIII in low and high intensity groups remained elevated even after 1 hour of rest at (*mean difference*= 34.85 iu/dl, 95% CI [7.8, 61.8], p 0.01) and (*median difference*=
52.2 iu/dl, p <0.001), respectively.

Overall, there were statistical changes in coagulation levels especially in PT, APTT, and fibrinogen. However, none exceeded their normal ranges. In contrast, FVIII and D-Dimer levels (SHI only) in time point B had increased and exceeded their normal ranges.

4.2.3 Haematological markers

Figure 4.4

Haematological markers across three-time points



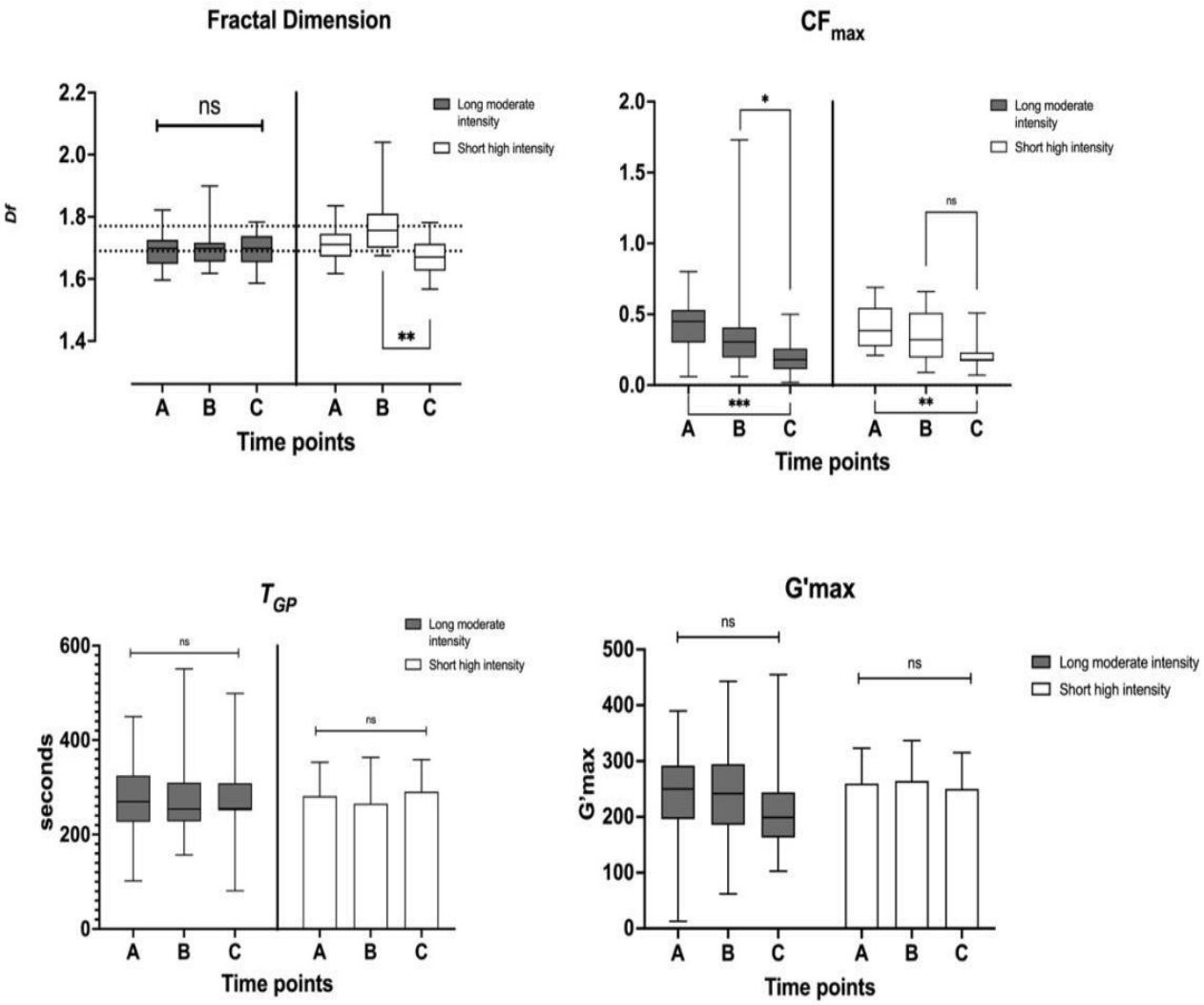
*Note: Haematological markers of LMI an SHI groups in between three time points. Time point A (before exercise), Time point B (immediately after exercise), Time point C (1 hour after recovery). Dashed line in the y-axis depicts upper and lower ranges. Error bars represent SDs for mean and IQRs for median values. *p <0.05), **<0.01, *** p <0.001 indicates significance level, ns not significant.*

The repeated measures ANOVA revealed significant main effects in both LMI and SHI group for **(A)** WBC- LMI F (1,24) = 55.5, p < .001), WBC-SHI F(2, 26) = 17.6, p < .001, **(B)** Hb- LMI F (2, 48) = 8,64, p < .001), Hb-SHI F(2, 26) = 18.35, p <0.001, **(C)** platelet- LMI F (2, 48) = 49.18 p < .001), platelet-SHI $\chi 2(2) = 19$, p <0.001, **(D)** RBC-LMI F(2, 48) = 9.5, p < .001), RBC-SHI F (2, 26) = 16.63 p <0.001, and **(E)** Hct-LMI F(2, 48) = , p .004), Hct-SHI $\chi 2(2) = 15.96$, p <0.001. Haematological markers graphs are clearly illustrated in Figure 4.4. The assumption of sphericity had not been violated in all data compared. Post hoc analyses showed significant changes in between time points in all variables analysed for each group. The statistically significant changes of the values may be the result of large sample size gathered, experimental sensitivity or measurement precision in between observations. However, no meaningful clinical assumptions can be made as variables did not exceed their pre-established normal range.

4.2.4 Hemorheological markers

Figure 4.5

Hemorheological markers across three time-points



*Note: Rheological markers of LMI and SHI groups in between three time points. Time point A (before exercise), Time point B (immediately after exercise), Time point C (1 hour after recovery). Dashed line in the y-axis depicts upper and lower ranges. Error bars represent SDs for mean and IQRs for median values. *p <0.05), **<0.01, *** p <0.001 denotes statistical level, ns not significant.*

Friedman tests were run to determine if there were changes in d_f, T_{GP}, G'_{max} *and* $CF_{max,}$ *which are clearly illustrated in Figure 4.5*. d_f remained stable across three time points in long moderate intensity group while a statistically significant reduction was detected in the SHI group at $\chi 2(2) = 9.5$, p 0.009. Post hoc analysis showed a decreased of d_f between bloods taken immediately after exercise and post 1 hour of recovery, at (*median*= 1.76, IQR of 0.1 [1.7-1.81] and (*median*= 1.67, IQR of 0.07 [1.62-1.71]) (*median difference*= 0.07, p 0.003), respectively. G'_{max} and T_{GP} were considerably stable across three time points and did not show any statistically significant change in both exercise groups. However, their trends remain relatively consistent with previous findings [254].

On the other hand, there was a statistically significant changed in Normal Force Difference across three time points in both exercise groups, LMI ($\chi 2(2) = 19.6$, p <0.001) and SHI ($\chi 2(2) = 10.3$, p <0.006). The trend was consistent in LMI and SHI participants. The reduction of clot contractile force was more pronounced between time point B and C in long moderate intensity group. Values were decreased from post exercise (*median*= 0.3, IQR of 0.2 [0.2-0.4]) to post 1 hour rest (*median*= 0.19, IQR of 0.14 [0.11-0.26]) (*median* difference= 0.1, p 0.014). Although post-hoc analysis revealed a statistically nonsignificant value between time point B and C in SHI group, at (*median*= 0.32, IQR of 0.3 [0.2-0.5]) and (*median*= 0.18, IQR of 0.06 [0.17-0.23]) (*median difference*= 0.07, p 0.15), respectively. However, the picture remains tilted to the downside. Moreover, Cf_{max} did not revert to baseline values, both groups shared a similar pattern when time point A and C were compared; Long moderate intensity group (*median difference*= 0.23, z= -3.78, p <0.001) and short high intensity group (*median difference*= 0.18, z= -3.06, p 0.002).

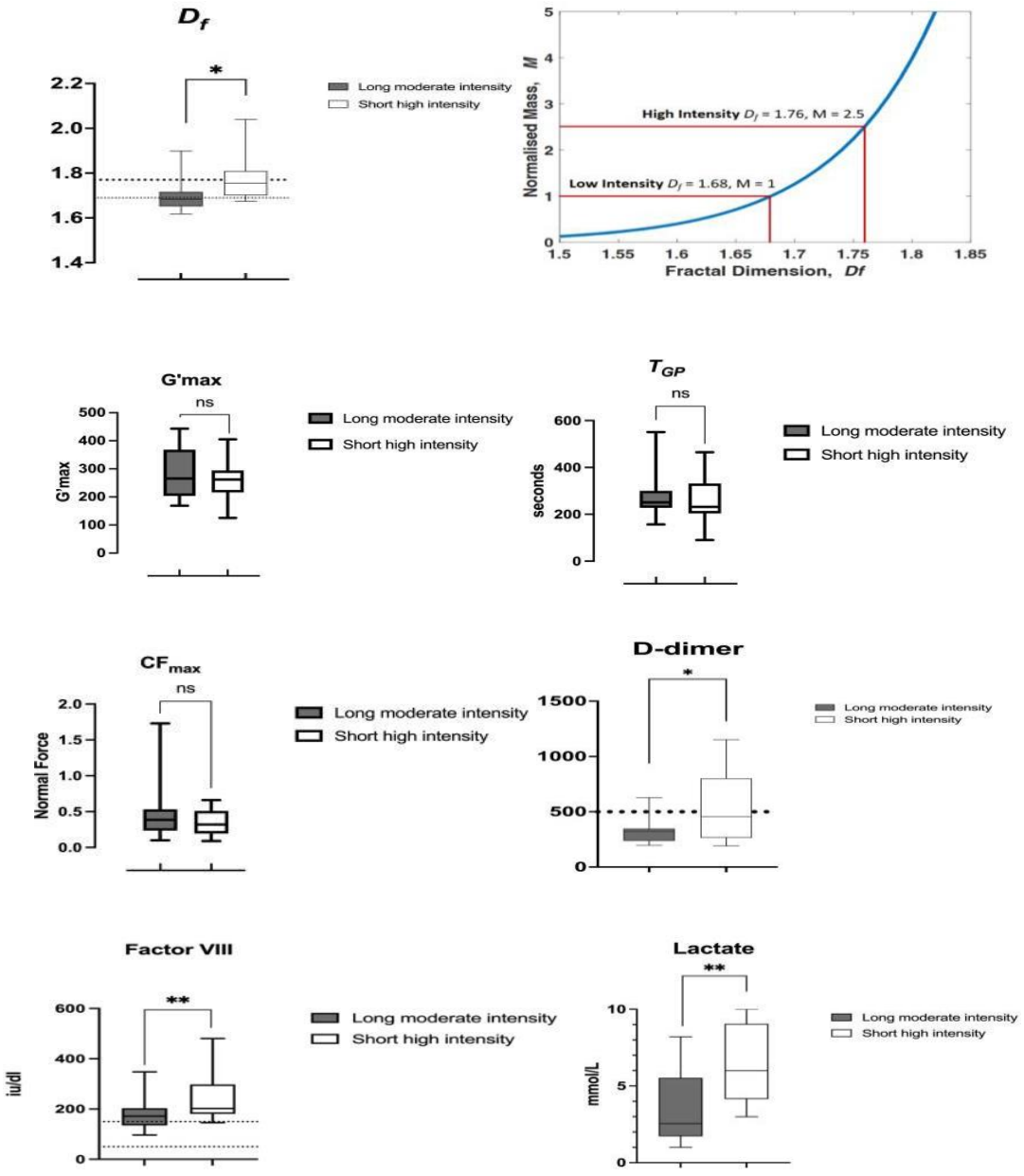
In summary, hemorheological markers d_f and Cf_{max}, detected a statistically significant reduction of clot microstructure and contractile forces, changes were more prominent after

exercise had occurred. The change in d_f was more pronounced in short high intensity group. Furthermore, the impact of exercise to previously mentioned hemorheological markers were noted to persist even after 1 hour of rest.

4.3 Immediately post-exercise paired variables between LMI and SHI groups.

Figure 4.6

Paired comparison of biological markers



*Note: Paired comparison of biological markers taken immediately after exercise between lower and higher exercise intensity. Dashed line in the y-axis depicts upper and lower ranges. Error bars represent SDs for mean and IQRs for median values. *p <0.05), **<0.01, *** p <0.001 denotes statistical level, ns not significant.*

Individuals belonging to short high intensity group elicited a much larger level of lactate concentration. There was a statistically significant difference of (*median*= 2.5 mmol/L) with short high intensity group (*median* = 6, IQR of 4.9 [4.15-9 mmol/L]) against long moderate intensity group (*median* = 2.5, IQR of 4.3 [1.65-5.95 mmol/L]), z = -2.7, p .008.

Among hemorheological markers analysed, only d_f had shown a statistically significant difference between groups. d_f values taken were much elevated in short high intensity group than its lower exercise intensity paired measurements. The median difference calculated at 0.08, with SHI group and LMI group, at (*median*=1.76, IQR of 0.12 [1.7-1.8]) and (*median*=1.68, IQR of 0.07 [1.65-1.72]) z=-2.28 p 0.02, respectively.

Similarly, FVIII in short high intensity group depicted larger concentrated levels than its paired counterparts. There was a statistically significant difference of (*median*= 27 iu/dl) with short high intensity group (*median* = 201, IQR of 116.9 [180-297.1 iu/dl]) against long moderate intensity group (*median* = 173, IQR of 70 [151.75-221.8iu/dl]), z = -2.8, p .005.

Additionally, fibrin degradation products (FDP) measured through d-dimer tests were observed to had been much more prominent in short high intensity group as expected. There

was a statistically significant difference of (*median*= 167 μg /L) with short high intensity group (*median* = 509, IQR of 553 [275-828.75] μg /L) against long moderate intensity group (*median* = 330, IQR of 88 [258.75-347 μg /L), z = 2.1, p .03.

In summary, the biological markers such as lactate, FVIII and d-dimer were seen to demonstrate a significant and consistent statistically changed when a much higher level of physical exertion was induced to the same participants, as illustrated in Figure 4.6. However, despite a statistically significant increase of d_f in short high intensity group, such level did not exceed its pre- established clinical range. Further details on each specific variables not mentioned above are listed in Table 4.2.

Table 4.2

Paired comparison of biological markers taken immediately after exercise between lower and higher exercise intensity.

Measured variable	**Long moderate intensity**	**Short high intensity**	**Mean/Median Difference**	**Test statistic**	**Significance value**
PT (sec.)	10.5 IQR0.75 [10.3-11]	10.5 IQR 0.42 [10.3-10.75]	-0.1	z=-5.37	p 0.59
APTT (sec.)	24.95 ±1.59	25.5 ±1.49	-0.57	t (11) =- 1.402	p 0.10
Fibrinogen (g/L)	2.89 ±0.46	2.88 ±0.42	0.17	t (11) =0.175	p 0.86
WBC (x10^9/L)	7.41 ± 2	7.93 ±2.3	-0.51	t (13) = - 1.01	p 0.3
Hb (g/L)	145.71 ±8.97	144.5 ±10.02	1.21	t (13) = 0.87	p 0.4
Platelets (x10^9/L)	289 IQR63 [249-312]	279 IQR54 [247.2-301.2]	-3.5	z= -0.88	p 0.38
RBC (x10^12/L)	4.8 ± 0.37	4.8 ±0.38	0.16	t (13) = 0.28	p 0.79
HCT (L/L)	0.42 ±0.02	0.44 ±0.26	-0.11	t (13) = -2.2	p 0.05

Chapter 5

Discussion and Conclusion

5.1. Dynamics of Endurance induced exercise in people over 40 years old

Endurance exercise in participants over 40 can induce a transient hypercoagulable phase, which is more pronounced with intensity. However, the relative lack of thrombotic events in trained athletes during or following exercise would indicate this is both short lived and mitigated by responses in endothelial function and fibrinolysis. This study suggests that d_f – a biomarker of thrombogenic potential and clot microstructure – was able to detect the hypercoagulable state induce by exercise, but also then the return back to baseline. Its potential utility as a marker of thrombogenicity has already been highlighted [260]. d_f was also able to show the increasing exercise intensities in this group may lead to increased tendency for clot formation with stronger mechanical properties, but that this returned to normal following a short period of rest as has been described previously. Indeed, in the short high intensity group this hypercoagulable state was seen to resolve even whilst lactate remained above baseline.

Endurance runners are known to have an improved haemostatic efficiency through response from the endothelial lining and vascular compliance. It is well known that exercise modifies endothelial cells by increasing production of Nitrous Oxide (NO) [80, 81], a potent vasodilator and anti-inflammatory. A lack of production of NO is known to be associated with increased cardiovascular risk [261]. As a result, reduction of intravascular shear stress occurs which effectively lessens platelet-vessel wall contact in the vasculature [262, 263]. This effectively dampens the hypercoagulable phase and reduces the likelihood of

thrombosis. It has previously been shown that as little as one month of endurance training is sufficient to generate this response [85]. Furthermore, compared to those with a sedentary lifestyle, regularly exercising individuals also show a reduced level of von Willebrand factor (vWf) antigen activity [86], and reduced platelet aggregation [47]. Altogether, regular exercise improves endothelial function providing a distinctive clot microstructure profile in these individuals, reducing thrombogenic risk in the transient hypercoagulable phase associated with exercise.

Another contributing factor to our findings could have been the efficiency and effectiveness of the fibrinolytic system in these individuals. Fibrinolysis occurs simultaneously alongside the primary development of the fibrin network in the vasculature [264]. This is supported by similar findings which described that acute endurance exercise generates immediate response from the fibrinolytic system [211, 265, 266]. However chronic adaptations in trained individuals enhanced fibrinolysis. This is supported by other studies demonstrating recently post-menopausal women completing a structured exercise training programme over 8 weeks had a reduction of clot mass of 40% following the training period [267]. Well-adapted individuals are known to have heightened resting fibrinolytic activity, attributed to an increased tPA release, decreased PAI-1 activity and decreased tPA-PAI-1 complex formation [210, 211]. Our data seems to support this in showing that d_f was relatively stable across the duration of the study in long moderate intensity exercise, but that this could be overcome by higher intensity activity. With higher intensity exercise, there is an associated increase in shear and blood flow, and this may have been sufficient to overcome the increases in fibrinolytic activity.

In a similar exercise study involving young, untrained participants, d_f was observed to have increased during the duration of exercise activity before returning to baseline with rest [254]. While our results did see an elevation with exercise, followed by a return to baseline after rest, this was only possible at higher intensities. The previous study demonstrated that a long moderate intensity exercise was sufficient to elevate $d_{f\,f}$ in young, untrained participants. Other studies have demonstrated that fibrinolytic response can vary with exercise intensity [211, 265, 268, 269]. Therefore, it is possible that the previous study protocol which utilised a bicycle ergometer and an incremental ramp protocol to exhaustion led to a greater exercise intensity, however, there is also a range of evidence to suggest that fibrinolytic response varies with level of fitness [37, 86, 268, 270-274], which we suggest is a more likely explanation as why long moderate intensity exercise in this study had less of an impact.

5.2 Reduced clot contraction post-exercise

The significance of clot contraction in thrombosis and hypercoagulable states is gaining widespread recognition [180]. Despite the importance of platelet driven mechanobiology in blood clots, there are limited accounts employing the most up to date techniques measuring clot contraction in exercise. For this reason, we investigated the response of clot contraction to exercise by measuring CF_{Max}. Overall, our results seemed to show a significant reduction in clot contractile forces following exercise. Additionally, this effect appeared to persist longer than other changes induced by exercise, as CF_{Max} remained low even after a period of rest, and it is not known how long this would have taken to fully return to pre-exercise level.

The hypothetical causes of a reduced CF_{Max} would include, a reduced platelet activity,

a lower d_f, increased fibrinolysis, or poorer adherence of the sample to the rheometer plate. Which of these factors is responsible for the changes in this study remains open for debate. Platelet activity was not measured. It is well recognised in trained athletes that both thromboxane and ADP aggregation is reduced in exercise [264]. For this to be responsible for CF_{Max} to be lowest following rest, there would need to be initial compensatory mechanisms, which are outlasted by the period of reduced platelet activity. d_f was not significantly lower at time point C than baseline values, so to be responsible for such a substantial alteration in CF_{Max} would seem unlikely. Were fibrinolysis to be responsible (or at least in part), clot degradation products such as D-dimer would be expected to be increased. And while D-dimer was elevated immediately following exercise, it had returned to normal levels following the period of rest. Clot adherence to the plate is perhaps the most difficult to measure. It remains an inherent assumption of the methodology that the clot adheres to the plate. If this were happening a sudden drop in G' would be expected at the point the clot begins to pull away from the plate, and this was not seen.

5.3 Limitations of this study and potential future work

One of the limitations of this study was the lack of a control sedentary group. There is a dichotomy between trained and untrained individuals in their response to exercise. However, evidence set out in other literature was substantial and comparisons with our data can be drawn from this body of evidence.

As this was a prospective observational study with no direct intervention from researchers, we aimed to ascertain the factors contributing to the reduction of contractile forces during exercise and its relationship to clot microstructure and the overall coagulation

system in an in vivo setting. A more focussed study designed to elicit the mechanisms responsible for these changes will be necessary to further explore this relationship. A separate in-vitro study will be designed to address this emerging conundrum.

Final Summary and Conclusion

We describe for the first time the utility of d_f throughout the hypercoagulable phase associated with exercise in trained over 40s. The validity of d_f as a biomarker in measuring exercise intensity has already been shown in untrained athletes. In this study, using additional markers we have shown that d_f accurately detected the differences in intensity in participants over 40 throughout the hypercoagulable phase seen in exercise and recovery.
Whether this ability to mirror these changes in healthy subjects can be used to detect patients with an excessive risk of thrombosis associated with a new exercise regime, most pertinently those undergoing a rehabilitation programme after conditions such as stroke or MI.

We suggest therefore that this biomarker may be used to monitor and guide commencement of exercise safely and determine appropriate intensity thresholds throughout the progression through an exercise programme. Further work is underway to determine this.

Appendices

6.1 Study Protocol

TITLE OF THE PROTOCOL: Responses of the coagulation system to exercise in healthy participants

Short title/Acronym:	***Coagulation in Exercise***
Sponsor:	**Swansea University**
Representative of the Sponsor:	**Paola Griffiths Research Governance**
REC reference:	**SUMS RESC2022-0056**

STUDY SUMMARY/SYNOPSIS

TITLE	Responses of the coagulation system to exercise in healthy participants
SHORT TITLE	Coagulation in Exercise
Protocol Version Number and Date	Protocol Version 1.4 (17.08.2022)
Methodology	Prospective observational Study
Study Duration	Until 31/07/2023
Study Centre	Swansea University
Objectives	To establish the changes in the coagulation system due to exercise seen in healthy trained participants aged over 40
Number of Subjects/Patients	30 healthy volunteers
Main Inclusion Criteria	Healthy volunteers: Aged over 40 No chronic health issues or regular medications Regularly participate in aerobic exercise
Statistical Methodology and Analysis	One way ANOVA testing across 3 time points

Protocol Agreement Page

The clinical study as detailed within this research protocol **(version 1.4 – 17/08/2022)**, or any subsequent amendments will be conducted in accordance with the Research Governance Framework for Health & Social Care (2005), the World Medical Association Declaration of Helsinki (1996) and the current applicable regulatory requirements and any subsequent amendments of the appropriate regulations.

Chief Investigator Name: Professor Phillip Adrian Evans

Chief Investigator Site: Morriston Hospital Contact details: Tel No: [redacted] Email: [redacted]

Signature and Date:

Principal Investigator: Professor Karl Hawkins

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Signature and Date:

Co-Investigator: Dr Suresh Pillai

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Co-Investigator: Professor Ylva Hellsten

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Co-Investigator: Dr Oliver Watson Email: [redacted]

Signature and Date:

Jun Cezar Zaldua (Research Assistant) Email: [redacted]

Signature and Date:

Janet Whitley (Honorary Research Assistant)

Email: [redacted] Signature and Date:

Glossary of Terms and Abbreviations

ANOVA	Analysis of Variance
CAD	Coronary Artery Disease
CI	Chief Investigator
CRF	Case Report Form
d_f	Fractal Dimension
DVT	Deep Vein Thrombosis
FBC	Full Blood Count
GP	Gel Point
HBRU	Haemostasis Biomedical Research Unit
MI	Myocardial infarction
PE	Pulmonary Embolism
PI	Principle Investigator
PIS	Patient Information Sheet
R&D	Research & Development
REC	Research Ethics Committee
SAE	Serious Adverse Event
SBUHB	Swansea Bay University Health Board
SOP	Standard Operating Procedure
TIA	Transient Ischaemic Attack

1. Introduction

1.1 Background

Exercise is promoted as a beneficial lifestyle intervention to reduce the risk of a wide range of cardiovascular diseases [1, 2]. Exercise has long been understood to induce a temporary and reversible hypercoagulable state in response to the physiological demands of exercise [3- 5]. This brief period of enhanced thrombogenicity is thought to last between 30 and 60 minutes [6]. However much of this literature has been focused on young and healthy volunteers. Whether these changes persist as people age or develop chronic medical conditions are not fully understood. Additionally, the recent development of novel coagulation tests which are able to better describe the coagulation system has led for the need for much of this work to be re-investigated.

This study is designed as a precursor to wider studies in which the changes in the coagulation system as a result of exercise can be better explored. The intention of these future studies is to investigate changes to coagulation in participants with chronic medical conditions such as hypertension, diabetes, stroke and coronary artery disease (CAD). Part of the pathophysiology of these disease states involves dysregulation of vascular and endothelial function which will have knock on effects on the coagulation system which ultimately cause the mortality and morbidity associated with these conditions [7]. Studies focusing on platelet activation have indicated similar changes in thrombogenic risk attributable to increased platelet activity following acute bouts of exercise [8]. However, this increased activity response was blunted in participants who regularly exercised [9]. Therefore, the results of this study will also be considered in the planning of further work to compare untrained and trained participants. The intended utility of this research would be to inform clinicians on how best to advise patients to introduce exercise into their daily life. The current literature and expert consensus are that there remains a range of unanswered questions with regards to the introduction of exercise, namely how age and co-morbidity influence best clinical practice [10].

Fractal dimension (d_f) is a novel biomarker which describes the structure of the incipient clot at the transition point between liquid and solid [11]. It has been shown the fibrin network at this point is the template for the subsequent clot. The complexity of the fractal network which is established directly affects the strength and elasticity of the clot with an established link to thromboembolic diseases [12, 13]. An increased fractal network is therefore indicative of a generally increased state of thrombogenicity in the body. Our previous work has demonstrated d_f is also raised as a result of exercise [14], however this focused on untrained volunteers who were much younger in age. As such the proposed study intends to investigate volunteers over the age of 40 to establish whether the changes described in younger participants can also be

Clot contraction is another recently developed novel biomarker which appears to have important implications in thromboembolic disease [15]. Contraction of a clot is a normal physiological mechanism designed to restore blood flow to the distal vessel once haemostasis has been established. Impairment of this contractile mechanism is implicated as a mechanism in ischaemic stroke [16]. While it is believed to be implicated in other thromboembolic diseases, the currently published literature is limited to healthy volunteers and patients with an acute stroke or transient ischaemic attack (TIA).

1.2 Rationale, Risks and Benefits

Participants who already exercise regularly have been selected in order to minimise the impact of the research study on participants. As this is exercise, they would have otherwise undertaken anyway, the net additional risk of the study is minimised to the blood sampling procedure. Nonetheless the risks of a single bout of aerobic cardiovascular exercise in a trained volunteer would include minor musculoskeletal complaints such as sprained ankles and injuries from simple trips or falls. The amount of blood taken is a small amount, which will be replaced by the body in a day to weeks' timeframe. The only common risks associated with these are mild discomfort, occasional light-headedness and bruising.

2. Study Objectives and Design

2.1 Study Objectives

Establish the changes seen in the coagulation system as a response to a single episode of aerobic exercise in a group of healthy participants aged over 40 who regularly exercise.

Compare these changes to those previously reported in younger participants.

The results of this study will be used to plan further studies involving patients with cardiovascular disease who are at risk of diseases such as stroke or myocardial infarction (MI). In these groups it is not known whether responses to exercise are the same as healthy groups, it is also unknown whether these responses change with age.

2.2 Study Design

A prospective observational study of adults over the aged of 40. Participants will undergo a single episode of aerobic exercise and undergo blood tests which measure coagulation activity before, immediately after and 1 hour after exercise.

3. Participant Selection and Recruitment

3.1 Participant selection

Main Inclusion Criteria:
Aged over 40

Regularly exercises upwards of 3 times per week over the past 3 to 6 months Able to complete

a 10km run at a self-selected pace

Main Exclusion Criteria:

Chronic medical conditions such as previous stroke, TIA, coronary artery disease (CAD), cancer, pulmonary embolism (PE) or deep vein thrombosis (DVT), hypertension, diabetes

Any regular medications to treat the above conditions

Have taken aspirin or other blood thinning medications within the last 7 days Musculo-skeletal

conditions that would be worsened by a 10km run

Know or think they are infected with a blood borne virus such as HIV, Hepatitis B or C Are

anaemic or receiving treatment for anaemia

Displaying signs or symptoms of Covid-19 infection, or have tested positive for Covid-19

3.2 Participant Recruitment

Local running clubs will be contacted via their club secretaries and club coaches to be distributed to other members. The Participant Information leaflet will be attached with contact details for the researchers. Eligible participants will be invited. The receipt of signed consents from these individuals will be the basis for their interest to participate.

4. Laboratories

4.1 Laboratory Assessments

- Rheology: 7ml of blood will be required for all rheological analysis that will give an indication of clot structure and quality. This will be performed immediately after blood sampling on an AR-G2 or an ARES-G2 Rheometer (TA Instruments) in the Rheology Lab, Centre for NanoHealth.

- Clot contraction analysis: 2.7ml of blood collected in a 3.2% sodium citrate vacutainer. 80µl will be used for analysis using a thrombodymanics analyser system (HemaCore, Moscow, Russia). Coagulation will be activated with thrombin and $CaCl_2$ and then added to Triton precoated cuvettes. Imaging analysis of blood is carried out at 37°C.

- Coagulation screen: 2.7ml of blood will be collected in 3.2% sodium citrate vacutainers. A total of two vacutainers will be used, one for Coagulation and d-dimer and the other one for aliquot samples.

- Full Blood Count (FBC): Up to 4ml will be collected in an EDTA vacutainer for FBC analysis.

- Lactate: 4.0 ml of blood will be collected in Gray top container and will be processed accordingly.

Total Sampling Volume: Approximately 25 ml. of blood will be collected at each time point.

4.2 Local Laboratories

Rheology, clot contraction and samples to be aliquoted will be processed in the Rheology Lab, Centre for NanoHealth at Swansea University. Coagulation screen, D-dimer, lactate and FBC will be analysed in Laboratory Medicine, Morriston Hospital.

4.3 Sample Labelling/Logging

Samples will be pseudo-anonymised with an allocated code: e.g. 'HEX##' for the healthy participants (where ## is an integer) and sent to the lab using a special study form.

4.4 Sample Receipt/Chain of Custody/Accountability

The blood samples from this research activity will be transported from Swansea University by a member of research staff within four hours after blood extraction to Laboratory Medicine, Morriston Hospital in compliance with the SBU Health Board/Public Health Wales Handling and Transport of Clinical Specimens Procedure

4.5 Sample Analysis Procedures

FBC, Lactate, D-dimer and clotting profile will be analysed by standard protocols in Laboratory Medicine, Morriston Hospital.

Rheological analysis will follow the standard procedure published in 2010 [11]. Clot contraction will be performed following the standard procedures published in 2016 [15].

4.6 Sample Storage Procedures

Aliquoted samples will be stored at -80^{o}C in Rheology Lab, Centre for NanoHealth at Swansea University and will be discarded after 12 months of study completion as per local laboratory procedures.

4.7 Data Reporting and Archiving

Data will be reported through the local laboratory reporting system in digital anonymized format. All study documents, including laboratory results, will be stored in locked cabinets and moved to SBU R&D Department for archiving 12 months after the main publication.

5. Safety reporting

Blood is taken by an experienced member of staff and where possible blood sampling for the study will take place at the same time as routine blood. Hence this study has very low risk.

6. Statistical Considerations

6.1 Sample Size

Healthy volunteer studies previously carried out at the Welsh Centre for Emergency Medicine indicate a normal clot contraction of 44% ± 7.9% standard deviation. Clot contraction in exercise has never been investigated, however studies investigating clot contraction in ischaemic stroke have indicated this can drop as low as 25% when contraction is impaired. We have determined a clinically meaningful difference would be at least 10% reduction in contraction, we have no reason to believe standard deviation would change. Using significance set at p=0.05 and power at 0.8, and comparing differences via a single sample ANOVA, we calculate at least 28 participants would be required to detect this difference. We will aim to recruit 30 participants. As the study takes place within a single visit in volunteers well used to running that distance, we do not expect significant drop out rates. As a subsidiary marker, we will compare d_f. This has a known normal value in healthy volunteers of 1.73 ± 0.03, and a clinically meaningful increase would be an increase to 1.77 ± 0.04.

6.2 Statistical Analysis

SPSS® and Graphpad Prism with their updated versions and licensed package will be used for statistical analysis. All eligible subjects will be included in the analysis. We will perform descriptive analyses to establish baseline characteristics. Correlations between $\underline{d_f}$ Gel Point and clot contraction with standard haemostatic markers will be investigated using Pearson's method for normally distributed data. Shapiro-Wilk testing will be performed to assess for normality and ANOVA or Kruskal-Wallis testing will be performed as appropriate.

7. Data Handling & Record Keeping

7.1 Confidentiality

There will be strict adherence to confidentiality as set out in the Caldicott guidelines. Confidentiality will be maintained by the use of a coding system. The data retrieved from the analysis will be stored in a secure place in a locked cupboard within a locked office. The participants will be reassured that all identifiable data from the study will not be reported. Participants will retain their right to revoke their authorisation for use of the data.

7.2 Study Documents

All essential documents will be contained in the Study Master File. The Chief Investigator will be responsible for the maintenance of the site file.

7.3 Case Report Form

Data will be collected using a standardised Case Report Form and then collated electronically and stored on a password-protected computer.

7.4 Record Retention and Archiving

All records are the responsibility of the CI and will be kept in secure conditions. When the study is complete records will be kept for a further 5 years in accordance with local R&D guidelines. Archiving is provided by Transmedia, Swansea Bay University Health Board's (SBUHB) archive facility under the control of the R&D Department.

7.5 Compliance

The Chief Investigator will ensure that the study is conducted in compliance with the principles of the Declaration of Helsinki (2008), and in accordance with all applicable regulatory requirements including the Research Governance Framework, Trust and R&D policies and procedures, and any subsequent amendments.

7.6 Data Handling and Processes

All data that will be collected throughout the research activity will be processed in accordance to UK General Data Protection Regulation and The Data Protection Act 2018 as set out in relevant sections.

7.7 Clinical Governance Issues

Ethical Approval

This has been given a favourable ethical opinion by Swansea University Research Ethics and Governance Committee (SUMS RESC 2022-0056).

8. Publication Policy

The findings of the study will be disseminated through international journals and conferences. No participant will be identifiable.

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End of Document

6.2 CONSENT FORM for Coagulation in Exercise

Name of Researcher: Prof. Phillip Adrian Evans Participant Identification Number for this trial:

Please initial box

1. I confirm that I have read the information sheet dated 17/08/2022 (protocol version 4) for the above study. I have had the opportunity to consider the information, ask questions and have had these answered satisfactorily. Yes/No ☐

2. I understand that my participation is voluntary and that I am free to withdraw at any time without giving any reason, without my medical care or legal rights being affected. Yes/No ☐

3. I understand that relevant sections of my medical notes and data collected during the study, may be looked at by individuals from Swansea University, from regulatory authorities or from the NHS Trust, where it is relevant to my taking part in this research. I give permission for these individuals to have access to my records. Yes/No ☐

4. I understand that the information collected about me will be used to support other research in the future, and may be shared anonymously with other researchers. Yes/No ☐

5. I agree to my General Practitioner being informed of my participation in the study. / I agree to my General Practitioner being involved in the study, including any necessary exchange of information about me between my GP and the research team. Yes/No ☐

6. I understand that the information held and maintained by Welsh Centre for Emergency Medicine Research/Swansea University/Swansea Bay University Health Board may be used to help contact me or provide information about my health status. Yes/No ☐

7. I agree to take part in the above study. Yes/No ☐

Name of Participant	Date	Signature
Name of Person	Date	Signature taking consent

6.3 Healthy Volunteers Questionnaire

Study Title: Coagulation in Exercise

Participant identification number:

Date of questionnaire: Date of Birth:

Personal Medical History

- Have you ever had a blood clot in the leg or lung?
  Yes / No
- Do you suffer from heart disease?
  Yes / No
- Do you suffer from any coagulation disorder (inherited or acquired)?
  Yes / No
- Do you suffer from cancer?
  Yes / No
- Do you suffer from any liver or kidney disease
  Yes / No
- Do you have diabetes?
  Yes / No
- List any other medical problems you have:

Family History

Does anyone in your immediate family have?

- Blood clot in the leg or lung?
  Yes / No
- Stroke or heart disease?
  Yes / No
- Cancer?
  Yes / No
- Clotting/ bleeding disorder
  Yes / No
- Any other inherited disease?
  Yes / No

Drug History

- Are you currently on any anticoagulant/anti platelet therapy?
  Yes / No
- Have you taken aspirin within the past 7 days]

Healthy Volunteers Questionnaire

- Current medication (please specify type and dosage)

Dietary History

- Are you a vegetarian?
  Yes / No
- Are you a vegan? Yes / No
- Other (please specify)

Social History

- Age _____
- Marital status _____
- Ethnicity _____
- Occupation _____
- Smoking- number on packets per day _____
- Alcohol-units per week and type _____

Exercise/ Mobility

- What type of exercise do you mainly do? _____
- Weekly training how often? (e.g. once, twice, thrice) _____
- Recent race (e.g. 5k, 10k marathon) _____

Signature: _____ Date: _____

6.4 CASE REPORT FORM

Participant ID: Date of Birth:

Inclusion Criteria	Exclusion Criteria
- Healthy volunteers: - Aged over 40 - No chronic health issues or regular medications - Regularly participate in aerobic exercise	- On anticoagulants - Disease process, liver disease, kidney disease, genetic disorders, cancer - Previous vascular events

RHEOLOGICAL ANALYSIS			
	SAMPLE A	SAMPLE B	SAMPLE C
GEL TIME			
PHASE ANGLE			
DF			
G PRIME MAX			
Normal Force			

CLOT CONTRACTION			
	SAMPLE A	SAMPLE B	SAMPLE C
CLOT CONTRACTION			

STANDARD LAB MARKERS			
	SAMPLE A	SAMPLE B	SAMPLE C
HB			
HCT			
PLATELETS			
WBC			
RDW			
PT			

APTT			
FIBRINOGEN			
D-DIMER			
LACTATE			
Approx. Volume of Fluid taken			

Exercise Background	
	SAMPLE A
Usual weekly mileage	
10km race time (recent)	
Time taken today	

6.5 Swansea University Ethics Approval

Swansea University
Prifysgol Abertawe

Approval Date: 07/06/2023

Research Ethics Approval Number: 1 2023 6945 5766

Thank you for completing a research ethics application for ethical approval and submitting the required documentation via the online platform.

Project Title	Responses of the coagulation system to exercise in healthy participants	Applicant name	Jun-Cezar Zaldua
Submitted by	Jun-Cezar Zaldua /		
Full application form link	https://swansea.forms.ethicalreviewmanager.com/Project/Index/8671		

The Medicine ethics committee has approved the ethics application, subject to the conditions outlined below:

Approval conditions

1. The approval is based on the information given within the application and the work will be conducted in line with this. It is the responsibility of the applicant to ensure all relevant external and internal regulations, policies and legislations are met.
2. This project may be subject to periodic review by the committee. The approval may be suspended or revoked at any time if there has been a breach of conditions.
3. Any substantial amendments to the approved proposal will be submitted to the ethics committee prior to implementing any such changes.

Specific conditions in respect of this application:

The application has been classified as Medium risk to the University. No additional conditions.

Statement of compliance

The Committee is constituted in accordance with the Governance Arrangements for Research Ethics Committees. It complies with the guidelines of UKRI and the concordat to support Research Integrity.

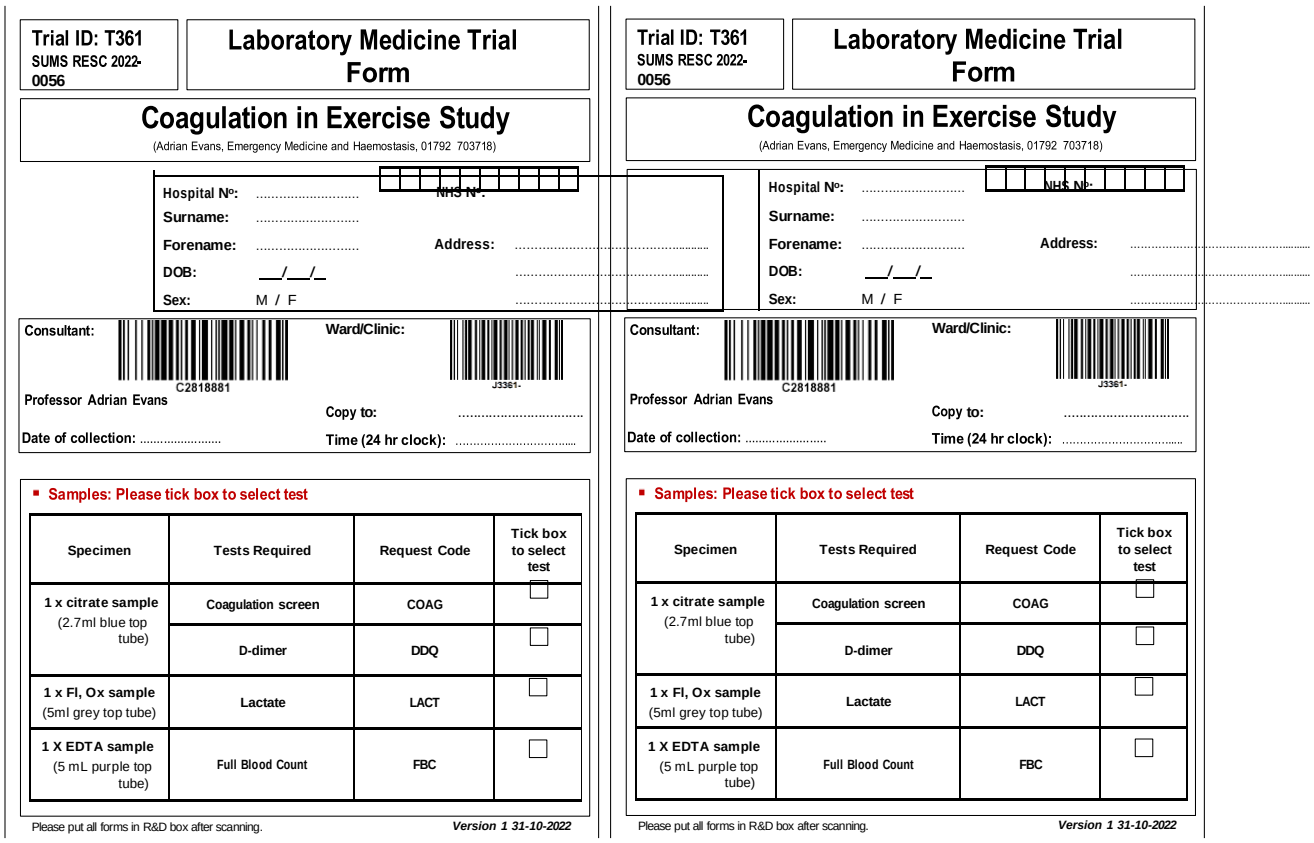
Best wishes, Ana Da Silva,

Medicine Research and Ethics Chair Swansea University.

If you have any query regarding this notification, then please contact your research ethics administrator for the faculty.

For Science and Engineering contact FSE-Ethics@swansea.ac.uk
For Medicine, Health and Life Science contact FMHLS-Ethics@swansea.ac.uk For Humanities and Social Sciences contact FHSS-Ethics@swansea.ac.uk

6.6 Laboratory Medicine Trial Form



Trial ID: T361
SUMS RESC 2022-0056

Laboratory Medicine Trial Form

Coagulation in Exercise Study

(Adrian Evans, Emergency Medicine and Haemostasis, 01792 703718)

Hospital Nº: ..... NHS Nº:
Surname: .....
Forename: ..... Address: .....
DOB: __/__/_ .....
Sex: M / F .....

Consultant: C2818881
Professor Adrian Evans
Ward/Clinic: J3361-
Copy to: .....
Date of collection: ..... Time (24 hr clock): .....

▪ Samples: Please tick box to select test

Specimen	Tests Required	Request Code	Tick box to select test
1 x citrate sample (2.7ml blue top tube)	Coagulation screen	COAG	☐
	D-dimer	DDQ	☐
1 x Fl, Ox sample (5ml grey top tube)	Lactate	LACT	☐
1 X EDTA sample (5 mL purple top tube)	Full Blood Count	FBC	☐

Please put all forms in R&D box after scanning. *Version 1 31-10-2022*

Trial ID: T361
SUMS RESC 2022-0056

Laboratory Medicine Trial Form

Coagulation in Exercise Study

(Adrian Evans, Emergency Medicine and Haemostasis, 01792 703718)

Hospital Nº: ..... NHS Nº:
Surname: .....
Forename: ..... Address: .....
DOB: __/__/_ .....
Sex: M / F .....

Consultant: C2818881
Professor Adrian Evans
Ward/Clinic: J3361-
Copy to: .....
Date of collection: ..... Time (24 hr clock): .....

▪ Samples: Please tick box to select test

Specimen	Tests Required	Request Code	Tick box to select test
1 x citrate sample (2.7ml blue top tube)	Coagulation screen	COAG	☐
	D-dimer	DDQ	☐
1 x Fl, Ox sample (5ml grey top tube)	Lactate	LACT	☐
1 X EDTA sample (5 mL purple top tube)	Full Blood Count	FBC	☐

Please put all forms in R&D box after scanning. *Version 1 31-10-2022*

6.7 Exercise study Welsh Format

Swansea University
Prifysgol Abertawe

WCEMR

MAE EICH

ANGEN CHI ARNOM !

Oes gennych ddiddordeb mewn cefnogi ein hymchwil?

- Rydym yn chwilio am unigolion iach >40 oed sy'n rhedeg yn gyson ac sy'n gallu rhedeg trac 10 cilomedr.
- Ceir profion gwaed cyn rhedeg, ar ôl rhedeg ac ar ôl 1 awr o orffwys i astudio effaith ymarfer corff ar geulo gwaed.
- Os oes gennych ddiddordeb, e-bostiwch ni ar WCEMR@wales.nhs.uk

Sganiwch y Côd QR i ganfod mwy

6.8 Exercise Study poster English Format

Swansea University
Prifysgol Abertawe

WCEMR

WE NEED

YOU!

Interested in supporting our research?

- We are looking for > 40 years old healthy individuals who run regularly and would be willing to take part in a study involving a 10km run at your own pace.
- Blood tests will be done pre, post- run and after 1 hour of rest to study the effect of exercise on blood clotting.
- If interested, drop us an email @ WCEMR@wales.nhs.uk

Scan the QR code to find out more

6.9 Image Attribution

Permission granted to use image in Figure 5 from Dr. Meghana Halkar

Jun Cezar Zaldua [redacted] Wed, 7 Feb, 18:06 (15 hours ago)
to Meghana

Dear Dr. Halkar,

I hope this email finds you well. I am currently studying a postgraduate degree in the UK and have come across one of your journal articles titled "Dual antiplatelet therapy for acute coronary syndromes: How long to continue?"

Please, I would like to ask your permission to reproduce one of the images you have published on this article specifically, Figure 1. I am planning to use it in my dissertation explaining the role of platelets in our coagulation system. Rest assured that proper credits will be used.

Many thanks and I look forward to hearing from you.

Best regards,

Jun Cezar Zaldua
Post graduate student
Swansea University

Meghana Halkar 09:27 (12 minutes ago)
to me

Good evening Jun,

Sure no problem to reproduce with credits.

Good luck with your dissertation.

Best
Meghana
Sent from my iPhone

> On Feb 7, 2024, at 4:07 AM, Jun Cezar Zaldua [redacted] > wrote:
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Institution name	Swansea University
Expected presentation date	Jun 2024

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Expected presentation date	Jun 2024

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Portions	1

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Requestor Location	Mr. Jun Cezar Zaldua Yr Helig Mynydd Gelliwastad Pantlasau Road, Morriston Swansea, SA66PX United Kingdom Attn: Mr. Jun Cezar Zaldua

Tax Details

Swansea University
Prifysgol Abertawe

GIG CYMRU NHS WALES
Bwrdd Iechyd Prifysgol Bae Abertawe
Swansea Bay University Health Board

6.10 Participant Information Sheet

Study Title: Coagulation in Exercise

Swansea University and the Welsh Centre for Emergency Medicine Research would like to invite you to take part in a research study. Participation is entirely up to you, and before deciding we will explain what the study involves and the intended benefits of the study. One of our team will go through this information sheet with you, to help you decide whether or not you would like to take part and answer any questions you may have. Please feel free to talk to others about the study if you wish. The first part of the Participant Information Sheet tells you the purpose of the study and what participation in the study would involve. Then we can give you more detailed information about the conduct of the study. We are very happy to answer any questions you might have.

Summary

Exercise is promoted as a beneficial lifestyle intervention to reduce the risk of a wide range of cardiovascular diseases. Exercise has long been understood to induce a temporary and reversible high clotting state in response to the physiological demands of exercise. This brief period of enhanced clot formation is thought to last between 30 and 60 minutes before settling to normal. However much of this literature has been focused on young and healthy volunteers. Whether these changes persist as people age or develop long term medical conditions, is not fully understood. Additionally, the recent development of new coagulation tests which are able to better describe the coagulation system has led for the need for much of this work to be re-investigated.

1) What is the purpose of the research?

The study aims to assess how blood clots change during exercise by investigating two aspects of clotting. First, how the template of a blood clot can change, and second how a clot shrinks down after formation. We know that balance in the coagulation system is affected by multiple factors. Other exercise studies have shown that exercise is one such factor. Exercise temporarily increases the tendency of blood to form a clot before settling back to a normal level, however these studies focused on young people. Our studies aims to examine the changes to healthy volunteers over the age of 40 before and after a 10km run.

2) What would taking part involve?

The study will take around two to three hours of your time either in the morning or afternoon on a Saturday. On a convenient date for you, you will be invited to the Institute for Life Sciences 2 (ILS2) Building at Swansea University. We will ask a series of questions about your health and lifestyle to confirm you are eligible for the study. A blood test will be taken before going for a run. Participants will then be taken over to the Swansea Bay walking and cycle path to go for a 10km run finishing back at the ILS2 Building. Immediately after the run another blood test will be taken. Participants will then be given an hour to rest and recover (during this time you will be able to shower, change and eat and drink as you like). After 1 hour a final blood test will be taken. After this is taken, the study is complete and you will be free to leave.

WCEMR
Canolfan Ymchwil Meddygaeth Brys Cymru
Welsh Centre for Emergency Medicine Research

3) Who has reviewed this study?

This study has been reviewed and given favourable opinion by Swansea University Research Ethics Committee with reference number **SUMS RESC 2022-0056.**

Data Protection Privacy Notice

The data controller tor this project will be Swansea University. The University Data Protection Officer provides oversight of university activities involving the processing of personal data, and can be contacted at the Vice Chancellors Office.

Your personal data will be processed for the purposes outlined in this information sheet. Standard ethical procedures will involve you providing your consent to participate in this study by completing the consent form that has been provided to you. The legal basis that we will rely on to process your personal data will be processing is necessary for the performance of a task carried out in the public interest.

The legal basis that we will rely on to process special categories of data **will** be processing is necessary for archiving purposes in the public interest, scientific or historical research purposes or statistical purposes.

Your data will be processed in accordance with the Data Protection Act 2018 and the General Data Protection Regulation (GDPR). **All** information collected about you **will** be kept strictly confidential and **will** be anonymous by allocating a unique study number to you. Your data will only be viewed by the researcher/research team.

All electronic data will be stored on a password-protected computer file in Welsh Centre for Emergency Medicine Research Emergency Department, Morriston Hospital, Swansea. All paper records will be stored in a locked filing cabinet in Welsh Centre for Emergency Medicine Research Emergency Department, Morriston Hospital, Swansea. Your consent information will be kept separately from your responses to minimise risk in the event of a data breach.

What are your rights?
You have a right to access your personal information, to object to the processing of your personal information, to rectify, to erase, to restrict and to port your personal information. Please visit the University Data Protection webpages for further information in relation to your rights. Any requests or objections should be made in writing to the University Data Protection Officer:

Ms Bev Buckley
University Compliance Officer (FOi/DP) Vice-
Chancellor's Office
Swansea University Singleton Park

Swansea SA2 8PP, Email: dataprotection@swansea.ac.uk

How to make a complaint?

If you are unhappy with the way in which your personal data has been processed you may in the first instance contact the University Data Protection Officer using the contact details above.

If you remain dissatisfied then you have the right to apply directly to the Information Commissioner for a decision. The Information Commissioner can be contacted at:

Information Commissioner's Office, Wycliffe House,
Water Lane, Wilmslow, Cheshire,
SK9 SAF
www.ico.org.uk

What if I have other questions?
If you have further questions about this study, please do not hesitate to contact us on 01792 703718:

Contact Details:

Jun Cezar Zaldua, RN Emergency Department Morriston Hospital, Swansea
Email: [redacted] Telephone Number: [redacted]

Dr. Oliver Watson Emergency Department Morriston Hospital, Swansea
Email: [redacted] Telephone Number: [redacted]

Prof. Adrian Evans Emergency Department Morriston Hospital, Swansea
Email: [redacted]

Chapter 7

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