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Haemostatic variables in African adolescents – The PLAY study

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OPSOMMING

Kardiovaskulêre siektes (KVS) is in beide ontwikkelde en ontwikkelende lande een van die hoofoorsake van morbiditeit en mortaliteit. In die nie-blanke populasie in Suid-Afrika is beroerte meer prominent as iskemiese hartsiektes. Dit kan toegeskryf word aan 'n kombinasie risikofaktore onder andere verhoogde hemostatiese merkers wat die ontwikkeling van beroerte bevorder. Dit is bekend dat versteurings in die hemostatiese balans (hiperkoaguleerbaarheid en hipofibrinolise) die ontwikkeling van KVS bevorder.

Dit word algemeen aanvaar dat genetiese-, omgewings-, en gedragsfaktore die basis lê vir die ontwikkeling van KVS gedurende volwassenheid. Een van die studies wat deel uitmaak van hierdie verhandeling, was 'n dwarsdeursnitstudie om vas te stel of hemostatiese abnormaliteite alreeds in adolessensie teenwoordig is en of daar alreeds volgens die hemostatiese faktore [fibrinogeen, trombien-anti-trombien-kompleks (TAT), faktor VIII-koaguleringsaktiwiteit (FVIIIk) en plasminogeen-aktiveerder-inhibeerder-tipe-1-aktiwiteit (PAI-1_{akt})] sekere hoë risikogroepe bestaan wat die ontwikkeling van KVS later in die lewe sal bevorder. In hierdie studie is indelings gemaak volgens geslag, liggaamsvetpersentasie, rypingstatus, lengte-vir-ouderdom en daaglikse aktiwiteitsvlakke.

Aangesien gedragsfaktore [dieet, fisieke aktiwiteit (FA), rook- en drinkgewoontes] beheerbare oorsake van KVS is, kan 'n mens in 'n mate die risiko vir KVS verminder. Die tweede studie wat deel uitmaak van hierdie verhandeling, het ten doel gehad om vas te stel of 'n FA intervensie hemostatiese faktore in 'n sub-steekproef van die deursnitstudie suksesvol kan verlaag.

Die leser word verwys na die abstrakte aan die begin van elk van die artikels (Hoofstukke 3 en 4) vir 'n beskrywing van die proefpersone, studie-ontwerpe en die metodes wat in elke studie gebruik is.

Die belangrikste resultate van die dwarsdeursnitstudie was dat (a) geslag onafhanklik bygedra het tot die variasie van PAI-1_{akt}, maar dat die geslagsverskille in fibrinogeen en TAT toegeskryf kan word aan die betekenisvolle verskille in vetmassa en FA-vlakke tussen die geslagte; (b) fibrinogeen was betekenisvol hoër in die dwerggegroeide kinders as in die kinders wat 'n normale lengte-vir-ouderdom het, wat dus moontlik kan aandui dat kroniese ondervoeding in die kinderjare onafhanklik kan bydra tot verhoogde KVS risiko; (c) dat fiksheid TAT-konsentrasies positief beïnvloed en dat (d) geen betekenisvolle verskille in FVIIIk tussen enige van die onderafdelings gevind kon word nie.

Uit die resultate van die intervensie blyk dit dat 'n 11-weeklange buitelug FA-intervensie geen betekenisvolle verbeterings op die hemostatiese merkers van nie-blanke adolessente gehad het

nie. Hierdie resulte moet egter versigtig geïnterpreteer word aangesien (a) seisoenale variasies die effek van die oefenintervensie kon oorskadu want basislynmetings het in die somer en eindmetings in die winter plaasgevind; (b) teenwoordigheid by die oefensessies nie noodwendig nakoming van die oefeninstruksies aandui nie; (c) basilynwaardes 'n prominente rol in die veranderings wat 'n mens te wagte kan wees speel en dus sal moontlike verbeterings duideliker gesien kan word wanneer die beginwaardes verhoog is. Soortgelyke studies op nie-blanke adolessente is nodig aangesien FA se effek op die hemostatiese veranderlikes 'n onderwerp van debat en spekulasie is en omdat verwante inligting in hierdie populasie beperk is.

Kernwoorde: dwerggroei, faktor VIII, fibrinogeen, fisieke aktiwiteit, hemostase, kardiovaskulêre siektes, plasminogeen-aktiveerder-inhibeerder-tipe-1, Suid-Afrika, trombien-anti-trombien-kompleks

ABSTRACT

Cardiovascular disease (CVD) is a major cause of adult morbidity and mortality in developed as well as in developing countries. In black population groups stroke is more prominent than ischaemic heart disease. This may be attributed to a combination of risk factors seen in this population group *inter alia* raised haemostatic markers, which favour the development of stroke since it is well known that a disturbance in the haemostatic balance (a hypercoagulable and a hypofibrinolytic state) predisposes to CVD.

It is generally accepted that childhood genetic, environmental and behavioural factors lay the groundwork for the manifestation of adult CVD. Therefore, one of the studies that form part of this dissertation was a cross-sectional study to determine whether haemostatic abnormalities are already present in black African adolescents and to determine whether high risk groups exist [in relation to the following haemostatic markers: fibrinogen, factor VIII (FVIII), plasminogen activator inhibitor type 1 activity ($PAI\text{-}1_{act}$), and thrombin anti-thrombin complex (TAT)] for the development of CVD later in life. The population subdivisions were made according to gender, body fat %, maturity status, height for age Z-score, and habitual PA levels. Since behavioural factors [diet, physical activity (PA), smoking and drinking habits] are controllable determinants, it could be possible to improve CVD risk to a certain degree. Therefore, the second study that forms part of this dissertation attempted to establish whether a PA programme will successsfully reduce haemostatic variables in a subset of the study population used in the first study.

The reader is referred to the abstracts at the beginning of each separate study manuscript (Chapters 3 and 4), for a description of the subjects, study design and methods used in each study.

The results of the cross-sectional study showed that in African adolescents (a) gender independently contributed to the variability in $PAI\text{-}1_{act}$, but that the gender difference in fibrinogen and TAT could be explained by the significant differences in fat mass and PA levels observed between the genders; (b) fibrinogen was significantly higher in the stunted compared to the non-stunted children indicating that childhood chronic malnutrition may possibly predispose independently to CVD; (c) fitness influences TAT concentrations positively and that (d) no significant differences in FVIII could be found between any of the subdivisions. As these determinants seem to be modifiable through behavioural changes and optimal nutrition status through early life, it raises a sense of urgency to develop strategies for the prevention and treatment of these risk factors.

The results of the intervention study showed that an 11-week outdoor PA intervention programme had no significant effect on the haemostatic markers of African adolescents, but the results of this study should be interpreted with caution since (a) seasonal variations could have clouded the effect of the PA intervention as baseline measurements were taken in the summer and end measurements in the winter; (b) attendance of the PA sessions does not necessarily implicate compliance to the exercises given; (c) baseline values seem to play a prominent role in the changes that could be expected during an intervention and, therefore, improvements in the haemostatic profile would most likely be more signifanct in individuals with raised baseline levels. Similar research on African children is warranted since studies investigating PA's effect on haemostatic variables remain a topic of debate and speculation and data on African population groups are scanty.

Keywords: cardiovascular disease, factor VIII, fibrinogen, fitness, haemostasis, overfatness, physical activity, plasminogen activator inhibitor type 1, South Africa, stunting, thrombin antithrombin complex

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ADDENDA

LIST OF ABBREVIATIONS

Ag	Antigen
ALA	α-linolenic acid
ANCOVA	Analysis of covariance
ANOVA	Analysis of variance
APCR	Activated protein C resistance
aPTT	Activated partial thromboplastin time
B	Baseline
BF%	Body fat percentage
BMI	Body mass index
CDL	Chronic diseases of lifestyle
CHD	Coronary heart disease
cm	centimeter
CON	Control group
CRP	C-reactive protein
CVD	Cardiovascular disease
D	Delta (change from Baseline to End)
DBP	Diastolic blood pressure
DHA	Docosahexaenoic acid
DM	Diabetes mellitus
E	End
EDTA	Ethylenediamine tetra acetic acid
ELISA	Enzyme-linked-immunosorbent assay
EPA	Eicosapentaenoic acid
ETP	Endogenous thrombin potential
ETPex	Extrinsic endogenous thrombin potential
ETPin	Intrinsic endogenous thrombin potential
EX	Experimental group
F	Factor
F1+2	Prothrombin fragment 1 + 2
FII	Prothrombin or factor II
FIIa	Thrombin or activated FII
FVIIc	Factor VII coagulant activity
FVIII	Factor VIII

FVIII Ag	Factor VIII antigen
FVIIIc	Factor VIII coagulant activity
FVIII PA	Factor VIII procoagulant activity
FDP	Fibrin degradation products
Fib	Fibrinogen
FPA	Fibrinopeptide A
g	gram
h	hour
HAZ	Height for age Z-score
HDL	High density lipoprotein
HDL-c	High density lipoprotein cholesterol
HOMA	Homeostasis model assessment
HR	Heart rate
HRT	Hormone replacement therapy
hs-CRP	High sensitivity C-reactive protein
IHD	Ischaemic heart disease
IL-6	Interleukin 6
IR	Insulin resistance
ISAK	International Society for the Advancement of Kinanthropometry
IU	International units
kDa	Kilodalton
kg	Kilogram
l	Liter
LA	Linoleic acid
LDL	Low density lipoprotein
LDL-c	Low density lipoprotein cholesterol
m	meter
MET	Metabolic equivalent
min	minute (60 seconds)
mmHg	millimeter mercury
mmol	millimole
mol	mole
MS	Metabolic syndrome
MSE	The mean square error
MUFA	Monounsaturated fatty acid

n	number
n-3	Omega 3 fatty acid
nmol	nanomole
OC	Oral contraceptive
PA	Physical activity
PAI-1	Plasminogen activator inhibitor type 1
$PAI\text{-}1_{act}$	Plasminogen activator inhibitor type 1 activity
PAI-1 Ag	Plasminogen activator inhibitor type 1 antigen
PAP	Plasminogen –antiplasmin complex
PAR	Protease activated receptor
PDPAR	Previous Day Physical Activity Recall
PLAY	PhysicaL Activity in the Young study
PT	Physical training
PTHR	Prothrombin or FII
PUFA	Polyunsaturated fatty acid
$\%VO_{2max}$	Percentage of maximal oxygen uptake
QUICKI	Quantitative Insulin Sensitivity Check Index
SBP	Systolic blood pressure
SD	Standard deviation
TAFI	Thrombin activatable fibrinolysis inhibitor
TAT	Thrombin-antithrombin complex
TC	Total cholesterol
TCT	Thrombin clotting time
TF	Tissue factor
TFPI	Tissue factor pathway inhibitor
TG	Triglycerides
TGF-β	Transforming growth factor β
THR	Thrombin or FIIa
TNF-α	Tumor necrosis factor α
t-PA	Tissue type plasminogen activator
t-PA act	Tissue type plasminogen activator activity
t-PA Ag	Tissue type plasminogen activator antigen
u-PA	urokinase type plasminogen activator
UWW	Under water weighing
VAT	Visceral adipose tissue

Vit	Vitamin
VLDL-c	Very low density lipoprotein cholesterol
VO_{2max}	Maximal oxygen uptake
vWf	Von Willebrand factor
W/O	Without
yr	Year

LIST OF SYMBOLS

α	Alpha
β	Beta
r	Correlation
°C	Degrees Celcius
$\downarrow$	Decrease
d	Effect size (Cohen)
=	Equal
♀	Female
γ	Gamma
>	Greater than
$\geq$	Greater than or equal to
$\uparrow$	Increase
♂	Male
μ	Micro
x g	Multiplied by gravitational force
%	Percentage
$\pm$	Plus minus
<	Smaller than
$\leq$	Smaller than or equal to
s_{max}	Maximum standard deviation of two means
Σ	Sum
x_1	Mean of one group
x_2	Mean of the other group

CHAPTER 1
INTRODUCTION

1.1 BACKGROUND AND MOTIVATION

Cardiovascular disease (CVD) is a major cause of adult death in developed as well as in developing countries (Murray & Lopez, 1997; Steyn *et al.*, 1992). A wealth of data has accumulated to suggest that a disturbance in the haemostatic balance (a hypercoagulable and a hypofibrinolytic state) predisposes to CVD (Ajjan & Grant, 2006; Kullo *et al.*, 2000; Sueishi *et al.*, 1998). As the haemostatic system is assuming a prominent role in CVD pathogenesis and progression, several questions arise regarding the haemostatic markers.

Questions regarding the optimal concentrations for the individual haemostatic markers to maintain good health remain unanswered. Currently existing normal reference ranges are limited for adults and even more so for children. According to Baron (2004), the existing standard reference ranges for blood testing are too broad to detect health problems adequately (Baron, 2004). Thus, research to determine haemostatic profile patterning is warranted.

Questions regarding the prevalence of adverse haemostatic profile patterning in adolescents are prominent since the process leading up to the critical stages of CVD [*i.e.* angina pectoris, myocardial infarction ("heart attack"), cerebrovascular accident (stroke) or ischaemic heart disease] in adult life may have started decades earlier during childhood or young adulthood (Cunnane, 1993; McGill *et al.*, 2002). Alarmingly, a great number of children and young adults are presenting risk factors associated with CVD (Decsi & Molnár, 2003; McGill *et al.*, 1998; McGill *et al.*, 2000) and CVDs are not uncommon in young adults (20-35 yr) (Davia *et al.*, 1974). Unfavourable CVD risk profiles seen in children give a sense of urgency to develop strategies for the prevention and treatment of modifiable risk factors as early in life as possible.

Several factors influence the haemostatic profile. These factors can be roughly divided into two groups, the uncontrollable factors *i.e.* genetics, race, gender and advancing age, as well as the controllable factors *i.e.* lifestyle habits and behavioural factors such as smoking habits, diet and physical activity (PA) levels. Since the latter factors are modifiable determinants, it could be possible to decrease CVD risk through managing these determinants early in life. The major strategy for preventing CVD and related disease is to modify the established risk factors before damage occurs. The efficacy of various approaches to manage haemostatic markers and reduce risk early in the life cycle must, therefore, be explored and should be investigated in controlled trials.

Of these modifiable factors, PA was chosen as an intervention modality to effectuate possible reductions in plasma concentrations of several haemostatic markers that have been shown to be affected by PA in the literature. PA was chosen for a number of reasons:

1) simple lifestyle changes as opposed to pharmacological intervention modalities are preferable making PA a prime candidate to alter the risk profile (Ernst, 1993);
2) it seems as if PA is successful in improving haemostatic profiles of adults [as reviewed by Lee and Lip (2003) as well as El-Sayed *et al.* (2004)];
3) despite PA's therapeutic value, there is a gap in the literature on the relationship between PA and/or physical fitness and haemostatic markers in children;
4) the existing studies investigating children's haemostatic markers in relation to exercise remain a topic of debate and speculation due to poorly designed studies where the intervention protocols were not standardised;
5) inactivity is a growing concern related to children due to evidence suggesting that PA among youth has declined over the past several decades (Pate *et al.*, 1994);
6) it seems as though inactive children are more likely to become inactive adults (Dennison *et al.*, 1988; Malina, 1996; Pate *et al.*, 1996), but the reverse is not always true, however, there is little reason to believe that inactive youth are more likely to become active adults (Luepker, 1999);
7) this declining level of exercise has the potential to increase the burden of chronic diseases since it is well-known that a sedentary lifestyle directly and indirectly fosters the development of these diseases (Thomas *et al.*, 2003); and
8) because evidence continues to mount regarding benefits of PA for children (Strong *et al.*, 2005), promoting PA and making it enjoyable and attractive to children have become a health priority.

If all the above are considered, it is clear that research on PA as a modality for changing the haemostatic profiles of children is warranted.

1.2 STRUCTURE OF DISSERTATION

This dissertation is in article format and consists of two chapters and two article manuscripts, one of which will be submitted for publication. The introductory chapter (Chapter 1) contains the problem statement, an enunciation of the dissertation, the aims and objectives and the author's contributions to the studies described in this dissertation. The ensuing chapter is a narrative literature chapter (Chapter 2), which provides additional background information for the interpretation of the data from the articles presented in Chapter 3 and 4. Chapter 3 consists of the first article, "Overfatness, stunting and physical inactivity are determinants of PAI-1_{act},

fibrinogen and TAT in African adolescents – The PLAY study", in which data from the PhysicaL Activity in the Young (PLAY) study were analysed. The first article investigates cross-sectional evidence in order to determine haemostatic profile patterning in young Africans and to establish whether certain population subdivisions of Africans are predisposed to CVD at a young age in terms of their haemostatic markers. The article will be submitted for publication in the European Journal of Clinical Nutrition. Chapter 4, "The effect of physical activity on the haemostatic profiles of African children – The PLAY study", is an experimental study done within the PLAY study. This article investigates PA as a modifiable factor for the haemostatic profile in the same African population used in the cross-sectional study. This study was unfortunately complicated by poor compliance and monitoring of the exercise sessions; subsequently no significant improvements were seen in the intervention group's fitness values and conclusions drawn from the results had to be made with great caution. The subjects raised the following reasons for their poor compliance: (1) some subjects had to look after their younger siblings after school while their parents were at work, (2) others helped their parents with household chores after school, and (3) others dislike exercise and therefore did not participate. For these reasons the results of this study will not be submitted for publication. Instead a critical discussion regarding these issues was included in the discussion section of the article in order to address the confounding factors critically and how they may have affected the results.

The relevant references of the chapters are provided at the end of each chapter. The technical style, dialect and references of Chapter 1, 2 and 4 are according to the mandatory style stipulated by the North-West University, but Chapter 3 is written according to the author's instruction for the European Journal of Clinical Nutrition, where the article will be submitted.

1.3 AIMS AND OBJECTIVES

The PLAY study is a multidisciplinary study in which several recognised CVD risk markers in the haemostatic system were measured [fibrinogen, factor (F) VIII coagulant activity (FVIIIc) and, plasminogen activator inhibitor type 1 activity ($PAI\text{-}1_{act}$) as well as thrombin-antithrombin complex (TAT), which is a global indicator of activation of coagulation reactions].

In the first article (Chapter 3) the main aims were to investigate the plasma concentrations of fibrinogen, TAT, FVIIIc and $PAI\text{-}1_{act}$ in African adolescents and to determine whether high risk groups exist in relation to their haemostatic markers. The objectives are to:

1) measure fibrinogen, TAT, FVIIIc and $PAI\text{-}1_{act}$ plasma concentrations in ± 196 African adolescents in the North West Province; and

2) subdivide the study population in categories based on what is known from the literature to be high risk goups and compare them with each other.

The aim of the second article was to establish whether a PA intervention will successsfully reduce plasma concentration of selected haemostatic variables in a subset of the study population used in the cross-sectional study. The objectives are to:

1) sucessfully present an effective PA intervention in a subset of the study population recruited in the cross-sectional study to which the subjects comply;
2) measure fibrinogen, TAT, FVIIIc and PAI-1_{act} plasma concentrations; and
3) establish whether a PA intervention will effectuate beneficial effects on fibrinogen, TAT, FVIIIc and PAI-1_{act} plasma concentrations by comparing the changes between the experimental and the control group with each other.

1.4 AUTHOR'S CONTRIBUTIONS

The two studies reported in this dissertation were planned and executed by a team of researchers. The contribution of the researchers involved in the two studies presented in this dissertation is given in Table 1.1 and 1.2.

Table 1.1: Research team's qualifications, affiliations and roles for the article: Overfatness, stunting and physical inactivity are determinants of PAI-1_{act}, fibrinogen and TAT in African adolescents – The PLAY study

Title, initials, and surname	**Affiliation**	**Role in the study**
Prof. H.S. Kruger (dietician and pharmacist)	School for Physiology, Nutrition and Consumer Science of the North-West University	Supervisor of the PLAY study (design, planning and conduct of the study, approval of final protocol) and involved in the interpretation of the results as well as writing up of the data. Co-supervisor of Cornelie Nienaber.
Miss C. Nienaber (post-graduate student)	School for Physiology, Nutrition and Consumer Science of the North-West University	Conduct of the study, blood sample analysis, anthropometric measurements, statistical analysis, interpretation of the results and writing up of the data.
Dr. M. Pieters (dietician)	School for Physiology, Nutrition and Consumer Science of the North-West University	Laboratory analysis, statistical analysis, interpretation of the results and guidance regarding the writing up of the data. Supervisor of Cornelie Nienaber.
Prof. H.H. Vorster	School for Physiology, Nutrition and Consumer Science of the North-West University	Interpretation of the results and guidance regarding the writing up of the cross-sectional data.

I declare that I have approved the above-mentioned article, that my role in the study as indicated above is representative of my actual contribution and that I hereby give my consent that it may be published as part of the M.Sc dissertation of Cornelie Nienaber.

Prof. H.S. Kruger | Dr. M. Pieters | Prof. H.H. Vorster

Table 1.2: Research team's qualifications, affiliations and roles in the article: The effect of physical activity on the haemostatic profiles of African children – The PLAY study

Title, initials, and surname	**Affiliation**	**Role in the study**
Prof. H.S. Kruger (dietician and pharmacist)	School for Physiology, Nutrition and Consumer Science of the North-West University	Supervisor of the PLAY study (design, planning and conduct of the study, approval of final protocol) and involved in the interpretation of the results as well as writing up of the data. Co-supervisor of Cornelie Nienaber.
Miss C. Nienaber (post-graduate student)	School for Physiology, Nutrition and Consumer Science of the North-West University	Conduct of the study, blood sample analysis, anthropometric measurements, statistical analysis, interpretation of the results and writing up of the data.
Dr. M. Pieters (dietician)	School for Physiology, Nutrition and Consumer Science of the North-West University	Laboratory analysis, statistical analysis, interpretation of the results and guidance regarding the writing up of the data. Supervisor of Cornelie Nienaber.

I declare that I have approved the above-mentioned article, that my role in the study as indicated above is representative of my actual contribution and that I hereby give my consent that it may be published as part of the M.Sc dissertation of Cornelie Nienaber.

Prof. H.S. Kruger | Dr. M. Pieters

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CHAPTER 2

LITERATURE

2.1 INTRODUCTION

This review of the literature facilitates the understanding and interpretation of the ensuing articles presented in this dissertation. The first article presented in Chapter 3 investigates cross-sectional evidence to determine the ranges for several of the haemostatic markers in a group of apparently healthy African children and to establish whether certain population subdivisions of these children are predisposed to cardiovascular disease (CVD) in terms of their haemostatic markers. The haemostatic markers' concentrations are dependent upon genetic, intrinsic and extrinsic influences. The latter being the controllable determinants, which could be possible modalities to improve the haemostatic risk profile. Of these controllable determinants, physical activity (PA) was used as a modifiable factor in a clinical intervention study (Chapter 4) to investigate whether plasma concentrations of the prominent haemostatic markers could be reduced.

The review will succinctly outline the role of several of the haemostatic factors (focusing on those measured and reported in the ensuing articles) in coagulation and fibrinolysis, followed by the influence of various environmental and lifestyle factors on their plasma concentrations. It appears that genetic factors have a major effect on the haemostatic proteins (De Lange *et al.*, 2001), but because the focus of this review is on body composition, PA and haemostasis, genetic haemostatic determinants will not be discussed. The last section of the review will be dedicated to the effect of PA on haemostasis preceded by an overview of the descriptors used in the literature of this field.

2.2 THE PATHOGENESIS OF THROMBUS FORMATION

2.2.1 An overview of haemostasis

The haemostatic system prevents blood loss and maintains blood in a fluid state under physiologic conditions by complex interactions between prothrombotic factors (coagulation), antithrombotic factors (fibrinolysis), platelets, other circulating cells, as well as the vascular wall (Colman *et al.*, 2000). Imbalances could lead to thrombosis (clot formation inside a blood vessel) and atherosclerosis, or a bleeding tendency (Vorster *et al.*, 1997). Hypercoagulability, hyperaggregability and hypofibrinolysis are part of the atherosclerotic process (Ajjan & Grant, 2006; Kullo *et al.*, 2000; Sueishi *et al.*, 1998). Hypercoagulability is associated with the following recognised CVD risk markers, increased fibrinogen, factor (F) VII, FVIII and von

Willebrand factor (vWf) while hypofibrinolysis is associated with increases in the recognised CVD risk markers plasminogen activator inhibitor type 1 (PAI-1) levels (Mertens & Van Gaal, 2002).

2.2.2 Coagulation

Blood coagulation involves a complex series of interactions between proteases, enzymes and co-factors that lead to the generation of thrombin [THR or FIIa (the 'a' following the haemostatic F indicates that the F is in an activated state)] and the formation of the fibrin-rich clot (Colman *et al.*, 2000). Several models of the coagulation cascade are described in the literature. The classical model describes three main pathways: (i) the intrinsic FX activation pathway; (ii) the extrinsic FX activation pathway; and (iii) the common pathway where the intrinsic and extrinsic pathways converge. The cell-based model developed to explain clinical observations that were not consistent with the classical model, describes coagulation not as a "cascade", but as a process occurring in three overlapping phases (Hoffman & Monroe, 2001). These phases (initiation, amplification and propagation) involve a series of reactions of trypsin-like serine proteases and their cofactors, which occur on two principal cell surfaces: the tissue factor (TF) bearing cells (subendothelial fibroblasts, epithelial cells of the skin and mucosa, stroma cells in the endometrium and astrocytes in the brain) and the platelets (Frédérick *et al.*, 2005; Hoffman & Monroe, 2001). The cell-based model will be discussed here in relation to atherothrombotic disease. Figure 1 schematically presents the cell-based model.

After vessel wall injury or atherosclerotic plaque disruption, TF-bearing cells are exposed to circulating blood (Hoffman & Monroe, 2001). The initiation phase starts with the combination of circulating FVII, a serine protease, with its cell-surface bound receptor TF (also called thromboplastin or CD142) (Frédérick *et al.*, 2005; Hoffman & Monroe, 2001). The TF/FVII complex is activated by conversion of bound FVII to FVIIa, and accelerates the activation of circulating FVII. The TF/FVIIa complex could also be the result of the direct binding of trace amounts of circulating FVIIa (arising from the action of FXa and FIX) with exposed TF (Frédérick *et al.*, 2005; Hoffman & Monroe, 2001). TF/FVIIa complex enhances the activation of TF/FVII dyadicly through an autocatalytic reaction by (i) directly catalysing the proteolytic cleavage of FX into FXa and (ii) *via* a proteolytic mechanism; the TF/FVIIa complex also contributes to the activation of FIX (Frédérick *et al.*, 2005; Hoffman & Monroe, 2001). FXa helps, alone or in combination with FVa, to cleave prothrombin (PTHR or FII) to generate THR. Clot formation remains limited to the site of injury and free FXa is rapidly inhibited by TF pathway inhibitor (TFPI) or antithrombin.

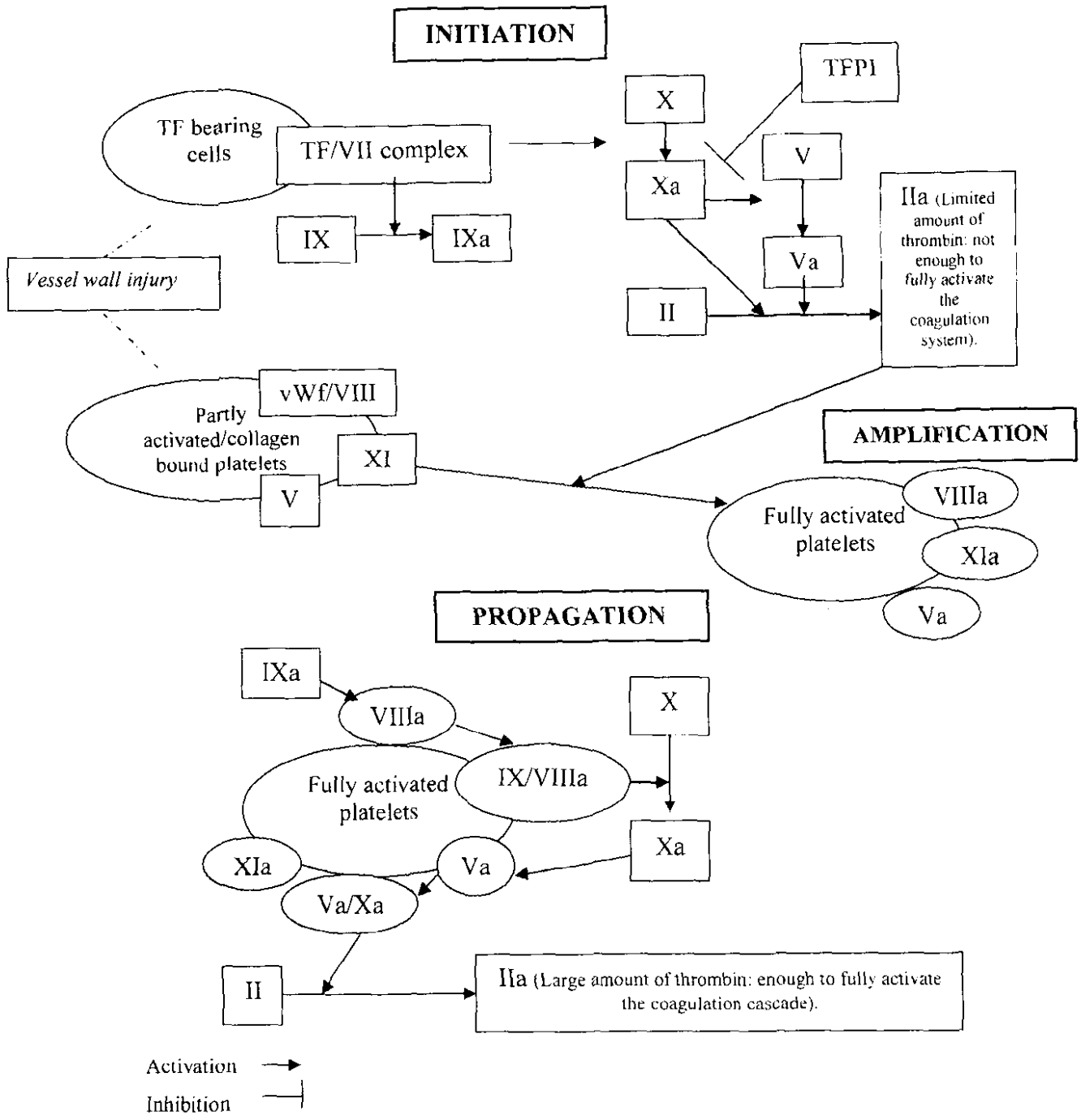

Figure 1: The cell-based model for coagulation [Adapted from Ajjan and Grant (2006)]

TF = tissue factor; TFPI = tissue factor pathway inhibitor; vWf = von Willebrand factor.

Initially, THR is generated in limited amounts and is not enough for the formation of a mature fibrin clot. During vessel wall injury platelets also adhere to the site of injury, mediated in part by the interaction of vWf with collagen and become activated (Hoffman & Monroe, 2001). During amplification the limited amounts of THR generated during initiation can activate collagen bound platelets further. In the propagation phase, fully activated platelets change shape and degranulate, which induces FV release. FV in turn

becomes fully activated by FXa or THR. In addition, THR cleaves FVIII releasing it from the vWf/FVIII complex and activates FXI bound to platelet surface (Frédérick *et al.*, 2005; Hoffman & Monroe, 2001). Platelet surface FXa/Va and FVIIIa/FIXa complexes result in the generation of sufficient THR from PTHR to form a stable haemostatic plug. THR feedback amplifies the system by activating FV, FVIII and FXI. The activation of FXI by THR is another amplification loop resulting in the generation of additional FIXa, which in turn activates more FX (Frédérick *et al.*, 2005).

The key function of the generated THR is the catalytic conversion of soluble circulating fibrinogen to insoluble fibrin. Once formed, fibrin also accelerates the activation of FXIII to FXIIIa by THR. FXIIIa stabilises the clot by covalent cross-linking of fibrin. THR also activates the thrombin activatable fibrinolysis inhibitor (TAFI), which prevents further fibrinolytic attack (Frédérick *et al.*, 2005).

2.2.3 Fibrinolysis

Blood clots formed at the end of coagulation play a temporary role and must be removed when normal tissue structure and functions are restored (El-Sayed *et al.*, 2004). The fibrinolytic system is designed to remove clots and to control the enzymatic degradation of fibrin (Colman *et al.*, 2000; Wu & Zhao, 2002). Figure 2 schematically outlines fibrinolysis. The dominant mechanism for fibrinolysis *in vivo* is the plasminogen-plasmin system in which plasmin breaks down fibrin into its degradation products (FDP) (Hoekstra *et al.*, 2004). Plasmin circulates in the blood in an inert form, plasminogen. Plasminogen and unbound or active tissue type plasminogen activator (t-PA) or urokinase type plasminogen activator (u-PA) bind to the surface of fibrin and cleaves inert plasminogen into active plasmin which in turn is responsible for the dissolution of fibrin (Wu & Zhao, 2002). In the fasting, resting steady state, the specific inhibitor of t-PA, PAI-1 is the main determinant for the intravascular amount of active t-PA and high concentrations of PAI-1 and t-PA antigen (Ag) coexist in the circulation together with low concentrations of active t-PA. Several proteins are involved in fibrinolysis inhibition *inter alia* α_2-antiplasmin, which binds plasmin in plasma and forms an irreversible stable complex; PAI-1 and -2, which inactivates t-PA and u-PA by forming an irreversible 1:1 complex; TFPI which binds to FXa and quenches the activity of FVII-TF complex; and TAFI, which influences plasminogen activation and can directly inhibit plasmin activity (Ajjan & Grant, 2006; Sagripanti & Carpi, 1998; Wu & Zhao, 2002). At the fibrin network level abnormal fibrin architecture with a dense and tight fibrin conformation also resists lysis and can contribute to hypofibrinolysis associated with CVD (Collet *et al.*, 2000).

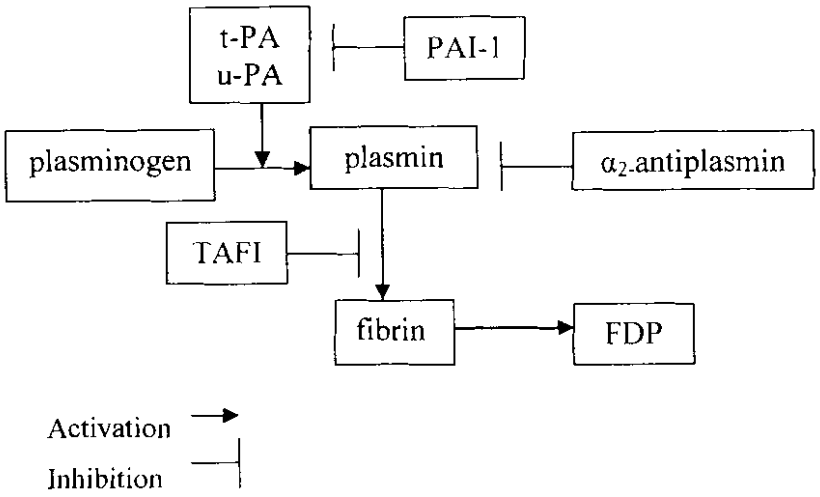

Figure 2: Fibrinolytic system [Adapted from Wu and Zhao (2002)]

t-PA tissue type plasminogen activator; u-PA urokinase type plasminogen activator; PAI-1 plasminogen activator inhibitor type 1; FDP fibrin degradation products; TAFI thrombin activatable fibrinolysis inhibitor

2.3 HAEMOSTATIC FACTORS AND THEIR PLASMA DETERMINANTS

2.3.1 Fibrinogen

Fibrinogen (also known as FI) with a computed molecular weight of 340 kDa, is a soluble glycoprotein synthesised in the liver and found in the plasma in 'usual' concentrations of 1.5 to 4.5 g/l (Hangtgan *et al.*, 2000; Kamath & Lip, 2003). Currently the recommended optimal range for fibrinogen is 2-3 g/l (Baron, 2004). Fibrinogen plays an important role in blood coagulation, determines blood rheology characteristics and acts as an acute phase protein (Hangtgan *et al.*, 2000; Kamath & Lip, 2003). A high plasma fibrinogen concentration has been shown to be associated with CVD and plays a pivotal role as a risk factor (Ernst & Ludwig, 1993; Kannel *et al.*, 1987; Meade *et al.*, 1986; Wilhelmsen *et al.*, 1984; Yarnell *et al.*, 1991). The precise role of fibrinogen in CVD pathology is not completely clear. Fibrinogen could merely be a marker of blood viscosity and risk marker for coronary heart disease (CHD), myocardial infarction and stroke (Vorster *et al.*, 1997), instead of being responsible for CVD. As an acute phase reactant, it might be a marker of inflammation, which probably plays a role in CVD since it seems as if a combination of inflammatory and thrombotic processes contributes to the development of CVD (Ajjan & Grant, 2005). Fibrinogen concentrations are dependent upon genetic, intrinsic and extrinsic influences.

2.3.1.1 Age

It seems as if fibrinogen concentrations increase with age (Barker *et al.*, 1992; Kannel *et al.*, 1987; Meade *et al.*, 1979; Tarallo *et al.*, 1992) independent of gender (Sagripanti and Carpi, 1998). Even in children fibrinogen progressively tends to increase with age (Cook *et al.*, 1999). A slower rate of disposal of fibrinogen and not an increased synthesis rate was suggested as a plausible mechanism for the age related increase in fibrinogen concentrations (Fu & Nair, 1998).

2.3.1.2 Gender

Crude fibrinogen values are consistently higher in women than in men of all ages, irrespective of pregnancy, the use of oral contraceptives (OC) (Cook *et al.*, 1999; Dotevall *et al.*, 1994; Giansante *et al.*, 1994; Krobot *et al.*, 1992; Laharrague *et al.*, 1993; Prisco *et al.*, 1996; Tarallo *et al.*, 1992) or differences in smoking habits (Mennen *et al.*, 1999).

2.3.1.3 Seasonal variations

CVD mortality shows a seasonal variation, with a peak during the winter season (Woodhouse *et al.*, 1994). Fibrinogen has a predictable seasonal chronobiological pattern of variation (Kelly, 2005) with significantly higher fibrinogen concentrations in the winter (Crawford *et al.*, 2003; Woodhouse *et al.*, 1994). This phenomenon can be attributed to fibrinogen's acute phase behaviour induced by infections occurring more often during the winter months (Woodhouse *et al.*, 1994), or by a complex negative relationship between temperature and pro-thrombotic markers (Cook *et al.*, 1999; Crawford *et al.*, 2003).

2.3.1.4 Diet

Nutritional factors (total diet, as well as specific dietary components, nutrients and overall nutritional status) appear to explain a small percentage of the variance in the distribution of haemostatic factors (James *et al.*, 2000; Vorster *et al.*, 1997; Vorster *et al.*, 1998).

The relationships of the different types of fatty acids with haemostatic markers are complex because a particular fatty acid may have both procoagulant and anticoagulant activities (Vorster *et al.*, 1997). In some studies, monounsaturated fatty acids (MUFAs) showed fibrinogen-lowering effects (Pérez-Jiménez *et al.*, 2002) while others did not find a decrease after MUFA (200 g avocado) substituted mixed dietary fat (Pieters *et al.*, 2005). Fish oils comprising of omega-3 (n-3) fatty acids [eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA)] and the antioxidant α-tocopherol [vitamin (vit.) E] seem to have fibrinogen-lowering effects (Haglund *et al.*, 1994; Oosthuizen *et al.*, 1994), but it is still controversial since some researchers found conflicting results (Allman-Farinelli *et al.*, 1999). A high-fat diet rich in MUFA and marine n-3 polyunsaturated fatty acids (PUFA) led to an increase in

fibrinogen, whereas a low-fat diet induced a nonsignificant decrease in fibrinogen (Junker *et al.*, 2001). Fat intake may influence haemostasis indirectly through its effects on the blood lipoproteins, which seem to correlate with the haemostatic markers (James *et al.*, 2000; Prisco *et al.*, 1996; Vorster *et al.*, 1997; Vorster *et al.*, 1998).

Data on fibre and fibrinogen are inconsistent (Silvis *et al.*, 1990; Turpeinen *et al.*, 2000) and complicated by the different types of fibres used in studies. Different types of fibre are hypothesized to have different physiological effects on the haemostatic system (Vorster *et al.*, 1997). Vorster *et al.* (1997) emphasized the difficulty to dissect effects of fibre from those of fat or other factors in dietary intervention studies.

Studies on specific foods containing antioxidants (flavonoids or polyphenols) and fibrinogen are still inconclusive (Vorster *et al.*, 1997). Black and green tea containing antioxidants of the flavonoid family did not reduce fibrinogen in smokers (De Maat *et al.*, 2000). After a 3-week vegetarian diet was followed, significant fibrinogen-lowering was seen (Høstmark *et al.*, 1993). This could be attributed to a higher fibre intake or the high antioxidant (polyphenolic) content of some vegetables.

Both undernutrition (poor iron, vit. E and vit. B6 status) and overnutrition are associated with high fibrinogen concentrations (James *et al.*, 2000). Lower fibrinogen concentrations were associated with dietary intakes compatible with prudent dietary guidelines in men and women (low intakes of animal protein; trans fatty acids and higher intakes of plant protein; dietary fibre, vit. E and iron and a high dietary polyunsaturated/saturated fat ratio) (James *et al.*, 2000). Dietary changes due to the nutrition transition could influence the haemostatic system negatively (Vorster, 2002). Urbanisation in developed, as well as in developing countries, has evoked large increases in the incidence of chronic diseases of lifestyle (CDL) such as hypertension, obesity, CVD, stroke and type II diabetes mellitus (DM) (Bourne *et al.*, 2002; Bradshaw *et al.*, 1999; Kruger *et al.*, 2001; Popkin *et al.*, 1996; Vorster *et al.*, 2000).

2.3.1.5 Alcohol consumption

Moderate alcohol consumption could be involved in protection against CHD through its effect on haemostatic mechanisms (De Gaetano *et al.*, 2003). Moderate alcohol consumption has been shown to reduce fibrinogen concentrations (Meade *et al.*, 1979; Mukamal *et al.*, 2004; Sierksma *et al.*, 2001; Sierksma *et al.*, 2002; Tarallo *et al.*, 1992). The mechanism of reduction was specific for fibrinogen and unrelated to the reduction in C-reactive protein, thus the fibrinogen decrease was not attributed to a reduced inflammatory state (Sierksma *et al.*, 2001). This is in keeping with other studies which also showed decreases (Dimmitt *et al.*, 1998; Hendriks & Van der Gaag, 1998; Krobot *et al.*, 1992; Pellegrini *et al.*, 1996). The mechanism behind this decrease remains to be determined, but it seems as though it is the

ethanol and not the non-alcohol components of beverages conveying this positive effect (Pellegrini *et al.*, 1996).

While moderate alcohol consumption is associated with decreased mortality from CVDs, drinking large amounts in a short period (binge drinking) and chronic alcohol abuse are associated with increased CVD morbidity (Puddey *et al.*, 1999). In the MONICA study self-reported moderate daily alcohol consumption up to 40 g was associated with lower fibrinogen, compared to non-drinking and heavy drinking, after adjustment for potential confounders (Imhof *et al.*, 2004). According to Denninger (1999), the liver produces most clotting factors and inhibitors, as well as proteins involved in fibrinolysis and clears activated enzymes involved in coagulation or fibrinolysis from the bloodstream. The liver protects against both bleeding and undue activation of coagulation. Liver diseases caused by alcohol abuse are commonly responsible for haemostatic abnormalities including decreased production of clotting factors, thrombocytopenia, platelet dysfunction and increased circulating fibrinolytic activity (Denninger, 1999).

2.3.1.6 Smoking and snuff

A plethora of studies indicate that cigarette smokers (active and passive) have higher fibrinogen concentrations proportionally to the amount smoked than persons who do not smoke and ex-smokers have lower values than continuing smokers (Barker *et al.*, 1992; Brunner *et al.*, 1996; Eliasson *et al.*, 1995; Iso *et al.*, 1996; Kannel *et al.*, 1987; Krobot *et al.*, 1992; Meade *et al.*, 1979; Tarallo *et al.*, 1992). This suggests a direct dose response, reversible relationship. The exact mechanisms behind this inverse association are not entirely understood, but may be related to an inflammatory process in the lungs evoked by smoking. Smokeless tobacco (snuff) taking or dipping, however, does not appear to affect fibrinogen concentrations (Eliasson *et al.*, 1995; James *et al.*, 2000).

2.3.1.7 Impaired growth

Impaired growth due to under-nutrition in foetal life, infancy and early childhood manifesting as low birth weight or stunting (low height for age) is strongly associated with high fibrinogen concentrations (Barker *et al.*, 1992; Brunner *et al.*, 1996; Martyn *et al.*, 1995). This could be explained through impaired liver development during critical early periods of life (Barker *et al.*, 1992; Martyn *et al.*, 1995). Research done on animals showed that influences which restrain growth during critical periods of early life permanently affect organ size and function (Barker *et al.*, 1992). The control of haemostasis in adults is partly programmed by the intrauterine and infant environments giving further evidence of the importance of foetal and infant development in the genesis of CVD (Barker *et al.*, 1992). However, results of a few

studies relating birth weight and fibrinogen concentrations are still contradictory (Cook *et al.*, 1999; Roseboom *et al.*, 2000).

Childhood stunting or growth retardation due to chronic malnutrition is a possible contributing factor to the alarmingly high prevalence of adult obesity [Barker *et al.*, 2002; Hoffman *et al.*, 2000(a); Hoffman *et al.*, 2000(b); Popkin *et al.*, 1996; Steyn *et al.*, 2005; Vorster *et al.*, 2000]. Hoffman *et al.* [2000(b)] found that short stature could be predictive of obesity because of impaired fasting fat oxidation seen in stunted people, predisposing them to excessive weight and fat gains at high energy and fat intakes found in typical Western diets. Adipose tissue *per se* can induce an inflammatory response which could lead to higher fibrinogen (Kamath & Lip, 2003). Early malnutrition in utero during infancy or during childhood together with genetic selection contribute to an increased vulnerability to CDL that traditional Africans experience when they adopt to a Westernised lifestyle (Barker *et al.*, 2002; James *et al.*, 2000). In South Africa rural exodus of Africans to urban areas are exposing them to Western lifestyles providing the ideal conditions for the complications of stunting to emerge (Popkin *et al.*, 1996).

2.3.1.8 Socio economic class / psychosocial factors / stress

Barker *et al.* (1992) and Cook *et al.* (1999) found no association between fibrinogen concentrations and current social class or social class at birth. Fibrinogen concentrations were unrelated to the town or the occupational social class of the parents (Barker *et al.*, 1992; Cook *et al.*, 1999). In a study conducted by Rodríguez-Larralde *et al.* (2005), Venezuela women in their control group's socioeconomic level had a significant effect on fibrinogen values. Brunner *et al.* (1996) and Morley *et al.* (2000) found discordant results. They found an inverse relationship between fibrinogen concentrations and employment grade, extent of education as well as the father's social class and social deprivation, respectively. The association between job stress and fibrinogen concentrations is a controversial subject with numerous confounders. The association between stress and fibrinogen concentrations may seem phylogenetically logical since an increased procoagulant state may be important to survive in physical fights (Theorell, 2002). Several studies have mirrored a relationship between job strain (high psychological demands and low decision latitude at work) and high fibrinogen concentrations (Brunner *et al.*, 1996; Su, 2001; Tsutsumi *et al.*, 1998) while others found no such relationship (Ishizaki *et al.*, 2001; Riese *et al.*, 2004). Low job decision latitude may be one of the factors contributing to the relationship between low socioeconomic group and high fibrinogen concentrations (Theorell, 2002). Conclusions must, therefore, be made with caution.

2.3.1.9 Hormonal status of women

Studies report both increased fibrinogen in OC users (especially those with a high oestrogen concentration) (Balleisen *et al.*, 1985; Cachrimanidou *et al.*, 1994; Ernst *et al.*, 1989; Famodu, 1997; Machado *et al.*, 2004; Meade *et al.*, 1979; Meade *et al.*, 1980) as well as no such change (Lee *et al.*, 1992). In a review by Ernst (1992), longitudinal studies demonstrated that OCs lead to a significant rise in fibrinogen concentrations within 1-3 months of medication, but upon discontinuation within approximately 3 months fibrinogen concentrations returned to normal. OC use might contribute to the CVD risk partly by elevating fibrinogen concentrations.

Menstruating women (premenopausal women) have lower fibrinogen concentrations than postmenopausal women (Brunner *et al.*, 1996; Lee *et al.*, 1992). Fibrinogen concentrations increased with age in men and in postmenopausal women but not in pre-menopausal women (Brunner *et al.*, 1996). Data on hormone replacement therapy (HRT) is contradictory including some studies showing a decrease in fibrinogen concentrations (Ciepluch & Czestochowska, 1995; Frohlich *et al.*, 1998; Gottsäter *et al.*, 2001), while some show an increase (Kroon *et al.*, 1994). Before the menopause women could partly be protected by sex hormones from the risk associated with raised fibrinogen concentrations (Brunner *et al.*, 1996).

2.3.1.10 Metabolic syndrome (insulin-resistance syndrome)

The metabolic syndrome (MS) comprises an array of CVD risk factors such as abdominal obesity, atherogenic dyslipidemia, hypertension, glucose intolerance or insulin resistance, proinflammatory state and a prothrombotic state (Grundy *et al.*, 2004). Fibrinogen concentrations consistently show direct associations with features of MS (Morange *et al.*, 2004). Surrogates for insulin resistance (IR) (raised fasting insulin concentration, hyperglycemia or DM) (Brunner *et al.*, 1996), high waist-hip ratio or visceral adiposity (Barbeau *et al.*, 2002; Brunner *et al.*, 1996; Krobot *et al.*, 1992), the anthropometry parameters including body mass index (BMI), body weight and body fat % (Balagopal *et al.*, 2005; Barbeau *et al.*, 2002; Brunner *et al.*, 1996; Cook *et al.*, 1999; Meade *et al.*, 1979; Morley *et al.*, 2000; Prisco *et al.*, 1996), raised serum triglycerides (TG) (Brunner *et al.*, 1996); low levels of high-density-lipoprotein cholesterol (HDL-c) (Brunner *et al.*, 1996; Eliasson *et al.*, 1994) and serum total cholesterol (TC) (Eliasson *et al.*, 1994; Prisco *et al.*, 1996) are positively associated with fibrinogen concentrations.

The relationship between fibrinogen concentrations and markers of glucose tolerance *e.g.* insulin (as seen in the first paragraph), is not independent of the accompanying inflammatory reaction (Mertens & Van Gaal, 2002). The notion is growing that fibrinogen concentrations

are determined by overall adiposity rather than insulin resistance (Morange *et al.*, 2004). Obesity induces a low-grade inflammatory state (Bastard *et al.*, 2006; Trayhurn & Wood, 2005) and because fibrinogen is an acute phase protein it is hypothesised that the concentrations of fibrinogen can increase. Fibrinogen concentrations are related to obesity *per se* [1.44 g/l rise in fibrinogen concentrations per kg increase in body weight (Morley *et al.*, 2000)] and weakly related to abdominal obesity and insulin levels (Morange *et al.*, 2004). Interleukin 6 (IL-6) represents the possible link for this abnormality as it is produced by adipose tissue and will directly stimulate the hepatic synthesis of fibrinogen (Morange *et al.*, 2004). An alternative explanation is that obesity may lead to increased production or decreased clearance of fibrinogen (Ferguson *et al.*, 1998).

Recent studies have suggested that low-grade systemic inflammation participates in the pathophysiology of CHD, MS and an abnormal coagulation process (Elisaf, 2001; Libby & Simon, 2001; Morrow & Ridker, 2000; Verheggen *et al.*, 1999).

2.3.2 Factor VIII

FVIII also known as antihemophiliac factor or fibrin stabilising factor, is a plasma coagulation protein acting as a cofactor in the clotting process (Baron, 2004). It is well established that elevated plasma levels of FVIII are independently associated with an increased risk for CVD (Kraaijenhagen *et al.*, 2000; Morange *et al.*, 2005; O'Donnell & Laffan, 2001; Rumley *et al.*, 1999). It is unclear whether elevated FVIII is a constitutional risk factor, an acquired risk factor or merely a reactive consequence of venous thrombosis (O'Donnell *et al.*, 2001). A functional relation exists between activated protein C resistance (APCR) and FVIII. Elevated FVIII levels contribute to APCR, which leads to increased thrombotic risk. The only receptor of FVIII identified so far is the lipoprotein receptor-related protein, which is thought to be involved in FVIII degradation. Genetic factors strongly contribute to the variability of FVIII levels while the environment poorly influences FVIII (Morange *et al.*, 2005).

2.3.2.1 Von Willebrand factor and the ABO blood group

The ABO blood group and vWf are known to be of the main determinants of plasma FVIII levels (Morange *et al.*, 2005). FVIIIc (the 'c' following the FVIII denotes coagulant activity) levels are significantly lower in group O than in group A or B individuals (O'Donnell & Laffan, 2001). FVIII requires vWf for stability and circulates as a complex with vWf. They are, therefore, positively related (Mertens & Van Gaal, 2002).

2.3.2.2 Age and gender

The normal aging process is paralleled by an increase in plasma FVIII concentration independent of gender (Corsaut *et al.*, 1990; Sagripanti & Carpi, 1998). FVIIIc seems to be higher in women than in men (Conlan *et al.*, 1993), but Blombäck *et al.* (1992) found no direct relation between FVIII and oestradiol, progesterone or testosterone levels.

2.3.2.3 Diet

Diet-related studies done to determine influences on FVIII are scarce and do not permit definite conclusions. FVIIIc seems to be effected by the fat load of a meal. FVIIIc levels were high after a PUFA-rich meal and low after a fat-free meal containing only carbohydrates, but these changes varied significantly between individuals (Salomaa *et al.*, 1993). Dietary fats such as n-3 fatty acids (EPA and DHA) have been demonstrated to alter coagulation and fibrinolysis variables. Allman-Farinelli *et al.* (1999) compared the effects of a cholesterol-lowering diet with a similar diet where 50% of the linoleic acid (LA) was replaced by α-linolenic acid (ALA), on selected hemostatic variables. The ALA-rich diet tripled the percentage of platelet EPA ($p < 0.0005$), but had little effect on FVIIIc or vWf. Allman-Farinelli *et al.* (1999) suggested that higher amounts of PUFA might be necessary to produce a lowering effect. Customary intakes of fish and n-3 fatty acids in populations that do not consume large amounts of fish are not associated with FVIII, or vWf (Archer *et al.*, 1998).

In a cross-sectional study on healthy elderly men plasma levels of the antioxidant vit. C, fruit intake, dietary vit. C intake or vegetable intake were not associated with vWf or FVIII levels (Wannamethee *et al.*, 2006) even though vit. C has anti-inflammatory properties that could influence FVIII's inflammatory characteristics.

2.3.2.4 Alcohol consumption

Moderate alcohol consumption is associated with lower levels of FVIIIc and various other inflammatory markers in older adults free of CVD (Conlan *et al.*, 1993; Mukamal *et al.*, 2004).

2.3.2.5 Smoking

FVIIIc levels seem to be negatively associated with smoking (Conlan *et al.*, 1993). The literature on FVIIIc and smoking is limited.

2.3.2.6 Hormonal status of women

No relationship between FVIII and oestrogen usage were found in healthy adults (Corsaut *et al.*, 1990). HRT seems to have no effect on FVIII Ag in healthy postmenopausal women (Post *et al.*, 2002).

2.3.2.7 Metabolic syndrome

Plasma levels of FVIII have also been shown to be associated with metabolic factors and inflammatory markers. Haemostatic abnormalities are abundant in people with the MS (Kamath & Lip, 2003). Associations between MS and increased haemostatic markers including raised FVIII and vWf are evident (Wannamethee *et al.*, 2005). Colan *et al.*, (1993) found that FVIIIc is positively associated with diabetes, BMI, waist to hip ratio, serum insulin and plasma TGs and negatively with HDL-c, but no correlations were observed between FVIII and plasma low density lipoprotein cholesterol (LDL-c) or lipoprotein(a). Studies on the relationship between insulin resistance and FVIII levels are inconsistent (Mertens & Van Gaal, 2002). Obesity, in particular abdominal obesity, tends to be associated with higher values of FVIII (Mertens & Van Gaal, 2002). Data on the effect of weight loss on FVIII are scarce, but it does not seem to have a significant effect (Mertens & Van Gaal, 2002).

2.3.3 Plasmin-α 2-plasmin inhibitor and thrombin-antithrombin III complex

Plasmin-α 2-plasmin inhibitor complex (PAP) is an overall marker of fibrinolytic activity as it is a product of fibrinolysis (Stegnar *et al.*, 2003; Vorster *et al.*, 1997). Thrombin-antithrombin III complex (TAT) is a product of THR generation and a parameter of the latent activation of coagulation (Asakawa *et al.*, 2000; Stegnar *et al.*, 2003; Uno *et al.*, 1989; Vorster *et al.*, 1997). Since TAT seems to be suitable as a laboratory marker of deep vein thrombosis, its use is becoming more widespread (Giansante *et al.*, 1994). The interpretation of crude TAT values are complicated if PAP values are unknown since coagulation and fibrinolysis occurs simultaneously. Therefore, if high TAT levels accompany high PAP levels the haemostatic system is in balance, but if one is raised significantly above the other, the scale is towards a haemostatic imbalance. The prognostic value of PAP and TAT to predict CVD remains to be fully defined in future epidemiological and clinical studies (Stegnar *et al.*, 2003). Currently data on these markers are considerably less abundant and more controversial than for the other haemostatic markers. Little is thus known about the environmental factors' associations with PAP and TAT.

2.3.3.1 Age and gender

TAT levels have an age related increase and are positively correlated with age (Sagripanti & Carpi, 1998). Hypercoagulability associated with aging appears to be compatible with health and longevity inasmuch as haemorrhage and thrombosis must be prevented in the elderly (Sagripanti & Carpi, 1998). Information on the association between advancing age and gender with relation to PAP and TAT is limited.

2.3.3.2 Diet

There is an information scarcity regarding the effects of diet on both PAP and TAT concentrations. Moderate weight loss induced by energy restriction in elderly, obese individuals increased PAP complex concentration by 20%, indicating augmented fibrinolytic activity (Calles-Escandon *et al.*, 1996). Further research in this area is merited.

2.3.3.3 Metabolic syndrome

MS and DM are associated with an increased incidence of vascular complications. A significant increase of plasma TAT and PAP levels in type 1 and 2 DM patients compared with healthy control subjects has been reported, which indicates that continuous activation of coagulation and fibrinolysis actually occurs in the majority of the patients with DM (Takahashi *et al.*, 1989). TAT seems to be higher in DM patients with retinopathy or nephropathy than in patients without these complications, suggesting that disorders of coagulation and fibrinolysis coexist with these complications of DM (Asakawa *et al.*, 2000).

2.3.3.4 Hormonal status of women

Cachrimanidou *et al.* (1994) found a significant increase of TAT complex after women used a desogestrel-containing OC. The onset of menopause is accompanied by a significant increase in antithrombin III plasma levels (Sagripanti & Carpi, 1998). The coagulation inhibitors, antithrombin III, protein C and protein S appear to be essentially unchanged with OC use (Cachrimanidou *et al.*, 1994).

2.3.3.5 Smoking

Enderle *et al.* (2000) compared endothelial function and variables of fibrinolysis (t-PA, PAP) and coagulation (TAT, fibrinogen) in smokers with healthy controls. The TAT complex, fibrinogen, PAP complex, t-PA and PAI-1 activity ($PAI\text{-}1_{act}$) did not differ between smokers and controls, but peripheral endothelial dysfunction appeared to be common in smokers.

2.3.4 Plasminogen activator inhibitor-type 1

In normal human plasma, PAI-1 (also known as the fast-acting inhibitor against t-PA a protein member of the serpin superfamily with a molecular weight of 52 kDa) levels can range from 0.5 to 1.5 nmol/l (Wu & Zhao, 2002). Vascular endothelial cells, hepatocytes, smooth muscle cells and adipocytes synthesise PAI-1 (Wu & Zhao, 2002). PAI-1 has a key role to inhibit t-PA and u-PA and, therefore, high levels of PAI-1 reduce fibrinolytic potential thereby increasing the risk for CVD (Morange *et al.*, 2004). Both $PAI\text{-}1_{act}$ and Ag are markers for fibrinolytic potential and function as well as atherogenic and thrombotic risk (Hoekstra *et al.*, 2004; Vorster *et al.*, 1997). In addition to CVD, PAI-1 appears to play a

role in the pathogenesis of inflammatory diseases, chemotherapy-induced pulmonary fibrosis and cancer progression (Wu & Zhao, 2002).

2.3.4.1 Age

The impact of ageing on fibrinolytic activity has not been fully elucidated. PAI-1_{act} seems to show some age dependence due to an association between advancing age and increased platelet aggregability and decreased fibrinolytic activity (Gleerup & Winther, 1995).

2.3.4.2 Blood lipids

Väisänen *et al.* (1997) found an independent association between small, dense LDL particles and PAI-1_{act} in middle-aged men. Salomaa *et al.* (1993) found strong associations between t-PA Ag and PAI-1 with total TG and very low-density lipoprotein cholesterol (VLDL-c) in the fasting state, but during lipemia, the associations were approximately similar or slightly weaker than in the fasting state. PAI-1 correlates with TC, LDL-c, VLDL-c and TG levels and inversely with HDL-c (as reviewed by Hoekstra *et al.*, 2004). VLDL-c induces a concentration-dependent increase in PAI-1 through stimulation of PAI-1 expression in endothelial cells mediated through transcriptional activation of the PAI-1 gene *in vitro* (Hoekstra *et al.*, 2004). According to Hoekstra *et al.* (2004), the effects of LDL-c on PAI-1 are discordant and are not dependent on interactions with the LDL-receptor. *In vitro* native-LDL does not stimulate PAI-1 synthesis, unless high concentrations, oxidized or glycated LDL-c are used (Hoekstra *et al.*, 2004).

2.3.4.3 Diet

Limited data are available on dietary factors' effect on PAI-1, but studies on the n-3 fatty acids are numerous. In several interventions n-3 fatty acids increase PAI-1_{act} and Ag levels (Grundt *et al.*, 1999; Haglund *et al.*, 1994; Oosthuizen *et al.*, 1994). This may be a nonspecific response to maintain vascular haemostasis (Haglund *et al.*, 1994). However, in other studies supplementation lowered or did not change PAI-1 (Prisco *et al.*, 1994; Toft *et al.*, 1997). Comparisons of the studies addressing this issue are complicated by small sample sizes, different control supplements as well as study designs. Hoekstra *et al.* (2004) concluded in their review that n-3 fatty acids may lead to an increase in PAI-1 levels, but the effects are slight and depend on the type of fat consumed. The mechanisms are still not fully understood.

Scanty data exist on the association between antioxidants and PAI-1 levels. No firm conclusions are allowed as yet although present data suggest that antioxidants have a possible PAI-1 lowering effect (Hoekstra *et al.*, 2004).

The effects of fibre on PAI-1 levels are only marginally researched and deserve further investigation. No significant differences between periods of high and low fibre intake were seen in PAI-1 levels (Turpeinen *et al.*, 2000).

Energy restriction sufficient to induce weight loss leads to diminution of elevated plasma PAI-1 in elderly, obese subjects (Calles-Escandon *et al.*, 1996). This decline in PAI-1 correlated with the decrease in body weight as well as the loss of fat mass (Calles-Escandon *et al.*, 1996). Junker *et al.* (2001) compared a high fat diet rich in MUFA and marine n-3 PUFA with a low-fat diet rich in complex carbohydrates and dietary fibre. The high-fat diet induced a significant lowering of *inter alia* PAI-1, but led to an increase in fibrinogen, whereas the low-fat diet lowered FXIIc values and induced a nonsignificant decrease in fibrinogen. Different fat loads seem to influence PAI-1 in middle-aged men. PAI-1 decreased during the day and this decline tended to be steepest after a fat-free morning meal (Salomaa *et al.*, 1993). Studies done on the diet as a whole and not on specific food are scarce and difficult to compare with each other due to the different objectives of the studies.

2.3.4.4 Alcohol consumption

Epidemiological surveys consistently indicate that PAI-1_{act} is increased with moderate alcohol consumption (Hoekstra *et al.*, 2004; Vorster *et al.*, 1997). In the Prime Study this trend was attributed to the effect of TG on PAI-1_{act} since TG levels are positively correlated with alcohol intake (Scarabin *et al.*, 1998). According to Hoekstra *et al.* (2004), the association between alcohol consumption and PAI-1 is dose-dependent (J-shaped). This association is consistent with experimental data showing an increase in PAI-1_{act} with alcohol consumption (Dimmitt *et al.*, 1998; Van de Wiel *et al.*, 2001). Future studies are required to clarify the specific mechanisms as well as the possible interactions between alcohol intake, antithrombotic and fibrinolytic profiles.

2.3.4.5 Smoking

PAI-1 levels are reported to be higher in smokers (Scarabin *et al.*, 1998; Yarnell *et al.*, 2000), although this remains an area of controversy because some studies found no association (Eliasson *et al.*, 1995). The reduced fibrinolytic potential may be relevant to increased CHD risk in smokers (Scarabin *et al.*, 1998).

2.3.4.6 Circadian pattern

PAI-1 concentrations follow a circadian oscillation with peak levels observed in the early morning (Van der Bom *et al.*, 2003). According to Van der Bom *et al.* (2003), some individuals show no apparent circadian rhythm, while others show up to a 10-fold variation in PAI-1 over one day. In a cross-sectional study Van der Bom *et al.* (2003) studied whether

diurnal variation of PAI-1 Ag differs for the genotypes of the 4G/5G polymorphism. They found that the morning increase in PAI-1 Ag concentration was more pronounced among subjects homozygous for the 4G allele compared with the morning increase among the other genotypes and found that homozygosity for the 4G allele is associated with increased PAI-1 levels during the morning only.

2.3.4.7 Hormonal status of women

There is evidence to suggest that sex hormones influence PAI-1 levels. Women using OCs tend to have lower PAI-1 concentrations (Cachrimanidou *et al.*, 1994; Scarabin *et al.*, 1995). Oestrogen and progesterone seem to lower PAI-1 levels, although progesterone's lowering effect is not significant (Winkler *et al.*, 1998). OC-induced lowering of PAI-1 is achieved after a short period and PAI-1 returns to baseline levels within 8 days after cessation (Winkler *et al.*, 1996).

PAI-1 levels seem to increase after menopause (Hoekstra *et al.*, 2004). Meilahn *et al.* (1996) showed that postmenopausal women receiving HRT had more favourable plasma PAI-1 levels than those not receiving therapy, but it could in part be explained by differences between the two groups in obesity and body fat distribution. Smaller HRT-induced lowering of PAI-1 was observed for transdermal HRT (Meilahn *et al.*, 1996), possibly because this does not pass the liver first. In a prospective study Ciepluch and Czestochowska (1995) found significantly decreased PAI-1 after 6 month of therapy. Randomised controlled trials with HRT consistently showed a decline in PAI-1 (Hoekstra *et al.* 2004). Evidence for a possible mechanism is as yet limited. According to Hoekstra *et al.* (2004), oestrogen may directly decrease PAI biosynthesis and secretion, or may increase the clearance rate, or the effect of sex hormones could be through effects on body composition and insulin resistance.

2.3.4.8 Metabolic syndrome

Hypofibrinolysis due to elevated plasma PAI-1 levels is being considered as part of the cluster of abnormalities seen in MS [Juhan-Vague *et al.*, 1989; Juhan-Vague *et al.*, 1991; Alessi & Juhan-Vague, 2006(a)]. MS is associated with an augmented risk of developing CVD and PAI-1 overexpression may participate in this process [Alessi & Juhan-Vague 2006(a)]. PAI-1 promotes fibrin accumulation and is involved in cell migration, angiogenesis and fibrosis, thereby contributing locally to atherosclerotic vessel wall and adipose tissue remodelling [Alessi & Juhan-Vague 2006(b)]. PAI-1 is associated with several components of MS including obesity, BMI and visceral fat (which characterises android obesity), blood pressure, plasma levels of insulin or proinsulin and an adverse lipid

profile (Hoekstra *et al.*, 2004; Juhan-Vague *et al.*, 2003; Morange *et al.*, 2004), as will be discussed below.

Body fat especially central (visceral) adiposity is associated with increased PAI-1 levels (Hoekstra *et al.*, 2004) and both modest and substantial weight loss has been shown to be effective in lowering PAI-1 levels (Mertens & Van Gaal, 2002). Obese subjects tend to have higher values of PAI-1 (Mertens & Van Gaal, 2002). Adipocytes can synthesise PAI-1 and might further increase plasma PAI-1 by increased hepatic PAI-1 production in response to adipocyte-derived cytokines [tumor necrosis factor α (TNF-α) and transforming growth factor β (TGF-β)] possibly explaining the high levels found in obesity, emphasising the role of adipose tissue in determining plasma levels of PAI-1, with a local contribution of TNF-α and TGF-β in PAI-1 production by adipose tissue (Hoekstra *et al.*, 2004; Mertens & Van Gaal, 2002; Morange *et al.*, 1999).

Studies *in vitro* show higher PAI-1 synthesis for visceral fat than for subcutaneous fat (Bastelica *et al.*, 2002; Cigolini *et al.*, 1999), but the message is discordant with some studies showing opposite results (Hoekstra *et al.*, 2004). Visceral fat contains more stromal cells than subcutaneous fat, thus suggesting that stromal cells, and not adipocytes, are the main source of PAI-1 within adipose tissue of obese subjects. According to Hoekstra *et al.* (2004), adipocytes from obese subjects produced more PAI-1 than adipocytes from lean subjects, even after adjusting for adipocyte size. According to Alessi and Juhan-Vague [2006(a)], the mechanisms of PAI-1 overexpression during obesity are complex and it is conceivable that several inducers are involved at the same time at several sites of synthesis. Recent *in vitro* and *in vivo* studies showed that besides its role in atherothrombosis, PAI-1 is also implicated in adipose tissue development and in the control of insulin signaling in adipocytes, suggesting that PAI-1 inhibitors serve in the control of atherothrombosis and insulin resistance [Alessi & Juhan-Vague 2006(a)].

Pannacciulli *et al.* (2002) demonstrated that PAI-1 concentrations are significantly higher in impaired glucose tolerance than in normal glucose tolerance subjects and suggested that the influences of total adiposity, central fat, IR and main determinants of PAI-1 concentrations are different according to the degree of glucose tolerance. Henry *et al.* (1998) found that levels of PAI-1 are primarily determined by the IR syndrome in a healthy population. This relationship is stronger in males.

2.3.4.9 Acute-phase response

PAI-1 is an acute phase protein and is directly associated with inflammatory responses (Trayhurn & Wood, 2004). The acute-phase response has a strong positive relationship with PAI-1 through pro-inflammatory cytokines (e.g. interleukin-1, TNF-α and TGF-β), which

stimulates PAI-1 transcription (Hoekstra *et al.*, 2004). Proinflammatory cytokines might have an important role in the overexpression of PAI-1, particularly in the adipose tissue (Mavri *et al.*, 2004).

2.4 PHYSICAL ACTIVITY

Epidemiological studies provide compelling evidence that PA is associated with a reduced risk of CVD (Lee *et al.*, 2003 Macera *et al.*, 2003). Findings have been consistent, showing that physically active or fit individuals experience lower CVD risk than those who engage only in light activities or are sedentary or unfit (Lee *et al.*, 2003; Sesso *et al.*, 2000). Several modifiable risk factors for CVD have been identified *inter alia* cigarette smoking, hypertension, dyslipidemia, obesity, type II DM and sedentarism (Thomas *et al.*, 2003). PA is regarded as a preventative modality for various CDL, including CVD (Hu *et al.*, 1999; Pedersen & Saltin, 2006; Thompson *et al.*, 2003). Several mechanisms may be responsible for the protective effects that PA confer. PA may have a direct effect on the heart: it increases myocardial oxygen supply, decreases oxygen demand and improves myocardial contraction and its electrical impulse stability (Dowell, 1983). Reduced oxygen demand and myocardial work are reflected in lowered heart rate and blood pressure at rest and a general reduction in sympathetic tone (Bowles & Wamhoff, 2003; Dowell, 1983; Thompson *et al.*, 2001). PA also increases the diameter and dilatory capacity of coronary arteries, increases collateral artery formation and reduces rates of progression of coronary artery atherosclerosis (Thompson *et al.*, 2001). Additionally, high levels of PA are associated with lower systolic (Braith *et al.*, 1994) and diastolic blood pressures (Asikainen *et al.*, 2003), elevated levels of HDL (Crouse *et al.*, 1997; Spate-Douglas & Keyser, 1999), low levels of LDL (Duncan *et al.*, 1991; Kraus *et al.*, 2002) and improved glucose homeostasis and increased insulin sensitivity (Ben-Ezra *et al.*, 1995; Eriksson *et al.*, 2004; Houmard *et al.*, 2004; Kang *et al.*, 2002). Preliminary data suggest that PA may also be associated with decreased levels of homocysteine (Gaume *et al.*, 2005; König *et al.*, 2003), a risk factor for CHD due to its prothrombotic and artherogenic properties. Physically active individuals are less likely to be overweight and body composition changes are induced by a PA intervention (Gutin *et al.*, 2002; Slentz *et al.*, 2004), thereby reducing CHD risk. The association of adiposity to CDL risk factors is corroborated by observations that multiple CVD risk factors are substantially higher in obese children than in non-obese age-matched control individuals (Decsi & Molnár, 2003; Ferguson *et al.*, 1998). Routine PA is also associated with improved psychological well-being (e.g. through reduced stress, anxiety and depression) [Dunn *et al.*, 2001; Warburton *et al.*, 2001(a); Warburton *et al.*, 2001(b)]. Other likely protective mechanisms

include reduced platelet aggregation and increased fibrinolytic activity, possibly resulting from lower levels of PAI-1 and fibrinogen (Elwood *et al.*, 1993; MacAuley *et al.*, 1996; Zanettini *et al.*, 1997).

2.4.1 Types of physical activities

To enhance clarity, the different descriptors and terminology used in the literature to describe PA will be defined in this section. Levine *et al.* (2000) enunciated PA as having a spontaneous component, such as fidgeting, sitting, standing and walking; an obligatory component, such as occupation, household and daily living activities; as well as a voluntary component, such as participation in sports related activities.

PA can be divided in aerobic or anaerobic activities according to the energy systems utilised. Synonyms for aerobic activities include endurance activities and involve sustained (5 to > 240 min) low levels of muscular activity [percentage of maximal oxygen uptake ($\%VO_{2max}$) of 65 to 100] using large muscle groups, such as walking, jogging or cycling. With aerobic training, the pulmonary, cardiovascular and neuromuscular systems become more efficient and result in improved delivery of oxygen from the atmospheric air to the mitochondria and enhance the control of metabolism within the muscle cells (Jones & Carter, 2000). Aerobic endurance is the length of time a muscle can continue to contract while supported by mitochondrial activities. Synonyms for anaerobic activities include circuit weight training, resistance, and strenuous or vigorous exercise and comprise frequent, brief, intensive activities ($\%VO_{2max}$ of 90-125%) that stimulate muscle hypertrophy and increase muscle strength, such as lifting weights. Anaerobic endurance is the length of time muscular contraction can continue to be supported by glycolysis and by the existing energy reserves of adenosine triphosphate and creatine phosphate.

According to Howley (2001), discrimination between occupational PA and leisure-time PA must be made. Occupational PA refers to approximately 8 h per day, whereas the time spent on leisure-time PA may vary considerably between individuals. Leisure-time PA comprises all forms of aerobic activities, structured endurance exercise programmes, resistance-training programmes and sports activities done for relaxation or enjoyment (Howley, 2001).

A distinction of the different exercise protocols used in studies must be made. When examining the acute response to exercise, the body's immediate response to an individual exercise bout is investigated. The chronic effect of exercise is examined after regular training over a period of weeks, when the body had time to adapt. The physiological adaptations that occur with chronic exposure to exercise are highly specific to the type of training. The term PA can be used interchangeably with exercise, training, conditioning or

any other sport specific activity. But the use of 'training' is preferred when referring to the chronic effect and 'exercise session' when referring to the acute effect. According to Thompson *et al.* (2001), acute and chronic exercise effects cannot be considered in isolation because chronic PA increases the capacity for exercise, thereby permitting more vigorous and/or more prolonged individual exercise sessions and, therefore, a more significant acute effect.

2.4.2 Chronic effects of physical activity and haemostasis

Physical inactivity is universally accepted as an important CVD risk factor, but the mechanism(s) through which it facilitates the development of cardiovascular complications is not fully understood. Exercise induced alterations on the haemostatic variables might offer a plausible explanation. According to Vorster *et al.* (1997), it is difficult to gather which haemostatic effects of exercise are caused by increased energy expenditure, usually coupled with increased dietary energy intake, which by improved energy balance and which by exercise and physical fitness *per se*. Data on haemostatic factors are important because the effect of PA on them seems large and because both have been shown to be strongly predictive of ischaemic heart disease (Elwood *et al.*, 1993).

2.4.2.1 Evidence from epidemiological studies

In a review Ernst (1993) concluded that cross-sectional and longitudinal data strongly suggest that regular PA over several months can reduce fibrinogen concentrations by ± 0.4 g/l, which corresponds to a substantial decrease in risk of CVD when extrapolated. Evidence from the Caerphilly Prospective Heart Disease Study showed that fibrinogen concentrations were lowered by 0.24 g/l in the third of the men who were the most active in leisure-time PA (Elwood *et al.*, 1993). Employed men had a smaller range of activity levels than unemployed men, therefore, significant interaction occurred with employment, the haemostatic factors and exercise (Elwood *et al.*, 1993). In the Northern Sweden MONICA as well as in the PRIME study a strong and dose-dependent association between PAI-1 and fibrinogen concentrations and regular leisure time PA was observed both in men and women (Eliasson *et al.*, 1996; Scarabin *et al.*, 1998). In children, fibrinogen concentrations were positively related to heart rate, which is a marker of fitness even after adjusting for possible confounders, but tended to be lower in children reporting fewer hours of PA, however, differences were small and insignificant (Cook *et al.*, 1999). Findings of cross-sectional studies suggest that regular PA reduced PAI-1 levels compared to sedentary subjects (Hoekstra *et al.* 2004). There seems to be little doubt about the protective effect of leisure-time PA and the evidence for occupational PA is, however, much less certain (Elwood *et al.*, 1993).

2.4.2.2 Evidence from experimental studies

2.4.2.2.1 Physical activity intervention alone

Regular PA has been reported either to improve, to impair or to have no effect on haemostatic factors. Several studies have explored the relationship between haemostatic variables concentrations and PA and/or fitness in adults, but findings are inconclusive. The consensus of opinion, however, suggests that an active lifestyle has a positive effect on the haemostatic variables. Table 2.1 presents a summary of the studies that investigated the effects of chronic training on haemostatic markers.

Table 2.1: Summary of studies that evaluated the effects of chronic training on haemostatic markers

Source	Study design	Subjects	Intervention	Main results	Summary
Chronic effects of exercise on haemostatic markers (long term)					
El-Sayed *et al.*, 1995	Controlled clinical intervention trial	25 young subjects	The EX (n = 13) exercised for 12 weeks [30 min, 3 x week at 70% (6 weeks) & 80% (6 weeks) of maximum heart rate]. CON maintained normal PA patterns. Haemostatic markers were ascertained in both groups before & after the 12 weeks both at rest & following maximal exercise.	Significant activation of coagulation was observed in response to maximal exercise before & after a PT programme in both groups in aPTT, TCT, FVIII PA, & FVIII Ag. Plasminogen activator showed a significant ↑ in response to maximal exercise before & after PT in both groups. Although VO_{2max} following the PT programme was significantly ↑ in the EX vs. CON, no significant changes were observed in either group in blood coagulation and fibrinolysis parameters at rest or in response to maximal exercise.	Maximal exercise transiently accelerates blood coagulation & activates blood fibrinolytic activity, however, PT appears not to influence the haemostatic & fibrinolytic systems at rest or in response to maximal exercise.
Ferguson *et al.*, 1999	Randomised controlled cross-over clinical trial	43 obese children (7-11 yr)	Group 1 participated in PT for 4 months & then ceased PT for 4 months, whereas group 2 did no PT for the first 4 months & then participated in PT.	D-dimer ↓ after 4 months of PT. Factors explaining individual differences in responsiveness to the PT showed greater reductions in Fib & D-dimer & that blacks showed greater ↓ in D-dimer than whites. Stepwise multiple linear regressions showed that only higher prephysical training concentrations of Fib, PAI-1 & D-dimer explained significant proportions of the	In obese children, 4-month period of PT did not lead to significant changes in haemostatic variables. Children with greater adiposity & concentrations of haemostatic factors before PT showed greater ↓ in haemostatic variables after PT

				variation in changes in these variables.	than did children with lesser values.
Gutin & Owens, 1999	Randomised cross-over clinical study	81 obese (7-11 yr)	Subjects were randomly assigned to engage in PT for the first or second 4-month period of the study. The PT programme was offered 5 days a week for 40 min/session.	PT influenced BF%, VAT, subcutaneous abdominal adipose tissue, insulin, TG & cardiac parasympathetic activity positively. Detraining led to unfavourable changes in BF% & associated risk factors.	A 4-month controlled PT, without dietary intervention, had a favourable impact on body composition and some obesity-associated CVD risk markers.
Karakoc *et al.*, 2005	Clinical trial W/O CON	10 regularly trained football players	During the last week of the football season, one day before a standard training session & 2 days after the previous league match, blood samples were taken (pre-exercise).	Fib ↑ insignificantly from pre- to postexercise. Blood clotting time ↓ significantly from pre- to postexercise.	Blood clotting time had shortened significantly after PT.
Stratton *et al.*, 1991	Experimental clinical trial W/O CON	10 young (24-30 yr) & 13 old (60-82 yr) ♂ subjects	6 months of intensive endurance exercise training	The young group had no significant changes in any of the measured variables, whereas the old group had a 39% ↑ in t-PA activity, a 141% ↑ in the % of t-PA in the active form, a 58% ↓ in PAI-1_{act} & a 13% ↓ in Fib.	Intensive exercise training enhances resting t-PA activity & ↓ Fib & PAI-1_{act} in older men.
Chronic effects of exercise on haemostatic markers (short term)					
Bodary *et al.*, 2003	Randomised controlled clinical trial	Sedentary 16 ♂ & 16 ♀ (50–70 yr)	10 d of moderate-intensity PT. Blood samples were collected on day 1, 2, 11, & 12. Subjects in EX performed 50 min of treadmill walking at 65% of maximum HR on the 10 consecutive days.	There were no significant changes in PAI-1, t-PA, or associated metabolic variables between EX and CON during the intervention period.	Short-term exercise PT does not change PAI-1 levels in normal, asymptomatic men and women.

Ag = antigen; aPTT = activated partial thromboplastin time; BF% body fat percentage; CVD cardiovascular disease; CON = control group; ↓ = decreased; EX = experimental group; FVIII = factor VIII; FVIII PA = factor VIII procoagulant activity; ♀ = female; Fib = fibrinogen; HR = heart rate; h = hour; ↑ = increased; ♂ = male; PA physical activity; PT physical training; PAI-1 = plasminogen activator inhibitor type 1; PAI-1_{act} plasminogen activator inhibitor type 1 activity; TCT = thrombin clotting time; t-PA = tissue plasminogen activator; TG = trigliseride; VAT = visceral adipose tissue; VO_{2max} = maximal oxygen uptake; W/O = without

2.4.2.2.2 Physical activity combined with diet energy restriction to induce weight loss

The effect of PA on haemostasis could in part be induced by weight loss. Therefore, the combined effect of dietary energy restriction and increased energy expenditure through exercise could impart a greater effect on weight and thus on the haemostatic markers.

After a 3-month PA-behavioural-diet based intervention, sedentary adolescents maintained their weight, but redistributed their body composition, aleviated their insulin resistance and reduced elevated concentrations of CRP, fibrinogen and IL-6 (Balagopal *et al.*, 2005). In a similar intervention Estellés *et al.* (2001) found increased fibrinolytic activity due to a decrease in PAI-1 in obese children who reduced their BMI opposed to the group of obese children who did not reduce their BMI. No significant differences in t-PA and fibrinogen between the groups were observed (Estellés *et al.*, 2001). Fibrinogen concentrations are not reduced by modest weight loss and it seems as though a substantial weight loss is necessary to lower fibrinogen concentrations (Mertens & Van Gaal, 2002). In contrast Barbeau *et al.* (2002) found that an 8-months intervention (PA and lifestyle education) did not influence haemostatic and inflammatory markers in obese adolescents.

2.4.3 Acute effects of physical activity and haemostasis

The intensity, duration and energy expenditure required to produce an acute exercise effect are not clearly defined and the timing of blood sampling seems to play a pivotal role in the results of studies investigating the effects of acute PA on haemostasis (Cooper *et al.*, 2004). Table 2.2 presents various studies that evaluated the acute effect of exercise on the different haemostatic markers. It seems as if a bout of exercise activates coagulation slightly, but that this activation is balanced by increased fibrinolysis (Baertsch *et al.*, 1995; Hilberg *et al.*, 2002; Hilberg *et al.*, 2003; Weis *et al.*, 1998). The increase in fibrinolysis is transient and decreases rapidly during the postexercise period (Cooper *et al.*, 2004). It is not certain whether the increase in coagulation decreases or remains elevated after an exercise bout since research is still limited. However, Prisco *et al.* (1998) found that clotting as well as fibrinolysis activation persisted up to 24 h after the end of the race and that haemostatic variables returned to baseline values after 48 h after a marathon race. The balance of anticoagulant and procoagulant and fibrinolytic effects in any individual may vary depending on quantity and type of exercise performed, as well as on genetic and other variables, all of which merit further study.

Table 2.2: Summary of studies that evaluated the acute effects of exercise on haemostatic markers

Citation	Study design	Subjects	Intervention	Main results	Summary
Acute effects of exercise on haemostatic markers					
Baertsch *et al.*, 1995	Clinical trial	10 ♂ subjects (19 - 38 yr)	Blood samples were collected before, immediately after, & 2, 8 & 21 h after a 128-163 min triathlon.	TAT, FPA, & t-PA Ag were maximally ↑ immediately after exercise & ↓ thereafter. F1+2, FDP & PAP ↑ similarly 0 & 2 h after exercise & ↓ thereafter.	Prolonged strenuous exercise leads to moderate activation of blood coagulation resulting in THR & fibrin formation accompanied by enhanced plasmin generation.
Bouix *et al.*, 1998	Clinical trial	19 ♂ professional & 13 leisure football players (17 – 33 yr)	25 min exercise-test to determine work capacity was performed.	Aerobic working capacity negatively correlated with Fib independent of blood rheology.	A strong negative correlation exists between Fib & fitness in football players.
Cooper *et al.*, 2004	Clinical trial	8 healthy ♂	A graded maximal exercise test on a treadmill was performed.	t-PA activity & Ag significantly ↑ from pre- to postexercise. t-PA activity did not change from 1 to 2 min postexercise but ↓ significantly at 4 min postexercise. t-PA Ag remained ↑ from 1 to 2 min postexercise but ↓ at 4 min postexercise. PAI-1 ↓ form pre-to postexercise but did not change during the 10-min postexercise period.	Although fibrinolysis ↑ with acute exercise, it ↓ rapidly during the postexercise period.
El-Sayed *et al.*, 1999	Clinical trial	8 moderately active ♂ (26.6 ± 3.6 yr)	Completed maximal VO_{2max} & 75% of VO_{2max} for 30 min separated by 7 d. Blood samples were obtained at rest, immediately postexercise & following 30 min of recovery.	Exercise resulted in ↓ Fib only when postexercise data were corrected for contraction of plasma volume.	Changes in plasma volume in response to exercise should be taken into account when interpreting exercise effects on Fib.
Hilberg *et al.*, 2002	Clinical trial	13 healthy ♂	Subjects underwent exhaustive treadmill or cycle ergometer tests & a control day in random order. Blood samples were taken, repeatedly after a 30 min rest, immediately before &	In comparison to the prevalue taken immediately before the exercise, TTPin was significantly ↑ directly after exercise. ETPin remained unchanged after both exercises. Additionally for TTPex and ETPex, no changes after exercise were detectable. aPTT was significantly shorter after exercise, F1 + 2-concentrations were higher	The shortening of aPTT & the ↑ of F1+2 indicate an activation of the coagulation system during exercise. The unchanged intrinsic & extrinsic ETP led to the conclusion that thrombin generation is insignificant, is directly counterbalanced by α 2-

			after, & 1 h after exercise.	but TAT remained unchanged. Differences between the treadmill or cycle ergometer could not be determined.	macroglobulin & is independent of the type of exhaustive exercise done.
Hilberg *et al.*, 2003	Clinical trial	15 healthy ♂ nonsmokers (24 ± 2 yr)	3 isokinetic maximal tests on a cycle ergometry system with durations of 15, 45, & 90 s. Blood samples were taken after a 30 min rest, immediately before & after exercise 15 min, & 1 h after completion of exercise.	Immediately after exercise tests, only F1+2 (15– & 90-s test) & TTPin (45 & 90 s) showed a moderate ↑ while TAT & ETP was unchanged. In contrast, an ↑ in PAP & t-PA Ag already after 15 s maximal exercise in relation to the exercise duration time could be seen. These effects were not totally reversed to baseline 15 min after exercise; D-dimer & PAI-1 Ag still remained unchanged after these types of exercise.	Maximal short-term exercise does not lead to a relevant activation of blood coagulation in healthy young subjects; it is only slightly altered within the normal range. Fibrinolysis is activated & the ↑ is directly dependent on exercise duration.
Weis *et al.*, 1998	Clinical trial	12 ♂ subjects (mean 24 ± 4 yr)	Haemostatic markers were measured before & after running on a treadmill for 1 h at two different intensities corresponding to moderate (82% maximal HR, 68% VO_{2max}) & very heavy (94% maximal HR, 83% VO_{2max}) exercise.	During moderate exercise plasma levels of t-PA Ag & PAP complexes ↑, whereas F1+2, TAT complexes & FPA did not change significantly. In response to very heavy exercise, mean plasma levels of t-PA Ag & PAP complexes exceeded the upper limit of normal values, while significant ↑ of plasma levels of F1+2, TAT, & FPA occurred within the normal range.	In healthy young individuals, exercise-induced activation of coagulation is well balanced by activation of the fibrinolytic system, since moderate exercise results in ↑ plasmin formation only, while at very heavy exercise generation of plasmin seems to exceed that of thrombin & fibrin.

Ag = antigen; aPTT = activated partial thromboplastin time; ↓ = decreased; ETP = endogenous thrombin potential; ETPex = extrinsic endogenous thrombin potential; ETPin = intrinsic endogenous thrombin potential; F1+ 2 = prothrombin fragment 1 + 2; Fib = fibrinogen; FPA = fibrinopeptide A; FDP fibrin degradation products; HR = heart rate; h = hour; ↑ = increased; TTPex = extrinsic total thrombin potential; TTPin = intrinsic total thrombin potential; ♂ = male; PAP = plasmin-antiplasmin complex; PAI-1 = plasminogen activator inhibitor type 1; THR = thrombin; TAT = thrombin anti-thrombin complex; t-PA = tissue plasminogen activator; $VO_{2\,max}$ = maximal oxygen uptake.

2.4.4 Physical activity and haemostasis: conclusions and recommendations for future research

Findings of studies investigating PA's effect on haemostatic variables remain a topic of debate and speculation. Reasons for the inconclusiveness and reported inconsistencies are (i) the limitation of available PA epidemiological data; and in clinical trails the (ii) lack of PA control and compliance measured; (iii) the inability to draw valid conclusions from results of studies with different exercise protocols with varying intensities in different study populations (patients, normal sedentary individuals, habitual exercisers or athletes, children or adults) with a wide range of physical abilities and fitness levels as well as variables measured; (iv) inappropriate study designs such as uncontrolled studies and too small sample sizes; (v) uncertainties in defining optimal exercise; and (vi) the analytical methods employed for the assessment of the haemostatic factors. Therefore, experimental protocols require extremely careful standardisation and more controlled randomised clinical trials with statistical power and proper designs with subjects of various ages, health status and fitness levels are warranted to evaluate and determine a dose-response and the preventive and therapeutic potential of regular PA. One concern is that in most of the studies the possible effect of concomitant potentially confounding factors has not been properly controlled. Studies have not been consistently sound from a methodological perspective, and further studies are needed with appropriate controls to reassess the putative favourable effect of PA on haemostasis.

2.5 CONCLUSIONS AND RECOMMENDATIONS

In summary, it seems as if various modifiable factors influence haemostasis including impaired liver development, diet (under and overnutrition), alcohol consumption, smoking, OC and HRT use and PA. Cessation of tobacco use will have a substantial positive effect on risk but diet, OC and HRT use seem to have surprisingly little influence. A prime candidate among the variables to alter the risk for CVD is PA and if an individual is overweight, weight loss. Despite considerable research endeavours to elucidate the effects of regular exercise on haemostasis, knowledge remains limited. Research in this field is preponderantly executed on adults and due to the lack of standardisation of PA protocols and differences in the type of subjects recruited, research on different adolescent subjects is warranted. Research to establish whether raised haemostatic variables are prevalent in adolescents, whether certain high risk groups within this age group exist and whether a structured PA intervention can lower haemostatic markers are recommended to increase knowledge in this field.

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CHAPTER 3

JOURNAL MANUSCRIPT OF CROSS-SECTIONAL STUDY:

OVERFATNESS, STUNTING AND PHYSICAL INACTIVITY ARE DETERMINANTS OF PAI-1_{act}, FIBRINOGEN AND TAT IN AFRICAN ADOLESCENTS – THE PLAY STUDY

Running title: Haemostatic variables in cardiovascular disease risk related subdivisions in African children

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3.1 ABSTRACT

Objective: To examine fibrinogen and thrombin anti-thrombin complex (TAT) concentrations, and factor (F) VIII coagulant (c) and plasminogen activator inhibitor-1 activity (PAI-1_{act}) in African children in order to determine haemostatic profile patterning and to identify possible subdivision at high risk for cardiovascular disease (CVD).

Design: Cross-sectional analysis of a convenience sample of children in the PhysicaL Activity in the Young (PLAY) study.

Subjects and setting: 117 girls and 78 boys aged 15.6 ± 1.35 yr from two public High Schools in a township in South Africa were investigated.

Methods: Haemostatic variables were investigated in the total group and sub-divisions for physical activity (PA) levels, maturity (Tanner staging), gender, fat% and height for age (HAZ).

Results: Overfatness (53.6%) coexisted with stunting (17.5 %). PAI-1_{act} differed significantly between the genders after adjustments for fat% and PA levels and explained 10% of the variance while muscle mass, which is also gender related, explained 1% indicating that gender contributes to PAI-1_{act} variability. C-reactive protein (CRP), gender and HAZ were the predictors of fibrinogen. Fibrinogen was significantly higher in girls than in boys (before adjustment for fat%), in overfat opposed to lean children and in stunted compared to the non-stunted children (before and after adjustments). TAT was significantly higher in girls than in boys, but after separate adjustment for PA and fat% there were no significant differences. In the multiple regression fitness and muscle mass explained TAT's variance the best. No significant differences were seen between the groups for CRP concentration and FVIIIc.

Conclusion: Overfatness, stunting and inactivity negatively influence PAI-1_{act}, fibrinogen and TAT respectively, possibly increasing future risk for CVD. These factors are modifiable through behavioural changes and optimal nutrition status through early life.

Sponsorship: National Research Foundation, SA Sugar Association and North-West University

Keywords: factor VIII, fibrinogen, overfatness, plasminogen activator inhibitor-1, physical activity, stunting, South Africa, thrombin anti-thrombin complex

3.2 INTRODUCTION

Disorders of both coagulation and fibrinolysis contribute to the development of cardiovascular diseases (CVD) (Ajjan & Grant, 2006). The role of the haemostatic system in relation to the manifestation of CVD has become more recognised as data accumulate suggesting that a disturbance in the haemostatic balance represents an independent CVD risk factor (Ajjan & Grant, 2006; Kullo *et al.*, 2000; Sueishi *et al.*, 1998). Prevention is the key to controlling the incidence of CVD (Stamler *et al.*, 1998). Since CVD is not uncommon in young adults and the pathologic processes associated with its development have been shown to begin during childhood (Davia *et al.*, 1974; Hughes *et al.*, 2006; McGill *et al.*, 2000; McGill *et al.*, 2002), it seems important to gather information regarding children's haemostatic risk profiles, which could be used in the screening and development of intervention strategies for high-risk groups. For children little data exist defining high-risk characteristics for the development of CVD.

In developing countries undernutrition co-exists with overnutrition (Vorster *et al.*, 1999). Both seem to be associated with haemostatic abnormalities (James *et al.*, 2000). Therefore, it is expected that the incidence of CVD will increase in developing countries (Vorster *et al.*, 1999). Malnutrition in utero (resulting in low birth weight) and during infancy or early childhood (manifesting as stunting) is hypothesized to contribute to an increased haemostatic risk due to impaired liver development during critical periods of early life (Barker *et al.*, 1992; Roseboom *et al.*, 2000). The normal aging process is paralleled by an increase in several haemostatic variables independent of gender (Corsaut *et al.*, 1990; Sagripanti & Carpi, 1998). Obesity in adulthood is also associated with elevated haemostatic variables (Krobot *et al.*, 1992; Mertens & Van Gaal, 2002). Even in childhood, adiposity is associated with unfavourable concentrations of haemostatic factors (Barbeau *et al.*, 2002; Cook *et al.*, 1999; Ferguson *et al.*, 1998; Gallistl *et al.*, 2000). Inactivity or low levels of physical activity (PA) have been associated with increased haemostatic markers (Cook *et al.*, 1999; Elwood *et al.*, 1993; Ernst, 1993).

Based on these facts, it can be expected that malnourished, inactive, stunted and/or overweight children may have altered haemostatic profiles associated with an early increased risk of CVD. Little is known of the haemostatic profile and the association between haemostatic factors and nutritional status in African adolescents in the above mentioned subdivisions. Therefore, the aim of this study was to examine fibrinogen, thrombin anti-thrombin complex (TAT) and factor (F) VIII coagulant activity (FVIIIc) and plasminogen activator inhibitor-1 activity ($PAI\text{-}1_{act}$) in African children in the total study sample, as well as in subdivisions in order to identify possible high risk groups. The data reported here are from a cross-sectional study within the

PhysicaL Activity in Youth (PLAY) study to investigate the haemostatic variables of black South African children.

3.3 SUBJECTS AND METHODS

3.3.1 Subjects and setting

A convenience sample of all grade 9 learners from Seiphemelo Secondary School and Boitshoko High School in the same township was used. All subjects lived in similar low-income living areas in a township in the North West Province, South Africa, and were of the Tswana, Sotho, or Xhosa ethnic groups.

The Ethics Committee of the North-West University approved this study (Ethics number: 04M01). Permission to conduct this study at the selected schools was obtained from the principals. Written consent from the parents/guardians and the children was obtained before a child could participate in this study.

3.3.2 Blood sampling

Blood samples were collected after a 12-h overnight fast. Registered nurses used 21-gauge scalp infusion sets to collect venous blood samples between 08:00 and 11:00. Collection tubes were inverted gently and put on ice until centrifugation. Citrated and clotted blood was centrifuged within 30 min after collection for 15 min at 2000 *g* at 4 °C to yield plasma for glucose, fibrinogen, PAI-1_{act}, TAT and FVIIIc, and serum for insulin and C-reactive protein (CRP) analysis respectively. Plasma and serum were aliquoted and then snap-frozen on dry ice before being stored at –84 °C until analysis.

3.3.3 Analysis of biochemical measurements

Plasma fibrinogen concentrations and FVIIIc were measured using the modified method of Clauss (1957) and the activated partial thromboplastin time assay respectively, on an automated coagulation analyser (ACL-200, Instrumentation Laboratories, Milan, Italy). PAI-1_{act} was measured using an indirect enzymatic method (Spectrolyse pL, Biopool, Umeå, Sweden, Cat. No.101201). TAT was determined by an Enzyme-Linked Immunosorbent Assay (ELISA) (Enzygnost® TAT micro, Dade Behring, Marburg, Germany, Cat. No. OWMG G15). High-sensitivity CRP (hs-CRP) was determined by rate turbidimetry with a high sensitivity C-Reactive Protein Kit (CRPH, IMMAGE ®, Immunochemistry Systems, Cat. No. 474630, California, USA) on the Synchron LX System (Beckman Coulter Inc., Fullerton, California, USA). Fasting insulin was measured with Microparticle Enzyme Immunoassay method on the

AxSYM ® system (ABBOTT Diagnostics Division, Tokyo. Japan). Fasting glucose was measured by an enzymatic method on a Vitros DT60 II Chemistry Analyser (Ortho-Clinical Diagnostics, Rochester. New York, USA).

3.3.4 Insulin resistance and insulin sensitivity calculations

The homeostasis model assessment (HOMA) was used for the calculation of insulin resistance {formula: HOMA = [fasting insulin (μIU) x fasting venous glucose (mmol/l)]/22.5} (Katz *et al.*, 2000). Insulin sensitivity was calculated using Quantitative Insulin Sensitivity Check Index (QUICKI) = 1 / log(fasting venous insulin) + log(fasting venous glucose) (Katz *et al.*, 2000).

3.3.5 Blood pressure measurements

Physiologists measured systolic blood pressure (SBP) and diastolic blood pressure (DBP) in duplicate under standardised conditions (National High Blood Pressure Education Programme Working Group on High Blood Pressure in Children and Adolescents, 2004) using a mercury sphygmomanometer (Tycos. USA) of appropriate cuff size (covering 80 to 100% of the arm circumference) and a stethoscope.

3.3.6 Anthropometric measurements

Qualified level 2 anthropometrists did the anthropometric measurements in triplicate. Subjects were examined in their underwear. Body mass was measured with a portable electronic scale (Precision Health Scale, A&D Company, Tokyo, Japan) to the nearest 0.1 kg. Heights were measured to the nearest 0.5 cm with a Stadiometer (IP 1465, Invicta. London. UK) without shoes, standing upright with their heads in the Frankfort plane (Norton & Olds. 1996). Skinfolds were measured with a John Bull calliper (British Indicators, London, UK) according to approved methods [International Society for the Advancement of Kinanthropometry (ISAK), 2001]. Air displacement plethysmography in the BodPod® measurement system (Life Measurement Inc. Concord, CA) using Boyle's law of the pressure/volume relationship was used to determine body fat mass and body fat %. Body density was calculated as mass divided by volume and corrected for lung volume. The Siri formula [under water weighing (UWW) = (4.95/density – 4.5) x 100] (Siri. 1993) and the Brozek formula [UWW = (4.57/density – 1.142) x 100] were used to calculate body fat % (Brozek *et al.*, 1963). Waist and hip circumferences were measured to the nearest 0.1 cm with the cross-hand technique and a Lufkin steel tape (Cooper Tools, Apex, NC, USA).

Anthropometric measures were used to determine stunting. The reference population used in calculating height for age expressed in Z-scores (HAZ) was that given by the National Centre

for Health Statistics (CDC, 2000). For the purpose of this study stunting was defined as a HAZ-score ≤ - 2 and is traditionally used as an indicator of poor nutritional status (CDC, 2000).

3.3.7 Questionnaires

3.3.7.1 Demographic and health questionnaire

Demographic data were obtained from a structured questionnaire, which gave information regarding the subjects' age, gender, home language, socio-economic class and general health data. Information on smoking and drinking and the habitual use of any pharmaceutical substance were obtained from a structured questionnaire.

3.3.7.2 Tanner staging of physical maturity

Maturation of the children was estimated according to the standards of Tanner and Whitehouse (1976) using pubic hair and breast development in girls and pubic hair and genital development in boys.

3.3.7.3 Habitual physical activity and fitness

Habitual PA was assessed for the previous weekday and a weekend day using the Previous Day Physical Activity Recall (PDPAR) questionnaire (Trost *et al.*, 1999). The reported activities were coded and each assigned a literature based metabolic equivalent (MET) min/wk value (Ainsworth *et al.*, 1993). The subjects were then classified according to the number of 30 min intervals with MET-values indicating high PA in three categories of PA.

Trained postgraduate Human Movement Science students administered the standard bleep test from which maximal oxygen uptake (VO_{2max}) was determined (Léger & Lambert, 1982).

3.3.8 Statistical analysis

The computer software package Statistica® version 7 (StatSoft, 2004) was used for the statistical analysis. The following population subdivisions were made: according to gender; according to body fat % where lean children had a body fat % of < 20% and < 25%, and overfat children a body fat % of ≥ 20% and ≥ 25% for boys and girls respectively; a subdivision according to maturity status where pre-pubertal children were in Tanner stage I-II and pubertal children in stage III-V; according to HAZs to establish a stunted (defined as a HAZ of ≤ -2) and a non-stunted group; and according to habitual PA levels as determined by the PDPAR questionnaire, where a classification of ≥ 2 was considered active and < 2 as inactive. Independent *t*-tests between the various population subdivisions were done to compare the haemostatic variables within the subdivisions. Spearman correlations were done for the total group as well as for boys and girls separately in order to identify possible confounders.

Analysis of covariances (ANCOVA) were then done to adjust for confounders found with the correlations. In order to determine practical significance, effect sizes were calculated for *t*-tests as well as for the ANCOVA's using Cohen's formulae $d = |x_1 - x_2| / s_{max}$ and $d = |x_1 - x_2| / \sqrt{MSE}$, respectively, where d = the effect size; MSE = the mean square error; x_1 = the mean of one of the groups; x_2 = the mean of the other group; and s_{max} = the maximum standard deviation of the two means (Ellis & Steyn, 2003). D-values of 0.2, 0.5 and 0.8 were interpreted as having a small, medium and large effect size (Ellis & Steyn, 2003). Factor analysis was used to determine whether haemostatic variables cluster with specific metabolic variables measured. A varimax raw rotation method was used. Only summary factors with an eigenvalue > 1 were selected for the analysis and only factor loadings of ≥ 0.4 were used for the interpretation of results. Based on the results from the factor analysis, variables that contributed most to each of the factors were selected for inclusion into multiple regression analysis in order to determine the best predictors of the haemostatic variables. A p-value of ≤ 0.05 was regarded as statistically significant.

3.4 RESULTS

Baseline characteristics of the subjects are given in Table 3.1. The children had a mean age of 15.6 ± 1.35 yr. Their average weight was 49.4 ± 8.97 kg with a resultant body mass index (BMI) of 19.8 ± 3.04 kg/m^2. The average fat % for boys and girls was 18.1% and 28.7%, respectively. The boys were within the healthy range but the girls were above the healthy range for body fat %. Alarmingly, 53.6% children were classified as moderately overfat. According to the HAZ, 31 children representing 17.5% of the total group were stunted. The median CRP-concentration was 1.36 mg/l after four children with a CRP value > 10 mg/l were excluded due to the possible presence of an acute infection (Pepys, 1996). None of the girls used oral contraceptives and a negligible amount of children smoked and if they smoked it was only occasional.

Table 3.2 presents the differences for several CVD risk markers in the respective subdivisions. BMI differed significantly between boys and girls (19.0 ± 2.60 vs. 20.3 ± 3.22 kg/m^2; p = 0.004; d = 0.40) and between the lean and overfat children (18.2 ± 2.09 vs. 21.2 ± 3.23 kg/m^2; p < 0.00001; d = 0.93). Body fat % differed significantly between the boys as opposed to girls (18.1 ± 6.74 vs. 28.7 ± 6.47 %; p < 0.00001; d = 1.57), the lean as opposed to the overfat group (17.2 ± 4.95 vs. 30.6 ± 4.91 %; p < 0.000001; d = 2.71), and the active as opposed to the inactive group (22.3 ± 8.49 vs. 26.7 ± 7.69 %; p = 0.001; d = 0.25). CRP concentrations did not differ significantly between any of the subdivisions. Fibrinogen was significantly higher in girls than in boys (2.80 ± 0.49 g/l vs. 2.59 ± 0.47 g/l; p = 0.004; d = 0.42); in overfat opposed to

lean children (2.82 ± 0.49 vs. 2.60 ± 0.47 g/l; p = 0.005; d = 0.45); and in the stunted compared to the non-stunted (2.89 ± 0.48 vs. 2.67 ± 0.49 g/l; p = 0.02; d = 0.46). PAI-1_{act} was significantly higher in girls than boys (3.98 ± 3.64 IU/ml vs. 1.79 ± 2.30 IU/ml; p = 0.000005; d = 0.60); in overfat as opposed to lean children (3.78 ± 3.63 vs. 2.28 ± 3.06 IU/ml; p = 0.007; d = 0.41) and in sedentary compared to physically active children (3.45 ± 3.55 vs. 2.39 ± 2.91 IU/ml; p = 0.04; d = 0.30). TAT was significantly higher in girls than in boys (6.10 ± 7.00 vs. 4.09 ± 4.26 µg/l; p = 0.03; d = 0.29). FVIIIc did not differ significantly between any of the subdivisions. No significant differences in the haemostatic variables between the prepubertal and the pubertal group were found.

Haemostatic variables correlated positively with body fat % and negatively with PA in the total group as well as in boys and girls separately, indicating a possible relationship between these variables and the measured haemostatic variables.

Due to known gender differences in body fat % (Kruger *et al.*, 2004) and PA levels (Goran *et al.*, 1998), adjustments were made for both of these when comparing the gender subdivision. Based on previously published results of black South African children adjustments were also made for body fat % in the HAZ subdivision, since it was found that the stunted children had significantly higher subcutaneous fat (Kruger *et al.*, 2004). Table 3.3 presents the adjusted means, MSE and effect sizes for these variables. After adjustments for PA and body fat %, PAI-1_{act} remained significantly higher in girls (d = 0.69; p = 0.002), but differences in fibrinogen (d = 0.31; p = 0.17), and TAT (p = 0.17; d = 0.31) were no longer significant. When adjusting for PA alone the significant difference in fibrinogen between the genders remained (p = 0.006; d = 0.46), but adjustment for fat % alone resulted in an insignificant difference in fibrinogen (p = 0.24; d = 0.26). Separate adjustment for fat % and PA both led to TAT not being significantly different between the gender groups (p = 0.10; d = 0.35 and p = 0.10; d = 0.28, respectively). After adjusting for body fat % in the stunted and non-stunted groups, fibrinogen remained significantly different (p = 0.03; d = 0.46).

Factor analysis carried out on various metabolic variables and the measured haemostatic variables revealed 8 factors that explained 77% of the total variance of the data. Table 3.4 presents the semi-partial correlations between the variables and the different factors they cluster into. The eight factors were termed: body-fat-correlates; glucose-insulin; gender related; physical fitness measures; acute-phase; FVIIIc-TAT; height for age; and a blood pressure factor. Tanner-staging and glucose did not cluster preferentially into a single factor indicating that their variance is explained in part by several different factors. Variables selected from the results of the factor analysis that was used in the multiple regression analysis were fat mass, BMI, PA, glucose, insulin, muscle mass, gender, fitness (according to the bleep test), VO_{2max},

HAZ, SBP, DBP and the haemostatic variables. These variables were entered in the forward stepwise procedure to select the best predicting model for each of the haemostatic variables. For TAT, the variables that entered the model were fitness according to the bleep test and muscle mass, which explained 6% and 4% of the total variance of TAT, respectively. The best predictor for the variance in FVIIIc was glucose explaining 17% of the variance. For CRP the selected model included fibrinogen and muscle mass, explaining 24% and 7% of the variance, respectively. The best predictors for fibrinogen were CRP, gender and HAZ, explaining 24%, 8% and 8% of fibrinogen's variance, respectively. The two variables that entered the model for PAI-1_{act} were gender and fat mass explaining 10% and 1% of its variance. Addition of other variables did not improve the models.

Table 3.1: General characteristics of the study population

Characteristic		**Total group** (n=190)
Age (yr)		15.6 ± 1.35
Body weight (kg)		49.6 ± 8.97
BMI (kg/m^2)	Total group Boys (n= 77) Girls (n = 112)	19.8 ± 3.04 19.0 ± 2.60 20.3 ± 3.22
Body fat %	Total group Boys (n = 78) Girls (n = 117)	5.2 ± 8.22 18.1 ± 6.74 28.7 ± 6.47
HAZ	*Stunted* (n = 31) *Non-stunted* (n = 146)	-2.51 ± 0.44 -0.77 ± 0.74
TAT (µg/l)		5.31 ± 6.11
FVIIIc %		152 ± 46.2
CRP (mg/l)		1.36 ± 3.01
Fibrinogen (g/l)		2.71 ± 0.49
PAI-1_{act} (IU/ml)		3.09 ± 3.33
Fasting serum insulin (µU/ml)		9.38 ± 6.52
Fasting plasma glucose (mmol/l)		5.07 ± 0.48
Insulin sensitivity (QUICKI)		0.35 ± 0.03
Insulin resistance (HOMA)		2.16 ± 1.64
SBP (mmHg)		97.9 ± 10.7
DBP (mmHg)		61.5 ± 10.8

Data expressed as mean ± standard deviation (SD).

BMI body mass index; CRP C-reactive protein; DBP diastolic blood pressure; FVIIIc factor VIII coagulant activity; HOMA homeostasis model assessment; HAZ height for age Z score; n amount. PAI 1_{act} plasminogen activator inhibitor type 1 activity; QUICKI quantitative insulin sensitivity check index; SBD systolic blood pressure; TAT thrombin anti-thrombin complex

Table 3.2: The anthropometric markers, haemostatic variables and CRP values for the total group, and the different subdivisions

Variable	Boys (n = 77)	Girls (n = 116)	d	Lean (n = 65)	Overfat (n = 95)	d	Prepubertal (n = 16)	Pubertal (n = 174)	d	Stunted (n = 31)	Non-stunted (n = 146)	d	Active (n = 72)	Inactive (n = 110)	d
BMI (kg/m^2)	19.0 ± 2.60§	20.3 ± 3.22§	0.40	18.2 ± 2.09§	21.2 ± 3.23§	0.93	18.7 ±3.26	19.8 ± 2.76	0.33	19.5 ± 2.86	19.8 ± 2.84	0.08	19.3 ±3.24	20.1 ± 2.80	0.52
Body fat %	18.1 ± 6.74§	28.7 ± 6.47§	1.57	17.2 ± 4.95§	30.6 ± 4.91§	2.71	22.2 ± 9.75	25.4 ± 7.92	0.32	26.3 ± 8.70	25.2 ± 7.97	0.13	22.3 ± 8.49§	26.7 ± 7.69§	0.25
TAT (µg/l)	4.09 ± 4.26*	6.10 ± 7.00*	0.29	5.32 ± 5.38	5.97 ± 7.16	0.09	6.32 ± 7.11	5.18 ± 6.02	0.16	3.85 ± 2.24	5.27 ± 5.56	0.23	6.00 ± 7.14	4.34 ± 4.35	0.23
FVIIIc %	156 ± 43.3	149 ± 48.1	0.14	155 ± 49.4	150 ± 46.4	0.10	156 ± 28.4	152 ± 47.6	0.07	156 ± 48.2	151 ± 46.6	0.11	154 ± 46.1	153 ± 47.2	0.03
CRP (mg/l)	1.45 ± 3.27	1.32 ± 2.87	0.04	1.18 ± 3.05	1.59 ± 3.24	0.13	1.48 ± 2.63	1.34 ± 3.07	0.05	0.73 ± 1.33	1.33 ± 2.80	0.22	1.34 ± 3.07	1.44 ± 3.10	0.03
Fibrinogen (g/l)	2.59 ± 0.47§	2.80 ± 0.49§	0.42	2.60 ± 0.47§	2.82 ± 0.49§	0.45	2.70 ± 0.46	2.71 ± 0.48	0.02	2.89 ± 0.48*	2.67 ± 0.49*	0.46	2.68 ± 0.49	2.75 ± 0.50	0.15
PAI-1_{act} (IU/ml)	1.79 ± 2.30§	3.98 ± 3.64§	0.60	2.28 ± 3.06§	3.78 ± 3.63§	0.41	2.66 ±2.69	3.16 ± 3.41	0.15	3.30 ± 3.08	3.02 ± 3.33	0.08	2.39 ± 2.91*	3.45 ± 3.55*	0.30

*Data reported as mean ± SD. * = $p \leq 0.05$ and § = $p \leq 0.01$ between the two groups within a subdivision.*

BMI body mass index; CRP C-reactive protein; d effect size (Cohen d value); FVIIIc % factor VIII coagulant activity percentage; PAI-1_{act} plasminogen activator inhibitor type 1 activity; TAT thrombin anti-thrombin complex.

Table 3.3: Adjusted means for haemostatic variables and CRP values

Gender differences after adjustment for body fat %						Gender differences after adjustment for PA					HAZ subdivision differences after adjustment for body fat %				
Variable	**Boys**	**Girls**	**MSE**	**d**	**p**	**Boys**	**Girls**	**MSE**	**d**	**p**	**Non-stunted**	**Stunted**	**MSE**	**d**	**p**
TAT (µg/l)	4.31	6.60	41.9	0.35	0.10	4.30	6.03	38.1	0.28	0.10	5.75	3.94	29.3	0.34	0.11
FVIIIc %	157	150	2270	0.14	0.50	155	152	2189	0.07	0.69	1.49	159	2308	0.20	0.35
CRP (mg/l)	1.73	1.19	10.1	0.17	0.42	1.51	1.33	9.60	0.06	0.73	1.35	0.79	7.29	0.21	0.33
Fibrinogen (g/l)	2.63	2.75	0.23	0.26	0.24	2.59	2.81	0.24	0.46	0.006	2.68	2.90	0.23	0.46	0.03
PAI-1 (IU/ml)	1.67	3.85	10.8	0.66	0.002	1.71	3.95	10.1	0.70	0.00003	3.11	3.29	10.9	0.06	0.79

CRP C-reactive protein; d effect size (Cohen d value); FVIIIc % factor VIII coagulant activity percentage; HAZ height for age Z-score; MSE mean square error; PA physical acitivity; PAI-1_{act} plasminogen activator inhibitor type 1 activity; TAT thrombin anti-thrombin complex

Table 3.4: Factor analysis between the variables and the factors they cluster into

Variable	Body fat correlates	Glucose-insulin	Gender related	Physical fitness measures	Acute phase response factors	FVIIIc-TAT	HAZ	Blood pressure
Waist girth	0.60		0.66					
Hip girth	0.80							
Triceps skinfold	0.93							
Body fat %	0.90							
Fat mass	0.95							
Physical activity	-0.49		0.40					
BMI	0.78		0.49					
Tanner		0.47						
Glucose		0.47	0.42			0.45		
Insulin		0.94						
Insulin resistance (HOMA)		0.93						
Insulin sensitivity (QUICKI)		-0.89						
Muscle mass			0.89					
PAI- 1_{act}			- 0.42					
$\%VO_{2\,max}$				0.96				
Bleep test				0.96				
Lungvolume				0.50				
hs-CRP					0.87			
Fibrinogen					0.75			
FVIIIc %						0.82		
TAT						0.53		
HAZ							0.84	
Age							-0.41	0.60
SBP								0.73
DBP								0.70

BMI body mass index; DBP diastolic blood pressure; FVIIIc % factor VIII coagulant activity percentage; HAZ height for age Z-score; HOMA homeostasis model assessment; hs-CRP high sensitivity C-reactive protein; PAI-1_{act} plasminogen activator inhibitor type 1 activity; % VO_{2max} percentage of maximal oxygen uptake; QUICKI quantitative insulin sensitivity check index; SBP systolic blood pressure; TAT thrombin anti-thrombin complex.

3. 5 DISCUSSION

Several haemostatic variables are recognised as independent risk markers for CVD (Ajjan & Grant, 2006) yet little is known about the age at which haemostatic abnormalities occur. To our knowledge, no data are available on the haemostatic markers in African adolescents and it is not known whether high risk groups in the population at this age already exists. Since under and overnutrition coexists in this population group and both were shown to affect the haemostatic markers adversely, research on this group is warranted.

The study population was still young (mean age 15.6 ± 1.35 yr). According to the anthropometric data 53.6% of the total group, 31.5% and 73.6% of the boys and girls respectively were moderately overfat. Obesity is common among African female adolescents and women but rare among African males (Monyeki *et al.*, 1999) and coexists with undernutrition as 17.5 % of these children were stunted. In 2004 Mukuddem-Petersen and Kruger (2004) found that stunting affected 10-15 yr old African children (23.7% girls and 26.7% boys in rural areas and 11.6% girls and 17.1% boys in urban areas) in the THUSA BANA study.

Smoking (Kelleher, 1992), alcohol, and oral contraceptive use (Ernst, *et al.*, 1989) are known predictors of the haemostatic profile concentrations and were not common in this group.

PAI-1_{act} was significantly higher in girls than in boys even after adjustments for fat % and PA levels, indicating that gender independently contributes to the variability of PAI-1_{act}. Results from the factor analysis support these findings as PAI-1_{act} clustered into the gender related factor. In the multiple regression, gender was also the most important contributor to the variability of PAI-1_{act}, but explained only 10% of the variance while muscle mass, which is also gender related, explained an additional 1%. Gender did, however, not contribute significantly to the variation in PAI-1 antigen when obese children and adolescents were investigated (Sudi *et al.*, 2000). According to Van Harmelen *et al.* (2000), the role that gender plays in regulation of plasma PAI-1_{act} is not due to differences in PAI-1 secretion from abdominal subcutaneous adipose tissue.

Fibrinogen was significantly higher in girls than in boys, in overfat opposed to lean children and in stunted compared to the non-stunted children. When adjusting for PA the significant difference in fibrinogen between the genders remained, but adjustment for body fat % resulted in an insignificant difference in fibrinogen, indicating that the gender difference observed may be attributed to the girls having a significantly higher body fat % than the boys. The literature shows that plasma fibrinogen concentration is positively associated with anthropometric markers for body fat in boys and girls (Ferguson *et al.*, 1998; Stensel *et al.*, 2001). Halle *et al.* (2004) found significantly higher concentrations of the inflammatory parameters such as

fibrinogen, interleukin-6 and tumor necrosis factor-alpha in obese children when compared to non-obese children. Therefore, this association could be attributed to the low-grade systemic inflammatory state associated with obesity (Bastard *et al.*, 2006; Halle *et al.*, 2004; Trayhurn & Wood, 2005; Ziccardi *et al.*, 2002). Ferguson *et al.* (1998) provide another possible explanation by attributing this association to increased production or decreased clearance of fibrinogen in obese subjects. After adjusting for body fat % in the stunted and non-stunted groups, fibrinogen, however, remained significantly different between these groups. This shows that early chronic malnutrition could possibly predispose independently to CVDs. These results concur with those previously reported by Barker *et al.* (1992) and Roseboom *et al.* (2000) who found that reduced growth early in life is strongly related to high fibrinogen concentrations and attributed this phenomenon to impaired liver development. In the factor analysis fibrinogen clustered with CRP and in the multiple regression CRP, gender and HAZ seem to be the most important predictors of fibrinogen. Fibrinogen's association with CRP is well-documented (Lowe *et al.*, 2001; Mendall *et al.*, 2000) and is attributed to their role as acute phase proteins indicating inflammation. CRP is a very sensitive marker of systemic inflammation and has emerged as a predictor of CVD (Koenig *et al.*, 1999). As subjects with high CRP (> 10 mg/l) were excluded one can, therefore, assume that acute inflammation, tissue damage or infection could not have influenced the fibrinogen values. The gender differences seem to be related to differences in body fat %.

TAT was significantly higher in girls than in boys, but after separate adjustment for PA and fat % there were no significant differences. In the multiple regression, fitness (bleep test) and muscle mass explained TAT's variance the best. Both fitness and muscle mass differed between boys and girls. This most likely explains the gender differences. Increased levels of this global marker of coagulation should, however, not be interpreted, without also taking the rate of fibrinolysis into consideration (Asakawa *et al.*, 2000; Stegnar *et al.*, 2003).

No significant differences between any of the subdivisions for FVIIIc could be observed. In the multiple regression, fasting plasma glucose seemed to be the variable that influenced FVIIIc the most. This result concurs with that of Conlan *et al.* (1993) who found that FVIIIc correlated positively with serum insulin, which regulates blood glucose levels. Haemostatic abnormalities including elevated FVIII are abundant in people with metabolic abnormalities (Wannamethee *et al.*, 2005).

The differences seen in haemostatic variables between the lean and overfat as well as the stunted and nonstunted subdivisions in the haemostatic markers were not only statistically significant, but had practical significance according to Cohen's d-values. Subdivision in pre-

pubertal and pubertal resulted in a small sample size in the pre-pubertal group, possibly explaining why no differences were observed for this subdivision.

3.6 CONCLUSION

In view of the results, inactivity and high body fat %, influenced TAT and fibrinogen concentrations and PAI-1_{act} negatively. High fibrinogen concentrations also seem to be associated with stunting. No significant differences were seen between the groups for CRP concentration and FVIIIc. The findings emphasise the overwhelming importance of childhood stunting, overfatness and inactivity on the haemostatic variables during childhood, probably increasing future risk for CVD especially stroke which is more prominent in black population groups than ischaemic heart disease (Vorster, 2002). These factors are modifiable through behavioural changes and optimal nutrition status through early life. These high-risk groups defined in children give a sense of urgency to develop strategies for the prevention and treatment of modifiable risk factors. Further research that will determine risk patterning in adolescents and enhance prevention strategies for CVD is warranted.

3.7 ACKNOWLEDGEMENTS

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CHAPTER 4

JOURNAL MANUSCRIPT INTERVENTION STUDY:

THE EFFECT OF PHYSICAL ACTIVITY ON THE HAEMOSTATIC PROFILES OF GRADE 9 AFRICAN CHILDREN – THE PLAY STUDY

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4.1 ABSTRACT

Objective: To assess the influence of a physical activity intervention on haemostatic markers in African adolescents.

Study design and methodology: Grade 9 subjects enrolled (n = 255) in a free-living quasi-experimental study. An outdoor exercise programme (1 h, 3 times/week) was presented to an intervention school while another school that underwent no intervention was also included. Fitness levels (bleep test), anthropometric variables, blood pressure, fibrinogen, factor VIII coagulant activity (FVIIIc), plasminogen activator inhibitor type 1 activity, thrombin antithrombin complex and C-reactive protein were determined at baseline and after 11 weeks. Attendance of the exercise sessions was monitored during the intervention.

Results: Only 81 children gave blood samples at baseline as well as at the end of the study. Of these, 24 children were from the reference school, 29 attended ≥ 40% and 28 attended < 40% of the exercise sessions. There was no difference at baseline in fitness between the three groups. The intervention also did not result in significant improvements in fitness levels. BMI in the ≥ 40% attendance group increased significantly from 19.3 ± 2.98 to 19.5 ± 3.24 kg/m^2 ($p = 0.04$), but fat mass decreased (from 12.5 ± 4.87 to 10.1 ± 4.65 kg; $p = 0.00008$). This decrease in fat mass was also seen in the < 40% attendance group (10.5 ± 5.57 to 8.87 ± 4.64 kg; $p = 0.009$), and in the reference group (15.9 ± 6.60 to 11.5 ± 3.91 kg; $p = 0.001$). In all the groups FVIIIc% increased, but significance was reached in the ≥ 40% and < 40% attendance groups only (from 150 ± 34.4 to 175 ± 64.3 %; $p = 0.01$, and from 154 ± 43.8 to 168 ± 63.7 %; $p = 0.02$, respectively). The intervention had no significant effect on the other haemostatic variables.

Conclusion: The physical activity intervention had no significant effect on any of the haemostatic variables measured. Plausible reasons for this outcome were that the compliance with the intervention was unsatisfactory, or that greater body fat losses are required to impart changes in the haemostatic markers, or that baseline values plays such a prominent role in the changes that can be expected during an intervention that improvements in the haemostatic profile can only be seen when the initial levels are raised. Seasonal variations in the haemostatic variables possibly clouded the effect of the PA intervention.

Keywords: factor VIII, fibrinogen, haemostasis, physical activity, plasminogen activator inhibitor type 1, South Africa, thrombin antithrombin complex

4.2 INTRODUCTION

Evidence is mounting to suggest that a disturbance in the haemostatic balance (a hypercoagulable and a hypofibrinolytic state) predisposes to cardiovascular disease (CVD) (Ajjan & Grant, 2006; Kullo *et al.*, 2000; Sueishi *et al.*, 1998). In black population groups stroke is more prominent than ischaemic heart disease and may be attributed to a combination of risk factors *inter alia* raised haemostatic markers in this population group which favours the development of stroke (Imam, 2002; Vorster, 2002). Several haemostatic markers including fibrinogen, plasminogen activator inhibitor type 1 activity ($PAI\text{-}1_{act}$), factor (F) VIII coagulant activity (FVIIIc), as well as the acute phase protein C-reactive protein (CRP) have been identified as independent CVD risk markers (Morange *et al.*, 2005; O'Donnell & Laffan, 2001; Thomas *et al.*, 2003).

In the previous work (submitted for publication in The European Journal of Clinical Nutrition) it was found that haemostatic markers in African adolescents are raised in certain risk groups at an early age. Overfatness and inactivity were two of the modifiable factors which were associated with adverse haemostatic profile patterning. Both are also independent risk markers for CVD themselves (Thomas *et al.*, 2003). It is well known that the prevalence of obesity is increasing in children and adolescents. Even in South Africa, children show trends of obesity and overweight similar to values in developed countries about 10 yr ago (Armstrong *et al.*, 2006). It is inappropriate to consider the issue of obesity in the context of food intake alone because low levels of physical activity (PA) are also associated with obesity. Alarmingly the prevalence of physical inactivity is also increasing during adolescence due to dramatic decreases in the amount of habitual PA in this age range (Armstrong & Van Mechelen, 1998; Pate *et al.*, 1994), which could enhance the development of CVD early in life. It is generally accepted that the onset of many chronic diseases lies in early childhood, therefore, preventive strategies should start as early in life as possible making children and adolescents the target populations for preventive strategies (Davia *et al.*, 1974; Hughes *et al.*, 2006; McGill *et al.*, 2000; McGill *et al.*, 2002).

Epidemiological studies provide compelling evidence that PA is associated with a reduced risk of CVD (Sesso *et al.*, 2000; Lee *et al.*, 2003; Macera *et al.*, 2003). Some research suggests that this positive effect could be attributed to changes in the haemostatic markers (El-Sayed, 1996; Ernst, 1993). Several epidemiological studies suggest that PA positively influence the haemostatic system (Eliasson *et al.*, 1996; Elwood *et al.*, 1993; Scarabin *et al.*, 1998; Zanettini *et al.*, 1997). A few comprehensive experimental studies exist concerning the influence of PA on blood haemostasis in adults, but findings are still inconclusive (Bodary *et al.*, 2003; El-Sayed *et al.*, 1995; Karakoc *et al.*, 2005; Stratton *et al.*, 1991). To

date, little attention has been given to this aspect amongst young people and the ambiguity is even greater in the few studies performed (Barbeau *et al.*, 2002; Estellés *et al.*, 2001). This inconclusiveness is due to variations in training programmes employed, populations studied and the analytical methods used. Until now no data were available on the influence of a PA intervention on haemostatic markers of African children. The PhysicaL Activity in the Young (PLAY) study was, therefore, performed in order to determine whether PA will improve the haemostatic profile of young adolescents. Haemostatic markers which have been shown in the literature to be modifiable through exercise were chosen for investigation.

4.3 SUBJECTS AND METHODS

4.3.1 Subjects and setting

The data reported here are from a convenience sample of apparently healthy grade 9 African children with CRP values < 10 mg/l from two public high schools.

The outdoor PA intervention took place at one high school. The non-participating reference group comprised of children in another high school in the same township. All participants lived in low-income living areas in the North West Province. South Africa, and were of the Tswana, Sotho, or Xhosa ethnic groups. The Ethics Committee of the North-West University approved this study (Ethics number 04M01). Permission to conduct this study at the selected schools was obtained from the principals. Written consent from the parents/guardians and the children was obtained before a child could participate in the study. The participants were informed of all aspects of the study and were asked to maintain their normal daily routine (eating pattern and PA level) for the duration of the study.

4.3.2 Study design

Due to practical and ethical considerations the study was parallel quasi-experimental. Drawing of blood samples was done during school hours in March 2005 (baseline) and after 11 weeks' intervention, in June 2005 at the North-West University.

4.3.3 Physical activity intervention

The PA programme comprised of three 60 min PA sessions per week and consisted of 20 min of aerobic exercise with music, 20 min strength and flexibility training and 20 min active play in ball games. After each session subjects received snacks and cold drinks to prevent their energy balance from fluctuating. The reference group received a multivitamin and mineral supplement with a micronutrient content similar to the foods given to the experimental group. An attendance register was used to monitor the subjects' compliance. An attendance of 40% was considered a substantial participation level to impart an exercise effect to the subjects

(Barbeau *et al.*, 2002). The exercise sessions were done under the supervision of post-graduate Human Movement Science students and members of the research team who ensured that each pupil followed the exercise instructions.

4.3.4 Blood sampling

Blood samples were collected after a 12-h overnight fast. Registered nurses used 21-gauge scalp infusion sets to collect venous blood samples between 08:00 and 11:00. Tubes were inverted gently and stored on ice until centrifugation. Citrated and clotted blood was centrifuged for 15 min at 2000 x *g* at 4°C to yield plasma for glucose, fibrinogen, PAI-1_{act}, and thrombin anti-thrombin complex (TAT), and serum for insulin and CRP analysis, respectively. Plasma and serum were divided into aliquots and then snap-frozen on dry ice before being stored at –84°C until analysis.

4.3.5 Analysis of biochemical measurements

Plasma fibrinogen concentrations and FVIIIc were measured using the modified method of Clauss (1957) and the activated partial thromboplastin time assay, respectively, on an automated coagulation analyser (ACL-200, Instrumentation Laboratories, Milan, Italy). PAI-1_{act} was measured using an indirect enzymatic method (Spectrolyse pL, Biopool, Umeå, Sweden, Cat. No.101201). TAT was determined by an Enzyme-Linked Immunosorbent Assay (ELISA) (Enzygnost® TAT micro, Marburg, Germany, Dade Behring, Cat. No. OWMG G15). High-sensitivity CRP was determined by rate turbidimetry with a high sensitivity C-Reactive Protein Kit (CRPH, IMMAGE ®, Immunochemistry Systems, California, USA, Cat. No. 474630) on the Synchron LX System (Beckman Coulter Inc., Fullerton, California, USA). Fasting serum insulin was measured with Microparticle Enzyme Immunoassay method on the AxSYM ® system (ABBOTT Diagnostics Division, Tokyo, Japan).

4.3.6 Insulin resistance and insulin sensitivity calculations

Fasting plasma glucose was measured by an enzymatic method on a Vitros DT60 II Chemistry Analyser (Ortho-Clinical Diagnostics, Rochester, New York, USA). The homeostasis model assessment (HOMA) was used for the calculation of insulin resistance {formula: HOMA = [fasting insulin (μ IU) x fasting venous glucose (mmol/L)]/22.5} (Katz *et al.*, 2000). Insulin sensitivity was calculated using Quantitative Insulin Sensitivity Check Index (QUICKI) = 1 / log(fasting venous insulin) + log(fasting venous glucose) (Katz *et al.*, 2000).

4.3.7 Blood pressure measurements

Physiologists measured systolic blood pressure (SBP) and diastolic blood pressure (DBP) in duplicate under standardised conditions (National High Blood Pressure Education

Programme Working Group on High Blood Pressure in Children and Adolescents, 2004) using a mercury sphygmomanometer (Tycos, USA) of appropriate cuff size (covering 80 to 100% of the arm circumference) and a stethoscope.

4.3.8 Anthropometric measurements

Qualified level 2 anthropometrists did the anthropometric measurements in triplicate (see Addendum B for the anthropometry data sheet). Subjects were examined in their underwear. Body mass was measured with a portable electronic scale (Precision Health Scale, A&D Company, Tokyo, Japan) to the nearest 0.1 kg. Heights were measured to the nearest 0.5 cm with a Stadiometer (IP 1465, Invicta, London, UK) without shoes, standing upright with their heads in the Frankfort plane (Norton & Olds, 1996). Skinfolds were measured with a John Bull calliper (British Indicators, London, UK) according to approved methods (ISAK, 2001). Air displacement plethysmography in the BodPod® measurement system (Life Measurement Inc, Concord, CA) using Boyle's law of the pressure/volume relationship was used to determine body fat mass and body fat %. Body density was calculated as mass divided by volume and corrected for lung volume. The Siri formula [under water weighing (UWW) = (4.95/density – 4.5) x 100] (Siri, 1993) and the Brozek formula [UWW = (4.57/density – 1.142) x 100] were used to calculate body fat % (Brozek *et al.*, 1963). Waist and hip circumferences were measured to the nearest 0.1 cm with the cross-hand technique and a Lufkin steel tape (Cooper Tools, Apex, NC, USA).

Anthropometric measures were used to determine stunting. The reference population used in calculating height for age expressed in Z-scores (HAZ) was that given by the National Centre for Health Statistics (CDC, 2000). For the purpose of this study stunting was defined as a HAZ-score $\leq$ - 2 and is traditionally used as an indicator of nutritional status (CDC, 2000).

4.3.9 Determination of physical fitness

Both at baseline and after the intervention trained postgraduate Human Movement Science students administered the bleep test to assess fitness by running to physical exhaustion (Stevens & Sykes, 1996). The number of completed laps was recorded for each child.

4.3.10 Questionnaires

Demographic data were obtained from a structured questionnaire which gave information regarding the subjects' age, gender, home language, socio-economic class and general health data. For the purpose of this study the data were merely used to describe the study sample since socio-economic status may have an effect on the haemostatic variables. Information on smoking and drinking and the use of any pharmaceutical substance was obtained from a structured habits and medication questionnaire.

4.3.11 Statistical analysis

The computer software package Statistica® version 7 (StatSoft, 2004) was used for the statistical analysis. Initially, data were tested for normal distribution using the Shapiro-Wilk's W-test. Parametric data were expressed as the mean ± standard deviation (SD) and non-parametric data as medians (lower; upper quartiles). Descriptive statistics for all baseline, end and changes from baseline to end (delta) variables were calculated. Changes within groups from baseline to end for parametric data were tested for significance by using the *t*-test for dependent samples and for non-parametric data the Wilcoxon matched pairs test was used. Differences in baseline and delta between the groups for parametric data were determined by using the analysis of variance (ANOVA) and for non-pararametric data with the Kruskall Wallis ANOVA. Where significant differences between the groups were indicated with the ANOVA, the Tukey honest significant difference test for unequal *n* for parametric and Bonferoni adjustment for non-parametric data were used to determine between which groups the differences occurred. Spearman's rank order correlations were done. A p-value of ≤ 0.05 was regarded as statistically significant.

4.4 RESULTS

All grade 9 adolescents in two township high schools were recruited for the study (n = 255), but only 81 children gave consent for blood drawing at baseline and after 11 weeks. All consenting grade 9 pupils in one school were exposed to an exercise programme while the reference group (n = 24) received only lifestyle advice in one session. Due to poor attendance of the supervised exercise sessions it was decided to divide the intervention group into a group with ≥ 40% attendance (n = 29) and a group with < 40% attendance (n = 28). According to Barbeau *et al.* (2002), 40% attendance is adequate to be considered compliant when investigating PA. According to the habits and medication questionnaire, none of the girls in the study population used oral contraceptives. Very few children smoked or used alcoholic beverages and if they did, it was only occasionally and was thus considered negligible.

Table 4.1 presents the baseline characteristics of the 3 groups. The intervention groups comprised of comparable amounts of boys and girls, but the reference group predominantly comprised of girls. The reference group had significantly higher subscapular skinfold thicknesses than both the intervention groups. The low-attendance group had significantly lower triceps skinfold thicknesses than both the reference group and the high-attendance group. The low-attendance group had significantly lower SBP than the high-attendance group. The three groups were comparable regarding age, BMI, waist circumference, DBP,

insulin and glucose levels, insulin sensitivity and resistance, and fitness according to the bleep test.

Table 4.1: Baseline characteristics of the intervention group with ≥ 40% and with < 40% attendance and the reference group, respectively

Variable	**Intervention group with ≥ 40% attendance** (n = 29)	**Intervention group with < 40%** (n = 28)	**Reference group** (n = 24)	**ANOVA p**
Age (yr)	15.7 ± 1.31	15.7 ± 1.46	15.1 ± 1.18	0.22
Boys/Girls ratio	13/16	14/14	3/21	-
BMI (kg/m^2)	19.3 ± 2.98	19.3 ± 2.95	20.3 ± 3.05	0.114
Waist circumference (cm)	64.3 ± 5.69	63.8 ± 4.80	63.4 ± 5.82	0.25
Triceps skinfold (mm)	12.0 (7.38; 15.8)	8.73 (6.65; 15.5)	13.7 (11.2; 21.3)	0.03[□]
Subscapular skinfold (mm)	8.05 (7.05; 11.9)	7.88 (6.53; 10.2)	11.2 (8.50; 14.2)	0.01[§]
SBP (mmHg)	100 ± 11.4	92.9 ± 9.01	94.7 ± 7.70	0.01[¤]
DBP (mmHg)	60.2 ± 9.08	58.7 ± 8.23	66.2 ± 9.52	0.10
Fasting serum insulin (μU/ml)	8.20 (4.80; 13.8)	8.00 (4.10; 9.70)	7.50 (4.80; 12.6)	0.45
Fasting plasma glucose (mmol/l)	5.14 ± 0.34	5.19 ± 0.55	4.85 ± 0.43	0.67
QUICKI	0.35 ● 0.04	0.36 ± 0.03	0.35 ± 0.04	0.054
HOMA	1.72 (1.13; 3.09)	1.84 (0.91; 2.22)	1.55 (1.08; 2.86)	0.57
Physical fitness (Bleep test level)	4.70 (3.30; 6.20)	3.60 (2.50; 5.40)	5.05 (3.20; 7.20)	0.25

Parametric data reported as mean ± SD and non-parametric data reported as median (interquartile range).

□ Post hoc test shows that the group with < 40% attendance differed significantly from the reference group and the group with ≥ 40% attendance.

§Post hoc test shows that the reference group differed significantly from the other two groups.

¤ Post hoc test shows that the group with < 40% attendance differed significantly from the group with ≥ 40% attendance.

BMI body mass index; DBP diastolic blood pressure; HOMA homeostasis model assessment; QUICKI quantitative insulin sensitivity check index; SBP systolic blood pressure

The changes within and between the groups during the intervention are presented in Table 4.2. The results of the bleep test indicate that there were no significant differences in cardiovascular fitness from baseline to the end neither in any of the groups nor in the changes from baseline to the end between the groups. In spite of the lack of change in the fitness values, BMI in the high-attendance group increased significantly from 19.3 ± 2.98 to 19.5 ± 3.24 kg/m^2 (p = 0.04). This increase cannot be attributed to the increase in fat mass as fat mass decreased significantly from baseline to end in this group (from 12.5 ± 4.87 to 10.1 ±

4.65 kg; p = 0.00008). This decrease in fat mass from baseline to end was, however, also seen in the other 2 groups (low-attendance group from 10.5 ± 5.57 to 8.87 ± 4.64 kg (p = 0.009), and in the reference group 15.9 ± 6.60 to 11.5 ± 3.91 kg (p = 0.001).

Table 4.2: Changes within and between the groups during the intervention

Marker		**Intervention group with ≥ 40% attendance** (n = 29)	**Intervention group with < 40%** (n = 28)	**Reference group** (n = 24)	**ANOVA p**
Bleep test	B	4.70 (3.30; 6.20)	3.60 (2.50; 5.40)	5.05 (3.20; 7.20)	0.25
	E	4.50 (4.20; 5.40)	4.95 (4.25; 6.65)	3.30 (3.30; 3.50)	
	D	0.41 ± 2.57	1.02 ± 3.33	-0.65 ± 2.42	0.83
BMI (kg/m^{-2})	B	19.3 ± 2.98*	19.3 ± 2.95	20.3 ± 3.05	0.11
	E	19.5 ± 3.24*	19.2 ± 2.49	19.9 ± 3.04	
	D	0.23 ± 0.57	0.22 ±0.69	-0.07 ± 0.78	0.40
Fat mass (kg)	B	12.5 ± 4.87*	10.5 ± 5.57*	15.9 ± 6.60*	0.07
	E	10.1 ± 4.65*	8.87 ± 4.64*	11.5 ± 3.91*	
	D	-1.26 ± 3.08	-1.29 ± 2.17	-2.17 ± 1.91	0.41
Fibrinogen (g/l)	B	2.75 ± 0.43	2.65 ± 0.47	2.78 ± 0:52	0.62
	E	2.79 ± 0.69	2.75 ± 0.47	2.80 ± 0.49	
	D	0.02 ± 0.71	0.17 ± 0.60	-0.05 ± 0.59	0.47
FVIIIc %	B	150 ± 34.4*	154 ± 43.8*	144 ± 41.8	0.68
	E	175 ± 64.3*	168 ± 63.7*	157 ± 55.4	
	D	31.8 ± 78.8	12.6 ± 26.8	12.6 ± 38.8	0.35
PAI-1_{act} (IU/ml)	B	3.32 ± 2.32	2.87 ± 3.15	4.31 ± 2.80	0.18
	E	3.58 ± 2.70	3.10 ± 2.44	3.73 ± 2.60	
	D	0.43 ± 2.35	0.006 ± 3.25	-0.86 ± 2.45	0.25
CRP (mg/l)	B	0.34 (0.15; 0.79)	0.39 (0.20; 0.53)	0.29 (0.15; 0.92)	0.68
	E	0.39 (0.19; 1.32)	0.66 (0.21; 1.31)	0.70 (0.15; 1.04)	
	D	0.52 ± 2.64	0.12 ± 1.65	0.42 ± 0.89	
TAT (µg/L)	B	4.40 ± 2.90	5.04 ± 5.54	7.35 ± 8.23	0.18
	E	12.5 ± 29.3	4.51 ± 6.88	11.7 ± 25.0	
	D	8.11 ± 31.2	-0.77 ± 8.52	3.19 ± 25.2	0.41

Parametric data are reported as mean ± SD and non-parametric as median (25^{th}; 75^{th} P).

** = $p \leq 0.05$ between baseline and end within a group.*

B baseline; CRP C-reactive protein; BMI body mass index; E end; D delta (change from B to E); FVIIIc % factor VIII coagulant activity percentage; PAI-1_{act} plasminogen activator inhibitor type 1 activity; TAT thrombin anti-thrombin complex.

FVIIIc was the only haemostatic variable that was significantly altered during the intervention. In both the high and low-attendance groups FVIIIc% increased significantly from baseline to end (from 150 ± 34.4 to 175 ± 64.3 %; p = 0.01, and from 154 ± 43.8 to 168 ± 63.7 %; p = 0.02, respectively). Although not significant, the increase in FVIIIc% in the reference groups was similar to the increase in the low-attendance group. The intervention had no significant effect on any of the other haemostatic variables.

Baseline correlations show that FVIIIc% correlated significantly with the triceps skinfold (r = -0.26; p = 0.02), BMI (r = -0.23; p = 0.04), and plasma glucose (r = 0.26; p = 0.02); that PAI-1_{act} correlated significantly with muscle mass (r = -0.25; p = 0.04), the triceps skinfold (r = 0.27; p = 0.02) and fat mass (r = 0.28; p = 0.02); and fibrinogen correlated with CRP (r = 0.47; p = 0.00002), and PAI-1_{act} (r = 0.27; p = 0.02).

Changes in the haemostatic markers from baseline to end correlated significantly with changes in some of the body composition variables. In the high-attendance group delta TAT correlated with delta fat mass (r = 0.53; p = 0.01); delta FVIIIc% with delta BMI (r = 0.38; p = 0.05); delta CRP with delta waist circumference (r = 0.44; p = 0.02), the triceps skinfold (r = 0.51; p = 0.006), and with the subscapular skinfold (r = 0.54; p = 0.003); and delta PAI-1_{act} correlated with delta BMI (r = 1.44; p = 0.02). In the reference group the change in TAT correlated negatively with waist circumference (r = -0.52; p = 0.02); delta FVIIIc% with delta waist circumference (r = -0.50; p = 0.03); delta PAI-1_{act} with the triceps skinfold (r = -0.46; p = 0.03) and the change in the triceps skinfold (r = 0.66; p = 0.001).

Changes in the haemostatic markers correlated significantly mainly with their respective baseline values and changes in the other haemostatic variables as well as with CRP and glucose. In the high-attendance group delta TAT correlated with baseline TAT (r = 0.66; p = 0.0004); delta FVIIIc% with delta CRP (r = 0.42; p = 0.03), and delta PAI-1_{act} (r = 0.48; p = 0.01); and PAI-1_{act} with delta FVIIIc% (r = 0.46; p = 0.01). In the low-attendance group the change in TAT correlated with baseline TAT levels (r = -0.60; p = 0.001); delta fibrinogen correlated with baseline fibrinogen (r = -0.43; p = 0.03), delta CRP (r = 0.45; p = 0 02) with baseline QUICKI (r = -0.40; p = 0.04); and delta PAI-1_{act} with baseline PAI-1_{act} (r = -0.43; p = 0.02). In the reference group delta FVIIIc% correlated with delta PAI-1_{act} (r = 0.51; p = 0.02), delta fibrinogen with delta glucose levels (r = 0.61; p = 0.008), and delta CRP (r = 0.66; p = 0.003); delta PAI-1_{act} with baseline PAI-1_{act} (r = -0.46; p = 0.04).

4.5 DISCUSSION

There is a gap in the literature on the relationship between PA and/or physical fitness and haemostatic markers in African children and since they are at a high risk for developing stroke early in life (Imam, 2002; Vorster, 2002), preventative strategies should be investigated. PA is a prime candidate to alter the risk profile as opposed to pharmacological intervention modalities (Ernst, 1993), although treatment dosages as well as the mechanisms by which PA confers its positive influence remain unclear and speculative.

The salient finding of this study is that an 11-week outdoor PA intervention programme (beginning in summer and ending in the winter) had no significant effect on the haemostatic markers of African adolescents.

The three groups were comparable regarding baseline characteristics. The higher triceps and subscapular skinfolds in the control group are most likely due to the gender ratio of the control group, which consisted mainly of girls. Girls have been shown to have greater skinfold thicknesses than boys in this group (Kruger *et al.*, 2004). Smoking (Kelleher, 1992), alcohol and oral contraceptive use (Ernst, *et al.*, 1989) are known factors that influence the haemostatic variables and were not common in this study group.

This study was unfortunately complicated by poor compliance of the exercise sessions. Subjects raised the following reasons for their poor compliance: (1) some subjects had to look after their younger siblings after school while their parents were at work, (2) others helped their parents with household chores after school, and (3) others dislike exercise and, therefore, did not participate.

According to the bleep test (a multistage fitness test which assess maximal oxygen uptake indirectly) there were no differences from baseline to end after following the conditioning programme in the 2 intervention groups regarding aerobic fitness. The change in fitness levels from baseline to end was similar in all three groups. The bleep test is designed for well-motivated, active individuals (Stevens & Sykes, 1996). There are several reasons why the results of the bleep test may not accurately reflect the effect of the intervention. An inherent disadvantage of this test is that if a subject was not equally motivated during baseline and the end measurements, the results will not accurately reflect cardiorespiratory fitness and hence the delta values will not mirror changes accurately. Another reason may be genetic endowment differences. Because genetics greatly determines aerobic capacity (Birrer & Levine, 1987), it is possible that children who did not exercise regularly could achieve better scores than those who exercised regularly but lacked the genetic makeup for aerobic capacity. The inability of the PA intervention to change fitness levels even in the intervention group with good compliance could possibly be attributed to subjects who attended the PA sessions (compliance of attendance), but did not partake optimally in the exercises (compliance of activity). Subjects were physically active outside the experimental setting but these activities most likely did not influence the fitness values since they would have been reflected in the baseline fitness values, but if changes in an individual's habitual acitivities occurred, the results would have been biased by the amount of habitual PA.

Despite the lack of improvement in fitness the intervention redistributed body composition. The BMI increased in the high-attendance group most likely due to an increase in muscle mass as fat

mass decreased significantly. This possible increase in muscle mass was not seen in the low-attendance group or the reference group indicating that although the intervention did not increase aeobic fitness, it may have increased muscle mass. The adolescent period is characterised by rapid growth of muscle and bone, which complicates interpretation of results from baseline to the end since it could influence body composition irrespective of an intervention (Tanner, 1963). The interpretation of the anthropometric variables is further complicated by the ranging ages of the subjects and even if the children's ages were the same, genetics also play a role in determining a child's age of maturation (Tanner, 1963). Differences in growth patterns between boys and girls could also influence the anthropometric variables (Beunen *et al.*, 2000). Because the intervention groups comprised of the same number of boys and girls but the reference group predominantly comprised of girls, bias may have been introduced in the reference group.

FVIIIc% changes from baseline to end of the reference group and the low-attendance group were similar although the increase in the low-attendance group was significant. The reason why the reference group's increase was not significant is most likely due to the great variance in FVIIIc% values. In the literature a single bout of exercise (an acute effect of PA) is associated with a transient increase in blood coagulation as evidenced by increased FVIII (El-Sayed, 1996; El-Sayed *et al.*, 2000), but resting levels of FVIII activity and antigen is not easily changed with chronic PA in either sedentary individuals (Boman *et al.*, 1994; El-Sayed *et al.*, 1995; Ponjee *et al.*, 1996; Prisco *et al.*, 1998; Van den Burg *et al.*, 1997; Van den Burg *et al.*, 2000) or in endurance-trained athletes (Watts, 1991). Limited studies exist concerning the influence of chronic PA on blood hemostasis, but these results suggest that FVIII activity and antigen levels at rest or after exercise remain unchanged in response to chronic exercise in normal healthy individuals (El-Sayed *et al.*, 1995), although not in cardiac patients (Suzuki *et al.*, 1992). The increase in FVIIIc and the increases in the other haemostatic markers though insignificant could possibly be due to a seasonal variation in the haemostatic markers. Research investigating seasonal variation on FVIII is limited, but several researchers found that prothrombotic markers (fibrinogen, FVIIc and mean platelet volume and count) peaked during winter time (Crawford *et al.*, 2003; Mavri *et al.*, 2001; Woodhouse *et al.*, 1994).

The exercise protocol (intensities and duration), the study population (patients, normal sedentary individuals, habitual exercisers or athletes, children or adults), the physical abilities of the subjects and their fitness levels influence study results when investigating the influence of exercise on haemostasis. Some research suggests that PA may reduce fibrinogen concentration in patients (Worsornu *et al.*, 1992) and in elderly males, but not in young males (Stratton *et al.*, 1991). However, Balagopal *et al.* (2005) found that a 3-month diet and

exercise intervention, which redistributed body composition in adolescents, resulted in the reduction of CRP, fibrinogen and IL-6 in response to the intervention but not in the controls. Similar to the findings of this study, however, exercise training for 8 months had no significant effects on haemostatic markers including fibrinogen and CRP in obese youths (Barbeau *et al.*, 2002).

The findings in this study on PAI-1 are compatible with earlier findings by Bodary *et al.* (2003), who found that after short term (10 day) moderate-intensity exercise training no change in PAI-1 levels occurred in normal, asymptomatic men and women. $PAI\text{-}1_{act}$ levels in previously sedentary subjects who followed a long term (8 month period) training regime seemed to decrease, but this decrease failed to reach the designated level of significance ($p > 0.05$) due to large group variances and possible seasonal variation (De Geus, *et al.*, 1992). In contrast, Estellés *et al.* (2001) found an increase in fibrinolytic activity due to a decrease in PAI- levels in the obese children who had reduced their BMI after a 3-month period of treatment to reduce weight. It is not clear whether it was the exercise *per se* or the reduction in BMI that led to the increase in fibrinolysis. Earlier studies suggested that the favourable effects of training on blood fibrinolysis appear to be age-related, as higher fibrinolytic potential was observed post-training in elderly, but not in young study participants (Stratton *et al.*, 1991; Schuit *et al.*, 1997). It may also be that PA has effects on other proteins in the fibrinolysis pathway than PAI-1, for example tissue plasminogen activator (t-PA) or plasmin. In an earlier study by El-Sayed (1996), an increase in fibrinolysis was found after exercise due to increases in t-PA and decreases in PAI-1. Another possible reason for the inability of the PA programme to change fibrinogen, PAI-1, TAT and CRP could be that fitness levels did indeed not change in the intervention groups as seen from the bleep test results.

The haemostatic variables correlated significantly with some of the anthropometrical variables at baseline and the changes in the haemostatic markers also correlated with changes in the anthropometric variables from baseline to end, thereby indicating that from a young age body composition seems to play a role in the haemostatic balance. It may be that decreases in the haemostatic markers measured with PA require pronounced decreases in adiposity before imparting changes on the haemostatic markers. It also seemed as though the baseline levels of the haemostatic variables were strong predictors of the change during the intervention. This is in keeping with a previous study in which the largest decrease in PAI-1 levels was observed in the obese children with the highest initial PAI-1 levels after a weight loss intervention (Estellés *et al.*, 2001). Barbeau *et al.* (2002) also found that indicators of body fat and baseline values of some of the haemostatic markers correlated significantly with their respective changes after a PA intervention.

4.6 CONCLUSION AND RECOMENDATIONS

No valid conclusion regarding the effect of PA on the haemostatic markers could be drawn from the above findings and further investigations are required. Plausible reasons for this outcome were that seasonal variations in the haemostatic variables clouded the effect of the PA intervention as baseline measurements were taken in the summer and end measurements in the winter, or that compliance with the intervention was unsatisfactory, or that greater body fat losses are required to impart changes in the haemostatic markers, or that baseline values play a prominent role in the changes that can be expected during an intervention. Therefore, improvements in the haemostatic profile are most likely to be seen when the initial levels are raised. There is a need for experimental studies with different frequencies, durations, modes and volumes of PA in which groups of children and adolescents of all races are compared with each other in relation to haemostatic markers in order to increase the knowledge on the relationship between haemostatic markers and PA.

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ADDENDA

Addendum A:

Informed consent form

PLAY project: information on the study

THE PRPOJECT HAS BEEN APPROVED BY THE ETHICS COMMITTEE OF THE NORTH-WEST UNIVERSITY (Potchefstroom Campus).

I CONFIRM THAT:

It has been explained to me, that:

1. The purpose of the research study is to collect information on growth and activity among Grade 9-10 schoolchildren in Seiphemelo and Boitshoko Secondary Schools, North West Province.
2. The measurements will be done at the beginning (February) and end (October) of the year at the North-West University and children will be transported by bus to the university.
3. I have been told that the researchers will measure me. The participant will be weighed and his/her height as well as circumferences and skinfolds of his/her arm will be measured without causing any pain to the child. For those measurements boys and girls in separate groups will be asked to undress in privacy of a room, because some measurements must be taken with the children dressed in underwear only, or a light shirt and pants/skirt. The researchers will also ask me to indicate my own level of physical maturation from pictures. The different age groups will be measured separately. The researchers and fieldworkers will work in a professional way, not to embarrass the children.
4. Fitness testing will be done.
5. The researchers will ask me about my home environment, the food that I usually eat and activities that I do. None of these questions will be to see if I am clever, or know correct answers. I can just tell them what I usually do.
6. Guidelines for appropriate, culture sensitive, practical and sustainable intervention programmes for children will be developed based on the results.
7. The information I will give shall be kept confidential, only to be used anonymously for making known the findings to other scientists.
8. It was also clearly explained to me that I can refuse to participate in this research study or I can stop answering the questions at any time during the interviews.

The information in this consent form was explained to me by professor Kruger in English and I confirm that I have a good command in this language and understood the explanations. I was also given the opportunity to ask questions on things I did not understand clearly.

I the participant (child) hereby agree voluntarily to take part in this research survey.

Signed/confirmed at_____on_____2006

Witness_____

Representative of participant (parent/guardian)_____

Addendum B:

Anthropometry data sheet

ANTHROPOMETRY DATA SHEET

Name and surname: _____ Gender:_____

DOB: ____/___/____ Test date: __/__/200_ Age: _____

Subject number: _____

			Measurement 1	Measurement 2	Measurement 3/Mean
1	Mass	kg			
2	Length	cm			
3	Arm span	cm			
4	Sitting height	cm			
Girths					
5	Upper arm (relaxed)	cm			
6	Waist (minimum)	cm			
7	Hip (maximum)	cm			
8	Calf	cm			
Skinfold:					
9	Triceps	mm			
10	Subscapular	mm			
11	Calf	mm			
12	Supraspinal	mm			
13	Abdominal	mm			
BodPod:					
14	Mass	kg			
15	Fat %	%			
16	Fat mass	kg			
17	% muscle mass	%			
18	Lung volume	L			

Addendum C:

PLAY study habits and medication questionnaire

PLAY STUDY HABITS AND MEDICATION QUESTIONNAIRE

Subject number:

Do you smoke or use any of the following?	YES = 1 (tick next to what you are smoking and write the amount per day next to it)			NO = 2
	TYPE	Tick here (√)	AMOUNT / DAY	
	Cigarettes			
	Tobacco / Pipe			
	Snuff			
	Chewing tobacco			
	Dagga			

If you answered YES in the previous question when (year AND month) did you begin smoking?
How old were you then? ..... ***years***

Have you smoked or used any of the following and then stopped smoking or using the substance?	YES = 1 (Tick next to what you were smoking and write the amount per day next to it)			NO = 2
	TYPE	Tick here (√)	AMOUNT / DAY	
	Cigarettes			
	Tobacco / Pipe			
	Snuf			
	Chewing tobacco			
	Dagga			

If you answered YES in the previous question when (year AND month) did you stop smoking?
How old were you then? .....***years***

Do you drink alcohol (beer, wine etc.)?	Yes = 1	No = 2	
If you answered YES for the previous question, state the average amount you drink during 1 week, for example: 3 x 750ml bottles / week	750ml bottles / week	340ml tins / week	200ml glasses / week
Do you drink homemade beer?	Yes = 1	No = 2	
If you answered YES for the previous question, state the average amount you drink during 1 week	500ml carton / week	250ml carton / week	200ml glasses / week

If you drink spirits (brandy, whisky, rum, vodka etc.) how many SHOTS do you drink per week?

If you drink alcohol, when did you start (year AND month) drinking? How old were you then? .....***years***	YES = 1	NO = 2
Have you drunk or used any alcohol and stopped drinking?	YES = 1	NO = 2

If you answered YES to the previous question, when (year AND month) did you stop?
How old were you then? .....***years***

Do you use any medication chronically (regularly / each day)?	YES = 1 (If YES, specify)	NO = 2

Do you use any birth control pill?	YES = 1 (If YES, tick next to the one you take.)	NO = 2
	Ovral	
	Triphasil	
	Nordette	
	Injection (Depo-Provera / Nur-isterate)	
	Other (specify)	
If YES when did you start (year AND month) using the contraceptives (birth control pill? How old were you then? ..... ***years***		

Addendum D:

Fitnessgram datasheet

PLAY STUDY DATASHEET 2006 (FITNESSGRAM)					
PLAY – DATA	**Subject no**				
Name:					
Age:			**Gender**	M	F
Birthdate:			**Grade**		
Test date:			**Arm span**		
			Sitting height		
ANTHROPOMETRIC MEASUREMENTS					
Stature			**Calf SF**		
Body mass			**Abdominal SF**		
Subscapular skinfold (SF)			**BMI**		
Triceps SF			**% Body fat**		
FITNESSGRAM					
Bleep-test (Levels)			**Pacer-test (Laps)**		
Aerobic capacity					
Curl Up			**Trunk lift**		
Push Up			**Standing long jump**		
Sit and reach	R		**Step up test**		
	L				
Modified sit and reach					
Bent armhang (girls)			**Pull-ups (boys)**		
Handgrip strength					

Any medical conditions/illnesses we should be aware of?

Addendum E:

Physical activity questionnaire of the previous weekend day

Physical activity questionnaire of the previous weekend day

Subject no

Name

Race: W B C I

Age

Gender M V

Grade

School:

Teacher:

Date

Classification

Think back about the weekend. For each of the 30 minutes periods, select a primary activity that you performed and write the type of activity in the type of activity column.

Mark the day of the weekend that you fill in this form

Saturday Sunday

Time	TYPE	Activity	METs		Very Light	Light	Moderate	Hard
7:00								
7:30								
8:00								
8:30								
9:00								
9:30								
10:00								
10:30								
11:00								
11:30								
12:00								
12:30								
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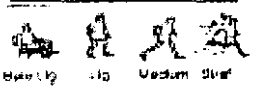

Very Light Light Medium Hard

Addendum F:

Physical activity questionnaire of the previous week day

Physical activity questionnaire of the previous week day

Subject nr

Name:

Race: W B C I

Age:

Gender M F

Grade

School:

Teacher:

Date

Classification

Think back about yesterday. For each of the 30 minutes periods, select a primary activity that you performed and write the type of activity in the type of activity column.

Mark the day of the week that you fill in this form

Monday | Tuesday | Wed | Thursday | Friday

Time	TIPE	Activity	METS			Very	Light	Mod	Hard
7:00				*					
7:30				*					
8:00				*					
8:30				*					
9:00				*					
9:30				*					
10:00				*					
10:30				*					
11:00				*					
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19:00				*					
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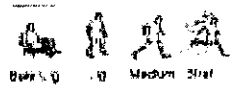
20:00				*					
20:30				*					
21:00				*					
21:30				*					
22:00				*					



Medium

Addendum G:

Male self-assessment of maturity characteristics

MALE SELF-ASSESSENT OF MATURITY CHARACTERISTICS

Subject nr:	Name:	Age:

1. Have you already experienced a voice change? Tick √ in the appropriate box.

No		Yes	
Unbroken	Signs of breaking	Definitely broken/ adult quality	

2. If applicable, circle the age/grade in which you experienced signs of breaking of your voice.

Primary school				Secondary school				
10 years Grade 4	11 years Grade 5	12 years Grade 6	13 years Grade 7	14 years Grade 8	15 years Grade 9	16 years Grade 10	17 years Grade 11	18 years Grade 12

3. **If applicable, circle the age/grade in which you experienced YOUR VOICE DEFINITELY BROKEN.**

Primary school				Secondary school				
10 years Grade 4	11 years Grade 5	12 years Grade 6	13 years Grade 7	14 years Grade 8	15 years Grade 9	16 years Grade 10	17 years Grade 11	18 years Grade 12

4. Do you think your voice broke at the same time, earlier or later than friends or boys of a similar age than you? Tick √ in the appropriate box?

EARLIER		LATER		SAME TIME	

5. If you have started shaving, in which grade dit it happen?

Grade:
Not Yet

6. Do you think you started shaving at the same time, earlier or later than friends or boys with a similar age than you? Tick √ in the appropriate box?

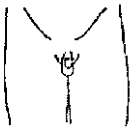
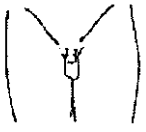
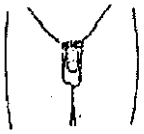
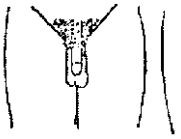
EARLIER		LATER		SAME TIME	

7. The description on this page describes different amounts of male facial hair. Please read each of the descriptions. Then tick √ the appropriate box that describes your stage of facial hair development best.

None	Increase in length, with pigmentation (darkening) at corners of upper lip, spreading medially to complete moustache.	Hair on the upper part of the cheeks and in the midline just below the lower lip.	Hair on the sides and lower border of the chin.

8. As you keep growing over the next few years, you will see changes in your body. These changes happen at different ages for different children and you may already be seeing some changes. Doctors use the set of drawings which is shown to you to determine stages of growth. These changes can be identified in 5 different phases. We want to determine how well you can select your stage of growth from the set of drawings. All you need to do is to pick the drawing and description that looks like you do know. Make a tick √ above the drawing that is closest to your stage of development, then put the sheet in the envelope and seal it so your answer will be kept in private.

The drawings on this page show different amounts of <u>male pubic hair</u>. Please look at each of the drawings and read the sentences under the drawings. Then tick √ the drawing that is <u>closest to your stage of hair development</u>

Picture 1	Picture 2	Picture 3	Picture 4	Picture 5
				
There is no pubic hair at all.	There is small amount of long, lightly coloured hair. This hair may be straight or a little curly.	There is darker hair, curlier and thinly spread out to cover a somewhat larger area than in stage 2.	The hair is thicker and more spread out, covering a larger area than in stage 3.	The hair now is widely spread covering a large area, like that of an adult male.

9. **In comparison to other boys of your age, how would you describe your development with regard to pubic hair development.**

Much earlier	Somewhat earlier	About the same	Somewhat later	Much later

10. **The drawings on this page show different stages <u>of growth of the testes, scrotum and penis</u>. A boy goes through each of the 5 stages shown. Please look at each of the drawings and read the sentences under the drawings. Then tick √ the drawing that is closest to your stage of growth**

Picture 1	Picture 2	Picture 3	Picture 4	Picture 5
The testes, scrotum and penis are about the same size and shape as they were when you were a child.	The testes and scrotum are bigger. The skin of the scrotum has changed. The scrotum (the sack holding the testes) has gotten lower. The penis has gotten only a little bigger.	The penis has grown in length. The testes and scrotum have grown and dropped lower than in picture 2.	The penis has gotten even larger. It is wider. The head of the penis is bigger. The scrotum is darker than before. It is bigger because the testes are bigger.	The penis, scrotum and testes are the size and shape of that of an adult man.

11. **In comparison to other boys of your age, how would you describe your development with <u>regard to growth of the penis, testes and scrotum</u>.**

Much earlier	Somewhat earlier	About the same	Somewhat later	Much later

THANK YOU FOR YOUR TIME!

Addendum H:
Female self-assessment of maturity characteristics

FEMALE SELF-ASSESSMENT OF MATURITY CHARACTERISTICS

Name:	Gender :		F	Age:
Subject number:				

2. **When was your last period? (Indicate the number of days, weeks, months).**

3. **If you have started menstruating, circle the age and the grade when you did.**

Primary school				Secondary school				
10 years Grade 4	11 years Grade 5	12 years Grade 6	13 years Grade 7	14 years Grade 8	15 years Grade 9	16 years Grade 10	17 years Grade 11	18 years Grade 12

4. **Do you think you started menstruating at the same time, earlier or later than friends or girls with a similar age than you? Tick √ in the appropriate box.**

EARLIER ☐ LATER ☐ SAME TIME ☐

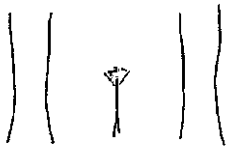
5. **If possible, try to recall the exact date when you started menstruating (year, month)**

Year:
Month:

6. **As you keep growing over the next few years, you will see changes in your body. These changes happen at different ages for different children and you may already be seeing some changes. Doctors use the set of drawings of pubic hair development which is shown to you to determine stage of growth. These changes can be identified in 5 different phases. We want to determine how well you can select your stage of growth from the set of drawings. All you need to do is pick the drawing and description that looks like you do know. Make a tick √ above the drawing that is closest to your stage of development. then put the sheet in the envelope and seal it so your answer will be kept in private.**

The following drawings show <u>different amounts of female pubic hair development</u>. Please look at each of the drawings and read the sentences under the drawings. Then tick √ the drawing that is **closest** to your hair developement.

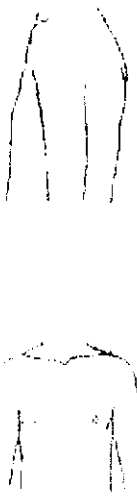
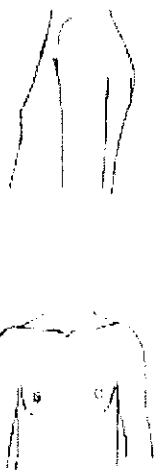
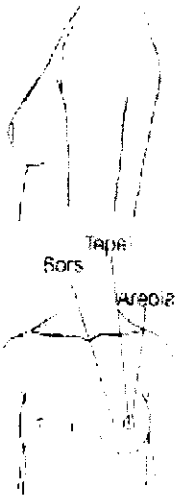
☐
☐

Figure 1	Figure 2	Figure 3	Figure 4	Figure 5
				
There is no pubic hair at all	There is small amount of long, lightly coloured hair. This hair may be straight or a little curly	There is darker hair, curlier and thinly spread out to cover a somewhat larger area than in stage 2.	The hair is thicker and more spread out, covering a larger area than in stage 3.	The hair now is widely spread covering a large area, like that of an adult female

7. **In comparison to other girls your age, how would you describe your development with regard to pubic hair development.**

Much earlier	Somewhat earlier	About the same	Somewhat later	Much later

8. The following drawings show the amount of breast development. Please look at each of the drawings and read the sentences under the drawings. Then tick √ the drawing that is **closest** to your breast developement.

Picture 1	Picture 2	Picture 3	Picture 4	Picture 5
				
The nipple is raised a little in this stage. The rest of the breast is still flat	This is the breast bud stage. In this stage the nipple is raised more than in stage 1. The breast is a small mound. The areola is larger than in stage 1	The areola and the breast are both larger than in stage 2. The areola does not stick out away from the breast.	The areola and the nipple make up a mound that sticks above the shape of the breast	This is the mature adult stage. The breasts are fully grown. Only the nipple sticks out in this stage. The areola has moved back to the general shape of breast

9 In comparison to other girls your age, how would you describe your development with regard to breast development.

Much earlier	Somewhat earlier	About the same	Somewhat later	Much later

Thank you for your time!

21 June 2004