THE ASSOCIATION BETWEEN LEVELS OF SOCIO-ECONOMIC STATUS AND FIBRIN NETWORK ARCHITECTURE IN WOMEN AGED BETWEEN 35 AND 44 YEARS

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DECLARATION OF INDEPENDENT WORK

I, MARIA ELIZABETH JOUBERT, identity number [redacted] and student number [redacted] do hereby declare that this research project submitted to the Technikon Free State for the degree MAGISTER TECHNOLOGIAE: BIOMEDICAL TECHNOLOGY, is my own independent work; and complies with the Code of Academic Integrity, as well as other relevant policies, procedures, rules and regulations of the Technikon Free State; and has not been submitted before to any institution by myself or any other person in fulfilment of the requirements for the attainment of any qualification.

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April 2003

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SUMMARY

Prospective epidemiological studies from 1980 to 1989 accumulated evidence of a possible relationship between cardiovascular disease and plasma fibrinogen concentration. It was soon evident that raised fibrinogen levels, causing hypercoagulable states, involve complex and multifactorial processes. Consequently it is important to realise that hypercoagulability is associated with other risk factors of cardiovascular disease. This elevates the increasing importance of studying the haemostatic variables together with these risk factors.

It is suspected however, that not only fibrinogen concentration, but also the quality of fibrin networks may contribute to coronary heart disease risk. It is known that other modulating factors in blood also affect the network structures as they are formed with otherwise constant fibrinogen and thrombin concentrations. Previous research extensively studied modulating factors such as albumin, glucose, smoking and diabetes.

Socially patterned accumulation of health capital and cardiovascular risk begins in childhood. In the Whitehall II longitudinal study conducted by Brunner, adult occupational position was inversely associated with fibrinogen, other metabolic risk factors and risk factors like leisure time physical inactivity. Childhood, social position was associated with adult fibrinogen levels.

The objective of this study was to determine the association between fibrin network architecture and socio-economic status in adult women. Three groups of adult women representing different socio-economic backgrounds were chosen randomly to voluntarily participate in the study. The study groups consisted of 27 white women (employees of the TFS), 30 "urbanized" black women (women living in an urbanized area for more than thirty years), and 30 "less urbanized" black women (women living in an urbanized area for less than thirty years).

Fasting blood samples were taken on the premises of the Technikon Free State by a registered nurse and volunteers had medical examinations by a registered general practitioner. Fibrin network architecture variables and plasma fibrinogen were determined on fresh essentially platelet free plasma by standardised laboratory techniques. Other metabolic variables were performed on serum and full blood counts were performed on EDTA whole blood using standardised laboratory techniques.

Results indicated that a association between socio-economic status and haemostatic profiles do exist. Many of the differences in analytical variables however, were expected and due to other relating factors such as ethnicity. The mean fibrinogen level of the white group of women was 3.54±0.24 g/L. The group of black women defined as "less urbanized" displayed lower mean fibrinogen levels (3.16±.0.19 g/L). In contrast, the levels of the "urbanized" black women were much higher (4.04±0.22 g/L). However, these differences were not significant. This confirms the effect of urbanization and thus socio-

economic status on plasma fibrinogen levels. Small differences were observed between network fibrin content and fibrinogen levels, and between mass length ratio and fibrinogen levels in all three the groups. It was unknown if these differences were static or in the process of development and an indication of future tendencies. Except for total protein values no significant differences were found between metabolic variables. This was expected as very strict inclusion/exclusion criteria were used to ensure that all volunteers were "apparently" healthy.

This study in a way contradicts the hypothesis that socio-economic class itself may be the main cause of differences in some metabolic parameters from individuals within different levels of socio-economic backgrounds seeing as such strict exclusion criteria were used. It is believed that the factors related to the different levels of socio-economic status, such as the prevalence of tuberculosis, HIV, diabetes mellitus, hypercholesterolaemia, may play an important role in the outcome of the health status of the individuals within different levels of society. This study implies that the metabolic variables associated with different levels of socio-economic status are not necessarily associated with socio-economic class itself, but rather with the associated factors related to the different levels of socio-economic status. The study group was also very small which might have contributed to the lack of significance between groups. This study emphasises the need for prospective epidemiological trails to evaluate the true effect of socioeconomic variables and associated conditions on metabolic risk factors.

OPSOMMING

Epidemiologiese studies wat gedoen is vanaf 1980 tot 1989 het bewyse versamel van 'n moontlike verwantskap tussen kardiovaskulêre siektes en plasmafibrinogeenkonsentrasie. Dit was gou duidelik dat verhoogde plasmafibrinogeenvlakke, wat hiperstolbaarheids toestande veroorsaak, komplekse en veelvuldige prosesse behels. Dit is dus belangrik om te besef dat hiperstolbaarheid geassosieer kan word met ander risikofaktore van kardiovaskulêre hartsiektes. Dit beklemtoon dan die toenemende belangrikheid van studies wat beide haemostatiese veranderlikes en hierdie risikofaktore behels.

Daar word egter vermoed dat dit nie net die fibrinogeenkonsentrasie is wat tot die risiko vir koronêre hart siektes bydra nie, maar ook die kwaliteit van fibriennetwerke. Dit is wel bekend dat daar moduleerende faktore in bloed is wat 'n invloed kan hê op die netwerk strukture, wanneer hul vorm by andersins konstante fibrinogeen en trombienkonsentrasies. Sommige van hierdie moduleerende faktore is al ekstensief ondersoek, van die faktore sluit in bv. rook, glukose, albumien, diabetes ens.

'n Patroon van sosiale versameling van rykdom, kapitaal en kardiovaskulêre risiko begin reeds as kind al vorm. In die Whitehall II studie onder leiding van Brunner is volwasse beroeps posisie omgekeerd geassosieer met fibrinogeen, ander metaboliese risikofaktore en risikofaktore soos fisiese onaktiewiteit in ontspannings tyd.

Die doel van die studie was om te bepaal of daar 'n verwantskap bestaan tussen die sosio-ekonomiese status en fibrien netwerk argitektuur in volwasse vroue. Die studie populasie het bestaan uit drie groepe volwasse vroue verteenwoordigend van verskillende sosio-ekonomiese agtergronde. Die proefpersone was almal vrywiligers en is lukraak gekies. Die groepe was sewe en twintig blanke vroue (werknemers van die Technikon Vrystaat), dertig "verwesterde" swart vroue (vroue wat al langer as dertig jaar in 'n stedelike gebied woon), en 30 "minder verwesterde" vroue (vroue wat vir minder as dertig jaar in 'n stedelike gebied woon).

Vastende bloed monsters is deur 'n geregistreerde verpleegkundige versamel op die gronde van die Technikon Vrystaat, en 'n geregistreede algemene praktisyn het 'n mediese ondersoek op al die vrywiligers uitgevoer. Fibriennetwerk struktuur veranderlikes en plasmafibrinogeenkonsentrasies is bepaal op vars issensieel plaatjie vrye plasma deur gestandardiseerde laboratorium tegnieke te gebruik. Ander metaboliese veranderlikes is op serum uitgevoer, en volbloedtellings is op EDTA heelbloed uitgevoer met die gebruik van gestandardiseerde laboratorium tegnieke.

Resultate wys dat daar 'n verwantskap bestaan tussen die sosio-ekonomiese status en haemostatiese profiele. Baie van hierdie verskille in die haemostaties analiete was egter te wagte, en as gevolg van ander verwante faktore soos etnisiteit. Die median fibrinogeevlakke van die groep blanke vroue was 3.54 ± 0.24 g/L. Die groep swart vroue beskryf as "minder verwesterd" het laer median fibrinogeenvlakke getoon (3.16 ± 0.19 g/L), in

teenstelling hiermee was die vlakke van die groep "verwesterde" swart vroue baie hoer (4.04 ± 0.22 g/L). Hierdie verskille was egter nie betekenisvol nie, maar dit bevestig die effek van verwestering en dus sosio-ekonomiese status op plasma fibrinogeen vlakke. Klein verskille is waargeneem tussen netwerk fibrien inhoud en fibrinogeen vlakke, en tussen massa-lengte verhouding en fibrinogeen vlakke in al drie die groepe. Dit was nie bekend of die verskille staties was of in die proses van ontwikkeling was as 'n indikasie van toekomstige neigings nie. Al die metaboliese veranderlikes het geen betekenisvolle verskille getoon nie met die uitsondering van totale proteine. Dit was te wagte siende dat baie streng insluitings/uitsluitings kriteria gebruik is om seker te maak dat alle proefpersone "oënskynlik" gesond was.

Die studie weerspreek tot 'n sekere mate die hipotese dat sosio-ekonomiese klas self die hoof oorsaak kan wees vir verskille in sommige metaboliese veranderlikes van individue met verskillende sosio-ekonomiese agtergronde na aanleiding van die steng uitsluitings kriteria wat gebruik is in hierdie studie. Dit word verwag dat faktore verwand aan verskillende vlakke van sosio-ekonomiese status soos die voorkoms van tuberkilose, HIV/VIGS, diabetes mellitus, hipercholesterolemie ens., 'n belangrike rol kan speel in die uitkoms van gesondheidsstatus van die individue in die verskillende vlakke van die gemeenskap. Hierdie studie impliseer dat metaboliese veranderlikes wat geassosieer word met sosio-ekonomiese status nie noodwending met sosio-ekonomiese klas self geassosieer word nie, maar eerder met geassosieerde faktore verwant aan die verskillende vlakke van sosio-ekonomiese status. Die studie groep was ook baie klein gewees wat kon bydra tot die beperkinge in

die interpretasie van die resultate. Die studie benadruk die belangrikheid van voornemende epidemiologiese ondersoeke om die effek van sosio-ekonomiese veranderlikes en geassosieerde toestande op metaboliese risikofaktore te evalueer.

TABLE OF CONTENTS

	PAGES
DECLARATION OF INDEPENDENT WORK	ii
SUMMARY	iii
OPSOMMING	vi
LIST OF TABLES	xiv
LIST OF FIGURES	xvi
LIST OF ABBREVIATIONS	xvii
LIST OF APPENDICES	xx
CHAPTER 1	
INTRODUCTION	1
1.1 Background	1
1.2 Hypothesis	3
1.3 Objectives	4
1.4 Structure of this dissertation	5
CHAPTER 2	
LITERATURE REVIEW	6
2.1 Plasma fibrinogen – Introduction	6
2.2 Synthesis and secretion of fibrinogen	8
2.3 Molecular structure of fibrinogen	10
2.4 Fibrinogen in blood coagulation and fibrinolysis	11
2.4.1 Blood coagulation	11
2.4.2 Fibrinolysis	13
2.5 Fibrinogen as a risk marker for Coronary Heart Disease	15

2.5.1 Hypercholesterolaemia 16
2.5.2 Dislipidaemia 17
2.5.3 Diabetes mellitus 18
2.5.4 Age 18
2.5.5 Hypertension 18
2.5.6 Obesity & Body mass index 19
2.5.7 Smoking 20
2.5.8 Oral contraceptives & Hormone replacement therapy 20
2.5.9 Physical activity 21
2.5.10 Psychological stress and social class 22
2.5.11 Ethnicity 23

CHAPTER 3
METHODS 25

3.1 Study design and workplan 25
3.2 Ethical approval 26
3.3 Subjects 26
3.4 Inclusion/exclusion criteria 27
3.5 Laboratory procedures 28
3.5.1 Sample preparation 28
3.5.1.1 Plasma 28
3.5.1.2 Serum 29
3.5.2 Measurement of biochemical variables 29
3.5.2.1 Compaction of fibrin networks 29
3.5.2.2 Mass length ratio from turbidity (μT) 30
3.5.2.3 Network lysis rate 31

3.5.2.4 Total plasma fibrinogen concentration and network fibrin content 32
3.5.2.5 Female sex hormones 33
3.5.2.6 Other metabolic risk markers 33
3.5.2.7 Full blood counts and HIV status 35
3.6 Other information 35
3.7 Statistical analysis 36

CHAPTER 4
RESULTS 38

4.1 Socio-economic status of the three different subject groups 38
4.2 Descriptive data of the metabolic variables for the three different subject groups 41
4.3 Descriptive data of the haematological variables for the three different subject groups 44
4.4 Correlations between baseline variables 48

CHAPTER 5
DISCUSSION, CONCLUSIONS & RECOMMENDATIONS 55

5.1 Introduction & limitations of this study 55
5.2 Metabolic variables 58
5.3 Haematological variables 61
5.4 Conclusion 63
5.5 Recommendations 64

ACKNOWLEDGEMENTS 65

APPENDIX A 67
BIOCHEMICAL VARIABLE UNITS AND NORMAL RANGES

APPENDIX B 68
QUESTIONNAIRE

APPENDIX C 73
CONSENT FORM (Black women)

APPENDIX D 76
CONSENT FORM (TFS staff)

REFERENCES 79

LIST OF TABLES

Table 2.1	Sex, race and age specific mean plasma fibrinogen levels.	23
Table 3.1	Methods used for the determination of different biomedical variables.	34
Table 4.1	Socio-economic status (level of education and employment status) of the three groups of women.	39
Table 4.2	Age, years spent living in an urban area and percentage lifetime spent in an urban area of the tree groups.	40
Table 4.3	The differences in percentage lifetime spent in an urban area.	40
Table 4.4	Physical and metabolic variables of all three groups of women from different socio-economic backgrounds.	43
Table 4.5	Haematological variables of all three groups of women from different socio-economic backgrounds.	44
Table 4.6	Spearman's correlation coefficients between variables of the group as total.	50
Table 4.7	Spearman's correlation coefficients between variables of the less urban black women.	51

3.5.2.4 Total plasma fibrinogen concentration and network fibrin content 32
3.5.2.5 Female sex hormones 33
3.5.2.6 Other metabolic risk markers 33
3.5.2.7 Full blood counts and HIV status 35
3.6 Other information 35
3.7 Statistical analysis 36

CHAPTER 4
RESULTS 38

4.1 Socio-economic status of the three different subject groups 38
4.2 Descriptive data of the metabolic variables for the three different subject groups 41
4.3 Descriptive data of the haematological variables for the three different subject groups 44
4.4 Correlations between baseline variables 48

CHAPTER 5
DISCUSSION, CONCLUSIONS & RECOMMENDATIONS 55

5.1 Introduction & limitations of this study 55
5.2 Metabolic variables 58
5.3 Haematological variables 61
5.4 Conclusion 63
5.5 Recommendations 64

ACKNOWLEDGEMENTS 65

APPENDIX A 67
BIOCHEMICAL VARIABLE UNITS AND NORMAL RANGES

APPENDIX B 68
QUESTIONNAIRE

APPENDIX C 73
CONSENT FORM (Black women)

APPENDIX D 76
CONSENT FORM (TFS staff)

REFERENCES 79

LIST OF TABLES

Table 2.1	Sex, race and age specific mean plasma fibrinogen levels.	23
Table 3.1	Methods used for the determination of different biomedical variables.	34
Table 4.1	Socio-economic status (level of education and employment status) of the three groups of women.	39
Table 4.2	Age, years spent living in an urban area and percentage lifetime spent in an urban area of the tree groups.	40
Table 4.3	The differences in percentage lifetime spent in an urban area.	40
Table 4.4	Physical and metabolic variables of all three groups of women from different socio-economic backgrounds.	43
Table 4.5	Haematological variables of all three groups of women from different socio-economic backgrounds.	44
Table 4.6	Spearman's correlation coefficients between variables of the group as total.	50
Table 4.7	Spearman's correlation coefficients between variables of the less urban black women.	51

Table 4.8 Spearman's correlation coefficients between variables of the urbanized black women. 52

Table 4.9 Spearman's correlation coefficients between variables of the white women. 53

LIST OF FIGURES

Figure 2.1	A molecular model of the Fibrinogen molecule.	9
Figure 2.2	Blood coagulation and fibrinolysis.	12
Figure 2.3	Summary of potential mechanisms by which increasing plasma fibrinogen levels may promote arterial disease and ischaemic events.	15
Figure 4.1	Lysis rates of fibrin networks of the three different groups of socio-economic status.	46

LIST OF ABBREVIATIONS

A	Alpha
β	Beta
γ	Gamma
%	Percentage
>	Larger
<	Smaller
±	Plus-minus
°C	Degree of Celsius
™	Trademark
®	Registered
µL	Microliter
µT	Mass length ratio
ALB	Albumin
BMI	Body mass index
CHD	Coronary heart disease
cm	Centimetre
CFN	Compaction of fibrin network
Da	Dalton
DIC	Disseminated intravascular coagulation
ESR	Erythrocyte sedimentation rate
E_2	Estradiol
FSH	Follicle stimulating hormone

Fb	Fibrinogen
g/L	Gram per liter
GGT	Gamma glutamyl transferase
g	Gram
g	Gravities
GLC	Glucose
g/dL	Gram per decilitre
HSF	Hepatocyte stimulating factors
HDL	High density lipoprotein
HIV	Human immune virus
Hb	Haemoglobin
ICD	Ischaemic heart disease
IDDM	Insulin dependant diabetes mellitus
IU/L	International units per liter
IU/mL	International units per milliliter
kg	Kilogram
kg/m^2	Kilogram per square meter
KIU/mL	Kilo-International units per milliliter
LDL	Low density lipoproteins
LH	Luteinizing hormone
mmol/L	Millimol per liter
mL	Milliliter
M	Molar
mM	Millimolar
nm	Nanometer

NaOH	Sodium hydroxide
NFC	Network fibrin content
nmol/L	Nanomol per liter
Prog	Progesterone
pmol/L	Picomol per liter
Q_1	Quartile one
Q_3	Quartile three
rpm	Revolutions per minute
RB	Rural black
RBC	Red blood cells
SD	Standard deviation
t-PA	Tissue-plasminogen activator
TP	Total protein
TC	Total cholesterol
TG	Triglycerides
UB	Urbanized black
VLDL	Very low density lipoprotein
X	Mean

Central University of Technology, Free State

LIST OF APPENDICES

APPENDIX A	Biomedical variable units and normal ranges
APPENDIX B	Questionnaire
APPENDIX C	Consent form (black women)
APPENDIX D	Consent form (Technikon Free State staff)

CHAPTER 1

INTRODUCTION

1.1 Background

Raised plasma fibrinogen is accepted as an important risk factor for coronary heart disease (CHD), atherosclerosis, and stroke (Kannel **et al.**, 1987; Meade **et al.**,1986; Stone & Thorpe, 1985; Wilhelmsen **et al.**, 1984; Yarnell **et al.**, 1991).

It is suspected, however, that not only fibrinogen concentration, but also the quality of fibrin networks may contribute to CHD risk (Blombäck **et al.**, 1992). It has also been shown that fibrin network structure can be directly affected by the fibrinogen concentration (Nair **et al.**, 1991a).

Knowledge of the structure of fibrinogen and of the properties of the fibrin polymers that are formed on activation of fibrinogen by thrombin or other enzymes has expanded considerably during the past decade. Evaluation of fibrin network properties has become easy and accessible in almost any standard laboratory. Methods based on turbidity and permeability have

become available for measurement of fibrin network structure properties (Nair **et al.,** 1991a).

Altered fibrin network structures are believed to play an important role in the development of CHD and thrombosis (Blombäck **et al.**, 1992). When examining cardiovascular risk, it is important to remember that fibrin concentration is an important risk factor, but there are many other aspects to consider, such as the complex relationship between fibrinogen and other risk factors producing altered network structures (Blombäck **et al.**, 1992). When thrombin and fibrinogen interact to generate fibrin monomers, the concentrations of both fibrinogen and thrombin play an important role in the fibrin network structure (Blombäck **et al.**, 1990). It was also found that clotting time is directly related to the gel structures of fibrinogen, thus events preceeding gel formation determine the gel structure (Blomäck **et al.**, 1982).

In a purified fibrinogen-thrombin system, under static condition, the gel structure is mainly determined by kinetic factors, i.e. the thrombin (Hantgan & Hermans, 1979) and fibrinogen concentrations (Donovan & Mihalyi, 1985; Hortin & Trimpe, 1991). Together these factors create the clotting potential. There are other factors that at the same clotting potential can modulate the network without perturbation of the general architecture. These modulating factors change the architecture of the network by either interacting with the water in the medium during fibrin formation or by binding to either fibrinogen

or to the fibrin strands in the established network. Some of these modulating factors have been studied extensively in the past such as fibrinogen composition (Fatah **et al.**, 1992), albumin (Galanakis **et al.**, 1987), cations, pH and temperature (Nair **et al.**, 1986), blood platelets (Van Gelder **et al.**, 1993), plasma antithrombin-III (AT) (Elgue **et al.**, 1994), dextran (Carr & Gabriel, 1980), glucose and antidiabetic drugs (Azhar **et al.**, 1990), fibronectin (Nair **et al**, 1991a) and social class (Markowe **et al,**. 1985). These modulating factors have remarkable effects on fibrinogen concentration in humans. This indicates directly that an associated effect on fibrin network architecture is evident (as described earlier, fibrinogen is a kinetic regulator of fibrin network architecture).

A variety of studies have indicated a definite relationship between plasma fibrinogen concentration and fibrin network architecture in human subjects. It has been shown that socio-economic status may have a direct effect on fibrinogen concentration, and associated cardiovascular risk. Little is known, however, about the relationship between fibrin gel properties and socio-economic status.

1.2 Hypothesis

The hypothesis tested in this study states that:

- H_1 socio-economic status will have a direct effect on fibrin network architecture in female human subjects.
- H_0 socio-economic status will not have a direct effect on fibrin network architecture in female human subjects.

1.3 Objectives

The main objective

The main objective of this study was to investigate the association between socio-economic status and fibrin network architecture in female subjects aged between 35 and 44 years of age.

The sub-objectives

The sub-objectives of the study were:

- to identify population groups with different socio-economic status
- to determine the prevalence of some cardiovascular risk factors, including fibrin network architecture variables in the defined population groups.

1.4 Structure of this dissertation

The structure of this thesis is as follows:

Chapter 2 is an extensive literature survey in which the most critical information needed to understand and interpret the hypothesis and results of this study is discussed. Chapter 3 provides detailed information about specimen preparation and all test procedures used in the study. The results for all tests are given in Chapter 4. Finally, in Chapter 5 the results, conclusion and recommendations made from this study are discussed.

CHAPTER 2

LITERATURE REVIEW

2.1 Plasma fibrinogen - Introduction

Over the last 20 years, an increasing amount of evidence has been published demonstrating that the plasma fibrinogen level has a major impact on arterial disease of the heart, brain, and limbs. This evidence comes from epidemiological, pathophysiological and clinical studies. These studies include the Northwick Park Heart Study (Meade **et al.**, 1986), the Göteborg Study (Wilhelmsen **et al.**, 1984), and the Framingham Study (Kannel **et al.**, 1987).

Fibrinogen is a high molecular weight glycoprotein (340,000 Da) which circulates in plasma at concentrations between 1.5 and 4.5 g/l. It is absent from serum, which is defibrinated plasma. Plasma fibrinogen has a half-life of approximately 100 hours. It's routes of catabolism are poorly defined, and known fibrinolytic pathways contribute little to its removal from the circulation. Fibrinogen crosses vascular endothelium both into the arterial wall and, through the microcirculation, into the tissues in inflammatory states, in which fibrin participates.

Traditionally, clinical interest in fibrinogen has been from haematologists dealing with bleeding disorders. Soluble fibrinogen is the precursor of insoluble fibrin, which is an important component of the haemostatic plug that develops after vascular injury. The minimum plasma fibrinogen level for haemostasis is 0.5 - 1.0 g/l. Low fibrinogen levels are associated with excessive bleeding after injury or surgery, in both rare congenital afibrinogenaemias and disfibrinogenaemias, and, more commonly, severe acute or chronic liver diseases due to reduced hepatic synthesis. Transient low fibrinogen levels may be associated with excessive bleeding in overt disseminated intravascular coagulation (DIC), primary pathological hyperfibrinolysis, and thrombolytic and defibrinating enzyme therapy (Lowe, 1993).

Fibrinogen is involved in acute-phase protein reactions to injury, surgery, acute infections and infarctions. Within a few hours of these events, hepatic synthesis of fibrinogen and other acute-phase proteins increase, probably due to hepatocyte stimulation by fibrin degradation products and/or activated monocytes (which produce cytokines such as interleukin-6) from damaged tissues. Such protein reactions presumably play a role in haemostasis and in inflammation after tissue injury (Dippel, 1992). Plasma fibrinogen levels usually peak at 2-4 times basal levels after 3-5 days, then gradually return to basal levels with resolution of inflammation. Plasma fibrinogen levels are chronically elevated in chronic infections, in

chronic inflammatory diseases such as rheumatoid arthritits, and in malignant diseases. Since it is a large, elongated molecule, increases in fibrinogen result in elevated plasma viscosity and increased red blood cell aggregation (e.g. an elevated erythrocyte sedimentation rate, ESR). Both plasma viscosity and the ESR are used to monitor plasma protein reactions as measures of disease activity (Lowe, 1993).

2.2 Synthesis and secretion of fibrinogen

Human fibrinogen (Figure 2.1) is a dimetric multichain protein. Each identical half molecule is composed of three distinct polypeptides (Aα, Bβ, and γ), synthesised primarily in the liver (Roy **et al.**, 1992). The two half molecules are joined together by symmetrical disulfide bonds between two Aα and two γ chains (Cook & Ubben, 1990). In addition, fibrinogen has a number of inter- and intrachain disulfide bonds. There are 29 in all, with no free cysteine residues (Roy **et al.**, 1992). Each fibrinogen chain is encoded by a separate gene, and synthesised in the granular endoplasmatic reticulum (Hartwig & Danishefsky, 1991).

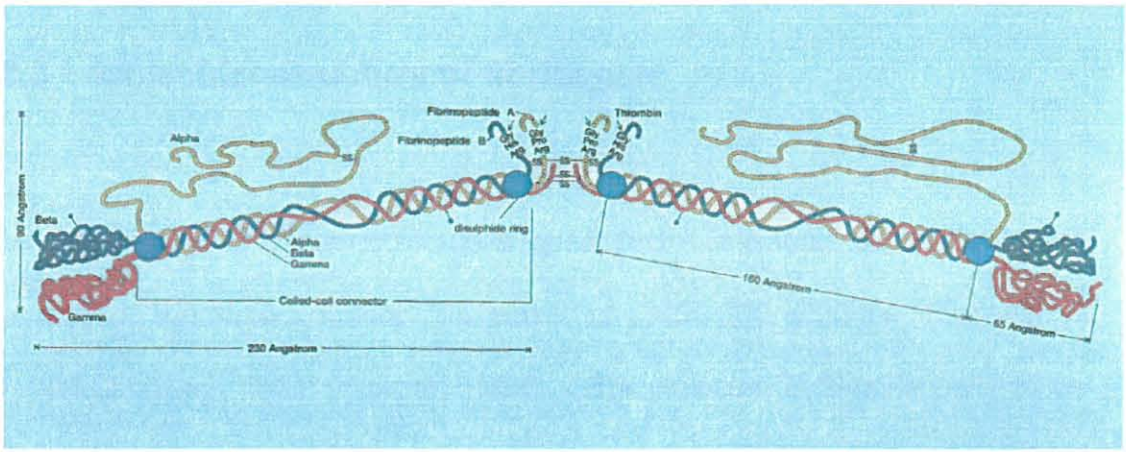

Figure 2.1 A molecular model of the Fibrinogen Molecule, as adopted from Doolittle (1981)

Hepatocytes are the principle site of synthesis of fibrinogen, and are controlled by hepatocyte stimulating factors (HSF) secreted by activated monocytes. Monocytes are activated by the products of fibrinolysis to produce cytokines (Wang & Fuller, 1991). At least three cytokines, interleukin-6, leukemia inhibitory factor, and oncostatin M contribute to HSF activity (Rose & Bruce, 1991). Both the γ and the Aα chain are stored in cells (Roy **et al.**, 1992) and released together by the Golgi cells when needed (Danishefsky **et al.**, 1990). Bβ chains of the fibrinogen molecule cannot be stored, and are immediately used after translation (Yu **et al.**, 1984).

2.3 Molecular structure of fibrinogen

Models of the fibrinogen molecule suggest that it is a long, slightly bent and coiled trinodular dimeric molecule of 340 000 Daltons (Hartwig & Danishefsky, 1991 ; Torbet, 1986). The molecule is simplistically to be perceived as a cylinder of 7 x 48 nm. The amino-termini of the six subunits form the E domain. This domain is central to the COOH-termini, which forms the two D domains (Dang & Bell, 1989). The E domain is linked to the two D domains by a coiled-coil triple helix structure of 111 amino acids (Huang **et al.**, 1993). Disulfide rings border each of the coiled-coil regions of the molecule and link the tree chains of the half-molecule (Blombäck **et al.**, 1968). Disulfide bonds at the amino-terminal region of the α and γ chains hold the two αβγ half-molecules together (Hoeprich & Doolittle, 1983). Disruption of these disulfide rings leads to the formation and secretion of two half-molecules (Zhang & Redman, 1994). Additional to this, another 29 disulfide bonds functional within the chains, exist in the molecule (Roy **et al.**, 1992). Disruption of these interchain disulfide bonds at the COOH-terminal end of the coiled-coil region allows dimer formation, but the 6-chain molecule which is assembled is not secreted (Zhang & Redman, 1994).

2.4 Fibrinogen in blood coagulation and fibrinolysis

2.4.1 Blood coagulation

The conversion of fibrinogen to fibrin by protease thrombin is the final step in the coagulation process. Coagulation can be regarded as a cascade-like amplifying reaction, in which most coagulation factors (factor xii, x, ix, vii and ii) have an enzymatic function in their activated form. Fibrinogen serves as a substrate in this process (Figure 2.2).

The plasma protein fibrinogen is converted to the insoluble fibrin matrix of blood clots by a multi-step process (Roy & Hermans, 1979). The fibrinogen molecule comprises of 2 Aα-, 2 Bβ-, and 2 γ-polypeptide chains (Roy **et al.**, 1992). It undergoes limited proteolysis and aggregation. Fibrin assembly is a highly ordered process which is initiated by thrombin-catalyzed hydrolysis at Arg-A16 to form fibrin I monomer and releases a 16 aminoacyl residue peptide (fibrinopeptide A) from the N-terminus of each Aα-chain (Lewis **et al.**, 1985). This process is followed by the end-to-end aggregation of the fibrin I monomers, resulting in the formation of fibrin I oligomers (protofibrils). This step causes lengthening of the fibrin strands. The fibrin protofibrils associate laterally (Roy **et al.**, 1979) and is then followed by thrombin-mediated hydrolysis at Arg-Bβ14 to form fibrin II and

release a 14-aminoacyl residue peptide (fibrinopeptide B) from the amino terminus of each of the Bβ-chains (Lewis **et al.**, 1985). This step causes thickening of the fibrin strands. The resulting fibrin II protofibrils have a greater propensity than fibrin I protofibrils to undergo lateral interaction which will result in fiber formation of the fibrin network.

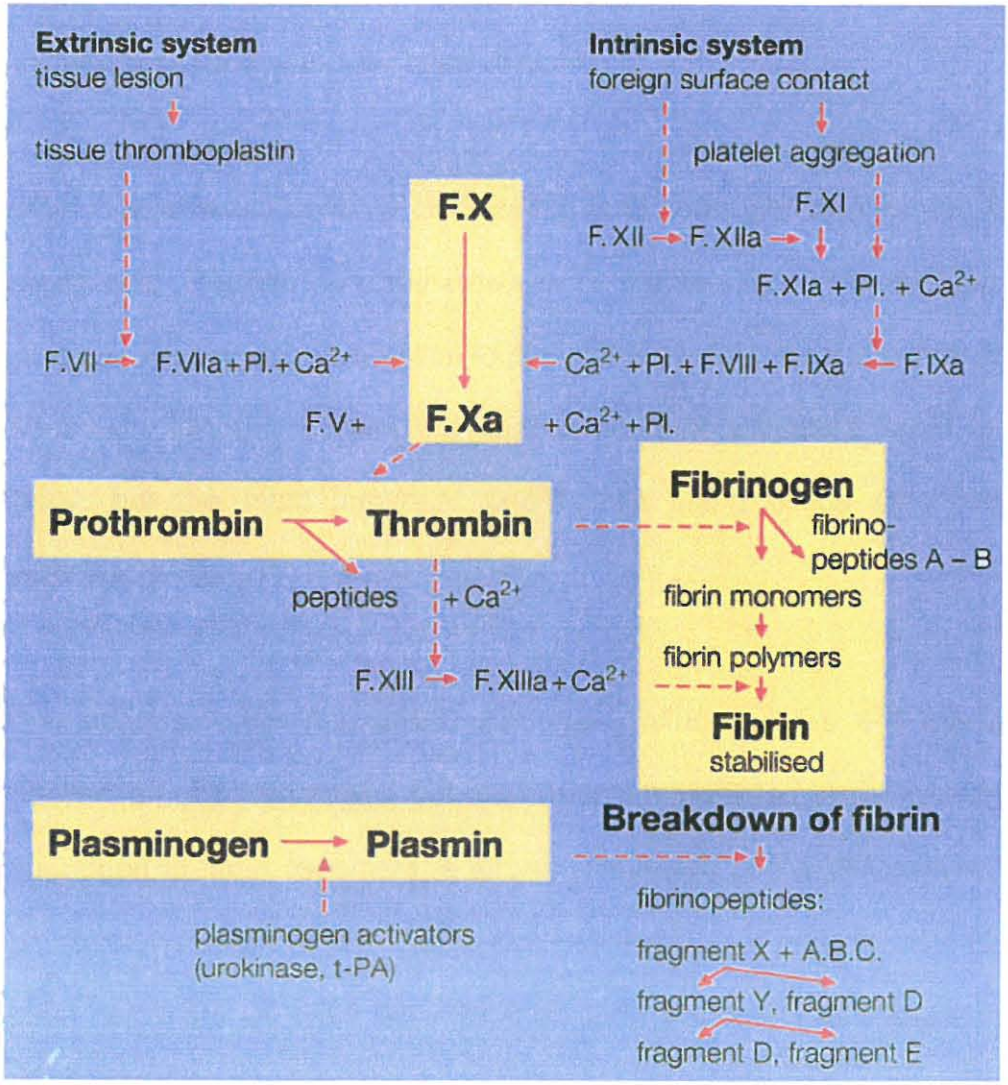

Figure 2.2 Blood coagulation and fibrinolysis (Dippel, 1992)

2.4.2 Fibrinolysis

Fibrinolysis, a specific process leading to solubilisation of fibrin formed, plays a key part in the natural antithrombotic mechanism (Gandrille & Aiach, 1990). The blood clotting system, both anabolic and catabolic, has been a subject of great interest for some years, and more so now with the higher incidence of cardiovascular heart disease.

Plasmin is a protease capable of digesting both fibrinogen and formed fibrin structures. Plasmin normally exists in its inactive form, plasminogen, in plasma. Plasminogen activation to plasmin is the first important step in the fibrinolytic process. There are various types of activators found in the body, but the most important one known being tissue plasminogen activator (tPA).

Neither fibrinogen nor monomeric fibrinogen influences the tPA mediated conversion of plasminogen, but after thrombin mediated transformation of fibrinogen to fibrin oligomers, a strong stimulation of the tPA activity can be observed (Grøn **et al.**, 1992). Consequently, on a mole to mole basis, fibrin oligomers are more powerful than fibrin monomers as stimulators of tPA activity (Grøn **et al.**, 1992).

Polymeric fibrin is a strong enhancer of the activation of plasminogen by tPA. At least two types of sites are involved in this enhancement, i.e. a site within Aα-(148-160), and a site within γ-(311-379). These sites are not accessible in fibrinogen but are exposed upon conversion of fibrinogen to fibrin (Nieuwenhuizen, 1994). The adsorption of tPA and plasminogen on the fibrin fiber surface allows the necessary contact needed to affect optimal activation rates for plasmin production and spatial proximity of the fibrin substrate. Thus the fibrin plays a dual role, not only does it enhance tPA activation, but it also acts as a substrate for plasmin (Gabriel **et al.**, 1992).

According to Gabriel **et al.** (1992) the fibrin structure also contributes to the regulation of the fibrinolytic rate. This explains the influence that albumin has on fibrinolysis seen as it also modifies clot structure, and facilitate further plasminogen uptake by preformed clots (Grandrille & Aiach, 1990). Inhibition of the firinolytic system can be accomplished by the actions of α -antiplasmin-2, plasminogen activator inhibitors and the fibrinogen molecule.

2.5 Fibrinogen as a risk marker for CHD

It is evident that raised fibrinogen levels, causing hypercoagulable states, involve complex and multi-factorial processes (Figure 2.3). According to Tarallo **et al.** (1992) the known risk factors for cardiovascular disease explain nearly 15% of the total variance in fibrinogen concentration.

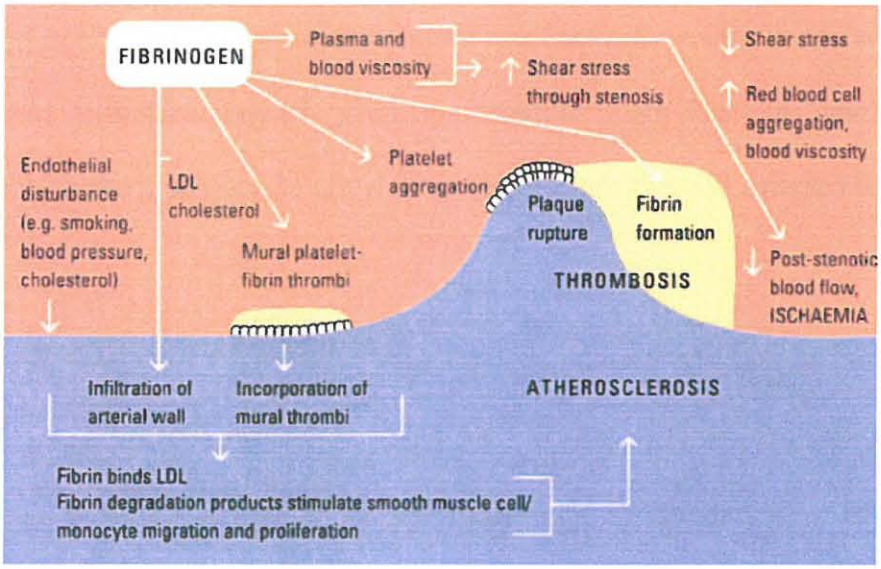

Figure 2.3 Summary of potential mechanisms by which increasing plasma fibrinogen levels may promote arterial disease and ischaemic events. LDL = low density lipoprotein (Lowe, 1993)

During the years 1980-1989, results were reported from four prospective epidemiological studies of fibrinogen as a possible risk factor for cardiovascular disease. These studies included the Göteburg Study (Wilhelmsen **et al.**, 1984), the Northwick Park Heart Study (Meade **et al.**, 1986), the Framingham Study (Kannel **et al.**, 1987) and the Leigh Clinical

Research Unit Study (Stone & Thorpe, 1985). The association between fibrinogen level and cardiovascular disease was present in all four of these studies. On the basis of their results, all the authors of these studies concluded that a high fibrinogen level was an independent risk factor for cardiovascular disease, and that fibrinogen plays an especially important role in the thrombotic processes in the pathogenesis of cardiovascular disease.

Fibrinogen is accepted as an independent risk factor for cardiovascular disease. It would therefore be of great importance, from both a scientific and preventive point of view, to know what factors influence fibrinogen levels.

2.5.1 Hypercholesterolaemia

A study on groups of men of similar mean age in communities of very low (Gambia), high (England and Czechoslovakia) and very high (Scotland and Finland) risk of ischaemic heart disease (IHD), showed a consistent gradient of higher cholesterol levels associated with higher fibrinogen levels. Consequently this association indicates that subjects with high cholesterol levels could even be more prone to development of IHD if high fibrinogen levels are present as well (Meade **et al.**, 1986).

2.5.2 Dislipidaemia

In a study performed on healthy men it was shown that elevated fibrinogen concentrations of >2.90 g/L were associated with increased levels of circulating small, dense LDL particles and reduced HDL cholesterol. Serum triglycerides and concentrations of triglyceride-rich lipoprotein particles (VLDL) were also higher (Halle **et al.**, 1996). This was also the conclusion of Simpson in a study on 18 patients with severe hypertriglyceridaemia, in which they also found that hyperlipidaemic patients had significantly lower fibrinolytic activity (Simpson **et al.**, 1983).

When comparing two groups of Africans it was found that the group who had a higher fat and fiber intake as well as a lower cholesterol intake, had a higher fibrinogen level than the group with a lower fat intake (Vorster **et al.**, 1997). Results from the THUSA study in South Africa showed that a small, but significant, positive correlation exists between serum cholesterol, LDL cholesterol and triglycerides (in women) with elevated plasma fibrinogen (James **et al.**, 2000).

2.5.3 Diabetes mellitus

The plasma fibrinogen levels are frequently elevated in diabetes mellitus and particularly in patients with vascular complications and impaired metabolic control (Ganda **et al.**, 1992). Altered properties of fibrin gel structure in patients with insulin dependant diabetes mellitus (IDDM) have also been demonstrated despite normal fibrinogen levels (Jörneskog **et al.**, 1996 and Nair **et al.**, 1991b).

2.5.4 Age

The Framingham Study showed a diminishing impact of CHD with advancing age only in women. It was also found that the risk of CHD increased progressively with fibrinogen levels in both sexes below the age of 60 years (Kannel **et al.**, 1987). Many other studies have also reported that fibrinogen levels increase with age (Armani **et al.**, 1992 & Avellone **et al.**, 1991).

2.5.5 Hypertension

Five decades of epidemiologic research have established that blood pressure elevation is a common and powerful contributor to all of the major

cardiovascular diseases, including coronary disease and stroke. Studies on the risk stratification in hypertension as part of new insights on the Framingham Study, showed elevated heart rate, left ventricular hypertrophy and an elevated fibrinogen concentration as factors that often accompany hypertension (Kannel, 2000). A statistically significant difference between fibrinogen levels was also found in a study performed on a control group of healthy women and pregnant women with severe chronic hypertension (Kendi **et al.**, 2000).

2.5.6 Obesity & body mass index

A study performed on obese women in South Africa who took part in a weight reduction program over eight weeks showed large fluctuations in plasma fibrinogen, which increased during the phases of substantial weight loss. The trend however, was for fibrinogen concentration to decrease over the eight weeks with a mean weight loss of 9.2 ± 2.9 kg (Vorster **et al.**, 1989). It was also found that the lifetime health and economic consequences of obesity are substantial and suggested that efforts to prevent or reduce this problem might yield significant benefits (Thompson **et al.**, 1999).

In obese patients (BMI > 30kg/m^2) the plasma viscosity and plasma fibrinogen levels are significantly increased compared to healthy (BMI < 25kg/m^2)

subjects (Lip 1995). In the THUSA study done on Africans in South Africa BMI showed a significant positive correlation with plasma fibrinogen levels (James **et al.**, 2000). Folsom **et al** (1991) developed two models to predict changes in fibrinogen for a 5 kg/m^2 increase in BMI. The influence of BMI on plasma fibrinogen level was greater in women than in men, with the first model showing a 1.23g/L increase and the second showing a 0.98g/L increase.

2.5.7 Smoking

Several studies concluded that elevated fibrinogen levels probably explained a considerable part of the causal association between smoking and cardiovascular disease (Meade **et al.**, 1987 & Kannel **et al.**, 1987). An association between smoking and fibrinogen levels was also described by Møller & Kristensen (1991).

2.5.8 Oral contraceptives & hormone replacement therapy

Women tend to have higher fibrinogen levels than men, especially after the menopause or with the use of oral contraceptive drugs. It seems that oral contraceptives with high oestrogen content have the strongest effect. Fibrinogen returns to normal within three months upon discontinuation of the

drugs (Ernst, 1991). In contrast to this, it was concluded that hormone replacement treatment with 17 beta-oestradiol for nine weeks significantly increased fibrinolytic potential in postmenopausal women with mild dyslipidaemia. This suggests that oestrogen may also have a cardioprotective effect that might be mediated in part by an increase in fibrinolytic potential (Gebara **et al.**, 1998). In the CARDIA study the use of oral contraceptives by women was associated with higher fibrinogen levels (Folsom **et al.**, 1993). However, the opposite was found to be true for the SHHS study (Lip 1995). Pregnancy is also often associated with a rise in plasma fibrin levels, but this is also accompanied by a compensatory rise in fibrinolytic potential (Yin **et al.**, 1998).

2.5.9 Physical activity

Fibrinogen levels increase with age. In a study performed on young (24-30 years) and old (60-82 years) men who followed an intensive six month endurance exercise program, a reduction in fibrinogen levels by 13% was demonstrated in the older group (Stratton **et al.**, 1991). A significant univariat and multivariat association between physical inactivity and fibrinogen levels was also described by Møller & Kristensen, in 1991. This association was also confirmed by Haddock **et al.** in 1998, when they concluded that cardiorespiratory fitness was an important independent

determinant of blood lipid and fibrinogen levels in non-smoking postmenopausal women, independent of the use of hormone replacement therapy. Therefore it seems that all studies which measured the relationship between long-term physical activity and plasma fibrinogen, found that physical fitness is associated with lower levels of the protein in blood. However, less is known about the acute effect of physical activity on plasma fibrinogen levels.

2.5.10 Psychological stress and social class

In a study performed on Danish men, it was found that fibrinogen levels were higher in men from low social class and among men living alone (Møller & Kristensen, 1991). Brunner concluded that socially patterned accumulation of health capital and cardiovascular risk begins in childhood and continues, according to socio-economic position, during adulthood. In this study, adult occupational position was inversely associated with fibrinogen, other metabolic risk factors and other risk factors such as smoking and leisure time physical inactivity. Childhood social position was associated with adult weight in both sexes and with current smoking and fibrinogen concentrations in women (Brunner **et al.**, 1999). The cause of higher plasma fibrinogen levels in lower socio-economic classes is not clear. It could be related to

environmental factors such as nutrition, smoking, alcohol and drug abuse and also the prevalence of chronic lower grade infections.

2.5.11 Ethnicity

There seems to be a co-existing variation between plasma fibrinogen levels and the prevalence of ischaemic heart disease across populations. It is known that fibrinogen levels are lowest among Asians, intermediate among English, Gzechs and North American whites, and highest among Finns, Scots, African Americans, Gambians and some American Indian tribes (Folsom 1995). In the Bogalusa Heart Study plasma fibrinogen levels did not differ essentially between Blacks and Caucasians or males and females, although an increase in levels with age or sexual maturation was noted in black females (Bao **et al.**, 1993). The fact that plasma fibrinogen levels are higher in post menopausal women and thus older women, is a well know fact, but in this case the authors also suggested a higher level in black females.

Table 2.1 Sex, race and age-specific mean plasma fibrinogen levels (mg/dL) and percentage of participants with fibrinogen levels > 350 mg/dL

Age	Men						Women					
	Whites (n=1.042)			Blacks (n=891)			Whites (n=1.123)			Blacks (n=1.137)		
	Mean	SD	%>350	Mean	SD	%>350	Mean	SD	%>350	Mean	SD	%>350
<30	238	44	2	246	48	4	263	52	5	287	59	13
>30	244	50	3	254	49	3	267	58	8	295	63	14

Table 2.1 shows data collected from the CARDIA study from 1990-1991. This data shows that the mean fibrinogen levels were higher in the older age group, in women rather than in men and in blacks rather than in whites (Folsom **et al.**, 1993).

CHAPTER 3

METHODS

3.1 Study design and workplan

This study used a randomized comparative design which formed part of a larger study (Nutritional Health of Women Stydy) performed by Dr C Walsh. All laboratory measurements and procedures were performed within the Research Laboratory of the Fibrinogen Unit, School of Health Technology, Technikon Free State, as well as the Laboratory of Drs Voigt, le Roux and Partners, Bloemfontein.

The contribution of the researcher included the following:

- The design and implementation of the questionnaire (Appendix B)
- Assistance in the obtaining of blood samples
- All laboratory investigations

For the purpose of this study the level of socio-economic status were linked to "urbanization", which were defined by the percentage lifetime spent living in an urban area and the years spent living in an urban area.

3.2 Ethical approval

Ethics Committee approval for the Nutritional Health of Women Study (Dr C. Walsh, Technikon Free State) was obtained from the University of the Free State (Etovs no. 02/00). Ethics Committee approval was also obtained for this particular study from the University of the Free State (Etovs no. 45/00).

3.3 Subjects

3 groups of volunteers from different socio-economic backgrounds were randomly chosen to participate in this study. The white women were all employees of the Technikon Free State and the black women came from the black suburbs in and around Bloemfontein.

- 30 "less urbanized" black women from the Free State area. This group was obtained from the Nutritional Health of Women Study performed by Dr C Walsh, School of Tourism, Hospitality and Sport, Faculty of Management, Technikon Free State. "Less urbanized" was defined as those subjects living in an urbanized setting for less than thirty years.

- 30 "urbanized" black women from the Nutritional Health of Women Study performed by Dr C Walsh, School for Tourism, Hospitality and Sport, Faculty of Management, Technikon Free State. "Urbanized" was defined as those subjects living in an urbanized setting for longer than thirty years.

- 27 urbanized white women obtained from the personnel corps of the Technikon Free State (TFS). The group consisted of only 27 subjects because all the subjects were volunteers from the TFS.

3.4 Inclusion/exclusion criteria

The inclusion criteria for the subjects were as follows:

- Women, aged 35-44.
- No history of any cardiovascular events, chronic disease states, familial abnormalities or any other physical abnormalities.
- Subjects should be non-smokers.
- Subjects should not be alcohol dependent. Liver enzymes were determined for confirmation (GGT assays).
- No medication (including oral contraceptives) may be used during the project, unless prescribed by a general practitioner.

- Subjects should not be glucose intolerant (fasting glucose levels < 6.0 mmol/L).
- Subjects should be HIV negative.
- Subjects should all be pre-menopausal and within day 21 - 30 of their menstrual cycle.

3.5 Laboratory procedures

Fasting blood samples were taken by a registered nurse from all subjects. Fibrin network architecture variables, serum glucose, hormone profiles (female sex hormones), HIV status and some other metabolic risk markers were measured on fresh blood samples using the described techniques.

3.5.1 Sample preparation

3.5.1.1 Plasma

For the preparation of essentially platelet-free plasma, 10 mL citrated blood (1 volume of 3.8 % tri-sodium citrate [Saarchem, South Africa, Cat. no. 582 25 00] in a 0.1 M sodium phosphate buffer, pH 7.4, with 35 µL of 10.000 KIU/mL Trasylol®/aprotinin per 9 volumes of whole blood) was centrifuged twice, for 10 minutes at 8000 g. Citrate acts as an inhibitor of early

activation of factor V and VIII. Aprotinin (Trasylol®, Bayer-Miles, Germany, Cat. no. H2912) acts as an inhibitor of fibrino(geno)lysis.

Determinations involving fibrin network structure properties were performed on fresh plasma samples. All remaining plasma was stored at -72 °C in Eppendorf® vials.

3.5.1.2 Serum

5 mL of whole blood was left to clot at room temperature. These samples were centrifuged at 3660 rpm for 10 minutes in order for the serum to separate. Samples were frozen and stored at -72 °C in Eppendorf® vials.

3.5.2 Measurement of biochemical variables

3.5.2.1 Compaction of fibrin networks

Compaction was measured in duplicate using the method described by Dhall **et al**. (1976). The compaction technique describes the tensile strength of fibrin fibers. The inverse correlation between compaction and Young's modulus, and between compaction and strength at break, indicates that the simple method of compaction depends on the number and strength of

the primary crosslinks and branch points in the network (Nair **et al.**, 1991a). 0.9 mL fresh plasma was pipetted into 1.5 mL Eppendorf® vials, pre-sprayed with lecithin-based aerosol (Spray-&-Cook)® to render the surface non-adhering. The plasma was clotted by introduction of 100 µL Thrombin Reagent (ICN, USA, Cat. no. 101141); 1 IU/mL Thrombin final concentration, 25 mM Ca^{2+} final concentration). Samples were left overnight for maximum polymerisation. After centrifugation at 8000 g for 45 seconds the volume of expelled sample from the fibrin networks was determined, and expressed as a percentage of the initial volume. The C.V. for this method was 2.3%.

3.5.2.2 Mass length ratio from turbidity (µT)

Mass length ratio from turbidity (µT) was determined (n=2 for each sample) using the method as described by Nair **et al.** (1991a). 0.9 mL of fresh platelet-free plasma was pipetted into micro-cuvettes of 1 cm path length. The plasma was mixed and clotted by introduction of 100 µL Thrombin Reagent (ICN, USA, Cat no. 101141); 1 IU/mL Thrombin final concentration, 25 mM Ca^{2+} final concentration).

The intercept, A, in plots of c/T(wavelength)3 as a function of 1/(wavelength)2 was used to calculate µT (mass length ratio) according to the equation :

$$\mu T = [10/1.48xA] \times 1012 \text{ daltons/cm} \quad (1)$$

Turbidity (optical density) was measured at a range of wavelengths between 600 and 800 nm (Shimadzu, UV-1201, Japan). The C.V. for this method was 5%.

3.5.2.3 Network lysis rate

The network lysis rate was measured in duplicate using a method developed in our own laboratory. 90 µl of plasma was mixed and clotted by the introduction of 10 µl Thrombin reagent (ICN, USA, Cat no. 101141; 1 IU/ml Thrombin final concentration, 25 mM calcium final concentration) in microtiter plates. After total polymerisation took place 50 µL of Streptokinase (ICN, USA, Cat no. 101114) with a final concentration of 100 U/ml was introduced to start lysis of networks. The lysis rate was measured with an EL 312e from Biotek instruments at 608 nm for 6 hours using 10 minute intervals. The lysis rate was determined by plotting the time versus change in absorbance. The C.V. for this method was 4.2%.

3.5.2.4 Total plasma fibrinogen concentration and network fibrin content

The method of Ratnoff & Menzies (1951) was used for duplicate determination of the fibrin content of fibrin networks for all fibrin network structure determinations.

The Ratnoff & Menzies method : 0.9 mL plasma for network fibrin content in test tubes filled with 1g glass beads (Saarchem, South Africa, cat. no. 267 02 50) was clotted under identical conditions as described for mass length ratio determinations. Samples were left overnight for total polymerisation to take place. All samples were centrifuged at 1300 x g. The isolated networks were washed three times with saline solution. 1 mL 2.5 M NaOH (Saarchem, South Africa, cat. no. 582 31 80) was dispensed into each test tube, the networks dissolved by heating at 95°C for 15 minutes. The samples were left to cool at room temperature. 7 mL water and 3 mL 1.9 M sodium carbonate (Saarchem, South Africa, cat. no. 582 20 40) was dispensed into each tube, containing 200 µL of the NaOH-fibrin suspension. The tubes were vortexed and mixed with 1.0 mL Folin & Ciocalteu's Reagent (Saarchem, South Africa, cat. no. 243 300). Samples were incubated at room temperature for 20 minutes and absorbances measured at 650

nm. Different concentrations of DL-Tyrosine (Sigma, U.S.A, St. Louis, cat. no. T-3379) dissolved in 25 M NaOH was used to prepare a standard curve. This curve was used to calculate the concentration of fibrin present in unknown samples. The C.V. for this method was 0.04%.

3.5.2.5 Female sex hormones

Female steroid hormones were measured using the Bayer ADVIA® Centaur™ System. For Luteinizing Hormone (LH) (Bayer Diagnostic, USA, cat no 110754) and Follicle Stimulating Hormone (FSH) (Bayer Diagnostic, USA, cat. no. 110756) the assay is a two-site sandwich immunoassay using direct chemiluminometric technology, and for Progesterone (Prog) (Bayer Diagnostic, USA, cat. no. 1199592) and estradiol (E_2) (Bayer Diagnostic, USA, cat. no. 110785) the assay is a competitive immunoassay using direct chemiluminescent technology.

3.5.2.6 Other metabolic risk markers

The haemostatic risk factor, fibrinogen concentration was determined using the Behring Coagulation Timer using Multifibren® U (Dade Behring, Marburg GmbH, Germany. Cat. no. OWZG 15). Fibrinogen-Standards 1-

4 (Dade Behring, Marburg GmbH, Germany. Cat. no. OWCS 11) was used to draw a calibration curve for fibrinogen concentration determination. Control Plasma N (Dade Behring, Marburg GmbH, Germany. Cat. no. ORKE 35) was used as a control for normal fibrinogen concentrations and Control Plasma P (Dade Behring, Marburg GmbH, Germany. Cat. no. OUPZ 13) was used as a control for pathological fibrinogen concentrations.

Other metabolic variables (Table 1) were determined using a Boehringer Mannheim/Hitachi 902 Automated Chemistry Analyser (Hitachi Ltd Japan).

Table 3.1 Methods used for the determination of different biochemical variables

Variable	Supplier & Cat. no.	Principle
Total protein (TP)	Roche Diagnostics GmbH, Mannheim, Germany. # 1553836	Colorimetric
Albumin (ALB)	Roche Diagnostics GmbH, Mannheim, Germany. # 1970569	Colorimetric
Total cholesterol (TC)	Roche Diagnostics GmbH, Mannheim, Germany. # 1489232	Enzymatic colorimetric
Glucose (GLC)	Roche Diagnostics GmbH, Mannheim, Germany. # 1448668	Enzymatic colorimetric
Triglycerides (TG)	Roche Diagnostics GmbH, Mannheim, Germany. # 1488872	Enzymatic colorimetric

* S.F.A.C. (Cat no. 759350, Roche Diagnostics GmbH, Mannheim, Germany.) was used as calibrator for chemistry measurements.

Precinorm® U (Roche Diagnostics GmbH, Mannheim, Germany. Cat. no. 171735) was used as a control for normal chemistry values and Precipath® U (Roche Diagnostics GmbH, Mannheim, Germany. Cat. no. 171760) as control for pathological range values.

3.5.2.7 Full blood counts and HIV status

Full blood counts were performed on EDTA blood using a Coulter Microdiff 18 Cell Counter. HIV tests were performed on an Abbott AxSYM ® System, using the Human Immunodeficiency Viruses (HIV-1/HIV-2): (Recombinant Antigens and Synthetic Peptides) reagent pack (Abbott, Germany, cat. no. 3D41-20). The HIV 1/2 gO reagent pack is for the *in vitro* qualitative detection of antibodies to human immunodeficiency virus type one and/or type two in human serum or plasma, by Microparticle Enzyme Immunoassay.

3.6 Other information

This project formed part of a bigger project. Other researchers also obtained information for their own purpose. However, for this study the following information was obtained:

- Questionnaires were completed to obtain pre-menopausal data, personal information and information on socio-economic status.
- A general medical examination was performed by a general practitioner to eliminate any gross pathology.
- Each subject signed a form of consent.

3.7 Statistical analysis

Percentage lifetime was described by medians and percentiles for each group. The median difference between the groups was compared by the non-parametric Kruskall-Wallis test and 95% confidence intervals for the median difference.

The number of respondents in each group was described by means of frequencies and percentages. For each group, the continuous variables were described by means and standard deviations in case of normally distributed data. Medians, and percentiles were used to describe skewly distributed variables.

Within each group, Spearman correlation coefficients were calculated between the different variables.

The differences in the values of clotting factors between the three subject groups were calculated by means of the Kruskall-Wallis test or the one-way analysis of variance, as applicable. Statistical differences found between the three groups for normally distributed variables were compared by the Scheffe-test between each pair. This was done in order to identify which pair of variables influences the overall statistical significance most.

Confidence intervals for the mean difference were also calculated. The intervals were clinically interpreted for significance.

CHAPTER 4

RESULTS

In this Chapter, the results of the study are given in table form with emphasis on the significant differences.

4.1 Socio-economic status of the three different subject groups

Table 4.1 shows frequencies and percentages for the level of education and employment status of the three different groups of subjects.

There was no significant difference between the level of education of the less urbanized and urbanized black groups, with more than 80% of the subjects in both these groups having an educational level lower than or equal to Grade 10. All of white women had an educational level of higher than Grade 10.

As with level of education, the employment status of the two black groups showed very little difference. In the less urbanized group 22 (73.3%) were unemployed and 8 (26.7%) employed, and in the urbanized group 21 (70%) were unemployed and 9 (30%) employed. All of the women in the white group were employed.

Table 4.1 Socio-economic status (level of education and employment status) of the three groups of women

Parameters	Less urbanized black (n=30)	Urbanized black (n=30)	White (n=27)
Level of education			
None – Grade 10	25(83.33%)	26(86.67%)	0(0%)
> Grade 10	5(16.67%)	4(13.33%)	27(100%)
Employment status			
Unemployed	22(73.33%)	21(70.00%)	0(0%)
Employed	8(26.67%)	9(30.00%)	27(100%)

Table 4.2 shows the median, median difference, confidence intervals and p-values of the level of "urbanization" (years living in an urban area, and percentage lifetime spent in an urban area) for the three different subject groups.

There was no significant difference between the median age of the three different subject groups, with all the groups having a median age of about 41 years. There was a significant difference between the years spent living in an urban area (Table 4.2) for the three groups, and thus also the percentage lifetime spent in an urban area (Table 4.3). The median years spent living in an urban area was 10.5 years for the less urbanized black group, 39.5 years for the urbanized black group and 23 years for the white group. The percentage lifetime spent in an urban area also differed significantly between

the three groups. The median percentage lifetime spent in an urban area was 26.08% for the less urbanized black group, 99.32% for the urbanized black women and 52.09% for the white women.

Table 4.2 Age, years spent living in an urban area and percentage lifetime spent in an urban area of the three groups.

	Group	Median	Kruskall-Wallis p-values
Age	LUB	40.68	0.5797
	UB	40.93	
	White	41.12	
*Years spent living in an urban area	LUB	10.5	<0.0001
	UB	39.5	
	White	23.0	
*Percentage lifetime spent in an urban area	LUB	26.08%	<0.0001
	UB	99.32%	
	White	52.09%	

LUB=less urbanized black, UB=urbanized black.
Means significant differences between the groups for the same variable.

Table 4.3 The differences in percentage lifetime spent in an urban area.

Group	Median difference	95% Confidence interval for median difference
UB - LUB	71.12%	[63.74% - 75.10%]
White - LUB	72.86%	[7.69% – 56.67%]
UB - White	39.76%	[1.05% - 63.14%]

LUB=less urbanized black, UB=urbanized black.

Table 4.3 shows the differences in percentage lifetime spent in an urban area by means of the median differences and non-parametric 95% confidence interval for median differences using the method as described by Altman **et al**, 2001. Significant differences were obvious between the urbanized black and less urbanized black women, as well as between the white and less urbanized black group.

4.2 Descriptive data of the metabolic variables for the three different subject groups

Table 4.4 shows the mean, SD, Q1, Q3, skewness and normal ranges of each of the metabolic variables for the three different groups of subjects. The mean BMI of the white women was only slightly higher than that of the less urbanized and urbanized black women, but the values of all three the groups are slightly above normal, and can be classified as being overweight (25-30 kg/m^2), but not obese (>30kg/m^2). However, this has no influence on the results of this study due to the fact that all three subject groups fell within the same weight category. Serum glucose, albumin, total cholesterol and triglycerides did not show any significant difference between the three groups or from the normal ranges. The mean total protein of the white women was significantly lower than that of both the urbanized and less urbanized black women, the mean of the two black groups being about 70% higher than that

of the white women. The values of both the urbanized and less urbanized black women were also above the normal reference ranges for serum total protein. This is a common occurrence in the clinical laboratory, however of no clinical significance.

Serum LH of the white women was lower than that of both the black groups. The mean values of all three these groups, however, fell within the normal ranges for day 21 – 30 of the menstrual cycle. FSH values of the white group also differed from the values of the two black groups, but in this case the mean values of the black groups were above the normal range, while the mean levels of the white women were within the normal ranges for day 21 – 30 of the menstrual cycle. The mean estradiol values of the urbanized black women were the lowest of the three groups. The mean values of the less urbanized black women, in contrast, were the highest of the three groups, with a 45% difference between the mean values of the less urbanized and urbanized black groups. This difference, however, was not significant. It is noted that the means of both these groups fell within the normal ranges for day 21 – 30 of the menstrual cycle. The mean progesterone levels of the white women were higher than that of the two black groups. The difference between the mean progesterone levels of the white group and the two black groups is not significant. However, the mean progesterone levels of the two black groups fell below the normal reference range given for day 21-30 of the menstrual cycle.

Table 4.4 Physical and metabolic variables of all three groups of women from different socio-economic backgrounds

Physical & metabolic variables	Group	X	SD	Q3	Skew	Q1	Normal Ranges
Body Mass Index/BMI (kg/m^2)	LUB	26.7	4.9	28.7	26.6	23.5	18.5 – 25
	UB	26.6	5.6	30.0	25.4	22.6	18.5 – 25
	White	28.2	8.0	33.1	25.2	22.8	18.5 – 25
Estradiol/E_2 (pmol/L)	LUB	502.3	825.5	559.0	260.0	73.4	121 – 551
	UB	346.4	226.8	535.0	289.5	146.0	121 – 551
	White	454.2	545.0	511.0	291.0	130.0	121 – 551
Luteinizing Hormone/LH (IU/L)	LUB	11.2	12.0	14.8	5.5	3.8	0.5 – 17
	UB	11.4	11.9	19.4	6.0	3.2	0.5 – 17
	White	5.17	7.5	5.4	3.5	2.3	0.5 – 17
Follicle Stimulating Hormone/FSH (IU/L)	LUB	17.7	20.4	18.4	7.7	5.4	1 – 9
	UB	13.5	15.6	15.8	8.7	5.5	1 – 9
	White	5.8	3.3	7.8	5.5	3.5	1 – 9
Progesterone/Prog (nmol/L)	LUB	9.2	19.2	2.2	0.8	0.5	10 – 89
	UB	9.8	21.7	3.2	0.9	0.6	10 – 89
	White	14.4	22.4	21.1	0.9	0.7	10 – 89
Glucose/GLC (mmol/L)	LUB	4.6	0.97	4.9	4.5	4.1	3.6 – 5.6
	UB	4.6	0.91	5.4	4.5	4.2	3.6 – 5.6
	White	4.2	0.93	4.7	4.4	4.1	3.6 – 5.6
Albumin/ALB (g/L)	LUB	43.1	3.1	44.7	43.0	41.6	35 – 52
	UB	43.2	3.0	44.2	42.7	41.4	35 – 52
	White	42.9	2.0	44.2	42.8	41.7	35 – 52
Total Protein/TP (g/L)	LUB	83.5	5.8	86.5	83.5	80.0	60 – 82
	UB	84.9	6.4	89.0	84.5	80.0	60 – 82
	White	75.3	3.0	77.0	76.0	72.0	60 – 82
Total Cholesterol/TC (mmol/L)	LUB	5.3	1.1	6.1	5.1	4.3	4.1 – 6.2
	UB	4.9	1.3	5.5	4.8	4.2	4.1 – 6.2
	White	5.5	0.9	6.3	5.1	4.8	4.1 – 6.2
Triglycerides/TG (mmol/L)	LUB	1.5	0.8	1.9	1.4	0.9	0.40 – 2.26
	UB	1.5	1.7	1.4	1.0	0.8	0.40 – 2.26
	White	1.3	0.5	1.7	1.3	0.9	0.40 – 2.26

No significant differences between metabolic variables were found between any groups.
LUB=less urbanized black, UB=urban black.

4.3 Descriptive data of the haematological variables for the three different subject groups

Table 4.5 shows the mean, SD, Q1, Q3, skewness and normal ranges of each of the haematological variables for the three different groups of subjects.

Table 4.5 Haematological variables for all three groups of women from different socio-economic background

Haematological variables	Group	X	SD	Q3	Skew	Q1	Normal Ranges
Red Blood Cells/RBC (10^6/µL)	LUB	4.42	0.39	4.64	4.42	4.17	4.2 -5.2
	UB	4.41	0.34	4.54	4.39	4.19	4.2- 5.2
	White	4.65	0.32	4.78	4.65	4.46	4.2 – 5.2
Hemoglobin/Hb (g/dL)	LUB	13.91	0.98	14.60	13.80	13.30	11.5 – 16.5
	UB	13.80	1.20	14.40	13.90	13.00	11.5 – 16.5
	White	14.10	0.80	14.70	14.10	13.30	11.5 – 16.5
Fibrinogen/Fb (g/L)	LUB	3.49	1.00	3.86	3.27	2.92	1.5 – 4.0
	UB	4.03	1.16	4.96	4.12	3.09	1.5 – 4.0
	White	3.76	1.11	4.71	3.38	2.96	1.5 – 4.0
Compaction of fibrin networks*/CFN (%)	LUB	46.50	10.00	51.0	45.00	40.00	NE
	UB	48.40	15.00	59.0	49.00	37.00	NE
	White	48.00	10.00	52.0	47.00	40.00	NE
Mass length ratio*/µT (Da/cm x 10^12)	LUB	58.70	15.60	69.7	60.20	49.20	NE
	UB	59.70	19.90	70.1	57.20	49.70	NE
	White	62.10	20.60	76.2	62.1	46.70	NE
Network fibrin content*/NFC (g/L)	LUB	0.13	0.04	0.13	0.12	0.11	NE
	UB	0.13	0.03	0.15	0.13	0.11	NE
	White	0.14	0.04	0.16	0.12	0.11	NE

No significant differences between haematological variables were found between any groups.

*=developed at a final concentration of 1 IU/mL thrombin and a final concentration of 25 mmol Ca^{2+}. LUB=less urbanized black, UB=urban black, NE=not established.

RBC and Hb mean values were within the normal reference ranges for all three the subject groups. This was expected, as all the subjects were HIV negative, were non-smokers and did not have any gross clinical abnormalities as specified in the strict inclusion criteria adhered to during recruitment of the volunteers. No significant differences between the mean plasma fibrinogen values of the urban black and white women (14% of the mean), less urbanized and urban black women (13% of the mean) and less urbanized black and white women (8% of the mean) were recorded. However, in contrast to the mean fibrinogen values of the white and less urbanized black women, the mean fibrinogen value of the urban black women fell just above the upper international cut-off value of 4 g/L for plasma fibrinogen. It is believed that this small elevation may be of no clinical significance within the black population group of South Africa, and will be discussed in the next Chapter. The mean values of the fibrin network compaction which is used as an index of the tensile strength of fibrin fibers within the fibrin networks were basically the same for the white and urbanized black women, while the mean values of the less urbanized black women were only slightly lower. The difference was also non-significant. The mean values for the mass length ratio of the fibrin fibers (an index used to describe the relationship of the ratio between the length and thickness of the fibrin fibers within the network structure) within the networks showed no significant difference between the less urbanized and urbanized black groups, as well as between the urbanized black and white groups, with only

a 5% difference between the values of the less urbanized black and white groups. No difference was recorded between the mean values of the network fibrin content of the three groups.

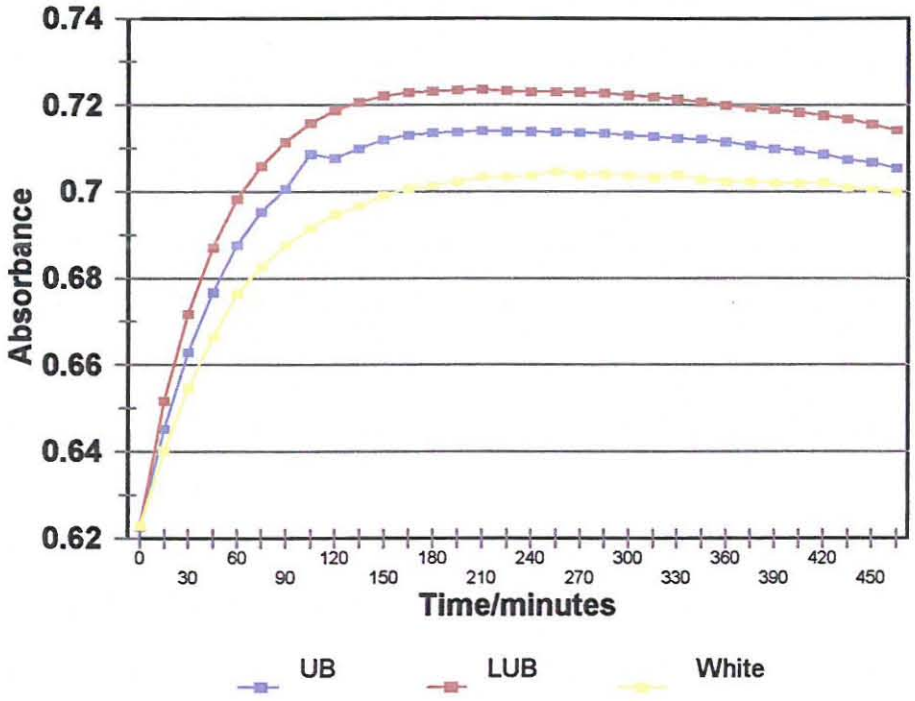

Figure 4.1 Lysis rates of fibrin networks for three different groups of socio-economic status. Developed at a final concentration of 1 IU/mL thrombin and a final concentration of 25 mmol Ca^{2+}.

The lysis rate of fibrin networks formed from human plasma explains the property of the fibrin networks to be broken down by the fibrinolytic enzymes present in blood. Under experimental conditions a standard lytic potential is developed by adding a fixed amount of excess streptokinase, an enzyme with fibrinolytic potential. These conditions urge the lysis potential to be

solely dependent on the structure/function characteristics of the fibrin strands within the network, including the concentration of the fibrin protein deposited within the fibrin network. During normal physiological functioning the rate would also depend on the concentrations of the different lytic enzymes, which may also vary from individual to individual. Consequently, during network studies a standard lytic potential for measuring network lysis rate is created.

As fibrin fibre size is decreased, the fibrinolytic rate also decreases. Thin fibrin fibres have a decreased rate of conversion of plasminogen to plasmin by tPA and thin fibres are lysed more slowly than thick fibrin fibres (Gabriel **et al.**, 1992). This phenomenon was explained by experiments which have shown that the number of tPA binding sites and fibrinolytic rate (Gabriel **et al.**, 1991) are dependent on fibrin structure. Since structural features at the end of fibrin assembly may determine the potential number of binding sites for the fibrinolytic enzymes, it is probable that thin fibres posess fewer plasmin binding sites, possibly as a function of the decreased surface of the thin fibres (Gabriel **et al.**, 1992). This may be due to (i) a decrease in the number of binding sites resulting from the smaller surface area of thin fibres or (ii) steric factors caused by the curvature of the thin fibres which prohibit stable interaction between tPA and its binding site (Gabriel **et al.**, 1992). Fibrin, with its ordered structure, appears to exert its rate-enhancing effect by presenting its binding sites for interaction with tPA and plasminogen, thus

concentrating and correctly orienting these two reactants on its surface and inducing conformational changes which lead to higher catalytic efficiencies (Nieuwenhuizen, 1994).

Figure 4.1 shows the lysis rates for the three different subject groups. It is evident from this graph that the lysis rate of fibrin networks of the group of less urbanized black women (red line) was the highest, and that the lysis rate of the fibrin networks of the group of white women (yellow line) was the lowest. The lysis rate of the fibrin networks for the group of urbanized black women (blue line) fell between the white and less urbanized black women groups.

4.4 Correlations between baseline variables

Tables six to nine show Spearman's correlation coefficients between the metabolic variables within each of the three different subject groups, as well as the correlation coefficients between the metabolic variables within the subject group as a whole.

In Table 4.6 Spearman's correlation coefficients of the variables that correlated with one another are given for the group in total. Positive correlations were found between serum LH and FSH, ALB and TP, RBC and Hb, as well as

between plasma fibrinogen and NFC. There was a negative correlation between μT and NFC.

In Table 4.7 Spearman's correlation coefficients of the variables that correlated with one another in the group of less urbanized black women are given. The following positive correlations occurred: between E_2 and both ALB and TP, between serum LH and FSH, ALB and both TP and Hb, between plasma fibrinogen and NFC and between CFN and μT. There was a negative correlation between μT and NFC.

Table 4.6 Spearman's correlation coefficients between variables of the group as total

	E_2	LH	FSH	Prog	GLC	ALB	TP	TC	TG	RBC	Hb	Fb	CFN	µT	NFC	BMI
E_2	1															
LH	NS	1														
FSH	NS	0.74	1													
Prog	NS	NS	NS	1												
GLC	NS	NS	NS	NS	1											
ALB	NS	NS	NS	NS	NS	1										
TP	NS	NS	NS	NS	NS	0.50	1									
TC	NS	NS	NS	NS	NS	NS	NS	1								
TG	NS	NS	NS	NS	NS	NS	NS	NS	1							
RBC	NS	NS	NS	NS	NS	NS	NS	NS	NS	1						
Hb	NS	NS	NS	NS	NS	NS	NS	NS	NS	0.53	1					
Fb	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	1				
CFN	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	1			
µT	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	1		
NFC	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	0.70	NS	-0.72	1	
BMI																

NS : Non-significant; Significant (p>0.5)

Table 4.7 Spearman's correlation coefficients between variables of the less urbanized black women

	E_2	LH	FSH	Prog	GLC	ALB	TP	TC	TG	RBC	Hb	Fb	CFN	µT	NFC	BMI
E_2	1															
LH	NS	1														
FSH	NS	0.85	1													
Prog	NS	NS	NS	1												
GLC	NS	NS	NS	NS	1											
ALB	0.50	NS	NS	NS	NS	1										
TP	0.57	NS	NS	NS	NS	0.59	1									
TC	NS	NS	NS	NS	NS	NS	NS	1								
TG	NS	NS	NS	NS	NS	NS	NS	NS	1							
RBC	NS	NS	NS	NS	NS	NS	NS	NS	NS	1						
Hb	NS	NS	0.60	NS	NS	0.55	NS	NS	NS	NS	1					
Fb	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	1				
CFN	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	1			
µT	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	0.52	1		
NFC	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	0.71	NS	-0.69	1	
BMI																

NS : Non-significant; Significant (p>0.5)

Table 4.8 Spearman's correlation coefficients between variables within the urbanized black women

	E_2	LH	FSH	Prog	GLC	ALB	TP	TC	TG	RBC	Hb	Fb	CFN	µT	NFC	BMI
E_2	1															
LH	NS	1														
FSH	NS	0.74	1													
Prog	NS	NS	NS	1												
GLC	NS	NS	NS	NS	1											
ALB	NS	NS	NS	NS	NS	1										
TP	NS	NS	NS	NS	NS	0.59	1									
TC	NS	NS	NS	NS	NS	NS	NS	1								
TG	NS	NS	NS	NS	NS	0.51	NS	NS	1							
RBC	NS	NS	NS	NS	NS	NS	NS	NS	NS	1						
Hb	NS	NS	NS	NS	NS	NS	NS	NS	NS	0.54	1					
Fb	NS	NS	NS	NS	NS	NS	NS	NS	-0.54	NS	NS	1				
CFN	NS	-0.62	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	1			
µT	NS	NS	NS	NS	NS	NS	NS	-0.53	NS	NS	NS	NS	NS	1		
NFC	NS	NS	NS	NS	NS	NS	NS	NS	NS	0.51	NS	0.77	NS	-0.68	1	
BMI																

NS : Non-significant; Significant (p>0.5)

Table 4.9 Spearman's correlation coefficients between variables in the white group

	E_2	LH	FSH	Prog	GLC	ALB	TP	TC	TG	RBC	Hb	Fb	CFN	µT	NFC	BMI
E_2	1															
LH	NS	1														
FSH	NS	0.78	1													
Prog	NS	NS	NS	1												
GLC	NS	NS	NS	NS	1											
ALB	NS	NS	NS	NS	NS	1										
TP	NS	NS	NS	NS	NS	0.63	1									
TC	NS	NS	NS	NS	NS	NS	NS	1								
TG	NS	NS	NS	NS	NS	NS	NS	0.60	1							
RBC	-0.51	NS	NS	NS	NS	NS	NS	NS	NS	1						
Hb	NA	NS	NS	NS	NS	NS	NS	NS	NS	0.74	1					
Fb	NA	NS	NS	NS	NS	NS	NS	0.58	0.57	NS	NS	1				
CFN	NA	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	1			
µT	NA	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	-0.64	NS	1		
NFC	NA	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	0.74	NS	-0.78	1	
BMI	NS	NS	NS	NS	NS	NS	NS	0.60	0.55	NS	NS	0.55	NS	NSW	0.95	1

NS : Non-significant; Significant (p>0.5)

In Table 4.8 Spearman's correlation coefficients of the variables that correlated with one another in the group of urbanized black women are given. The following positive correlations were found: between serum LH and FSH, serum ALB and both TP and TG, between RBC and both Hb and NFC and between plasma fibrinogen and NFC. Negative correlations were found between LH and CFN, between TC and µT, between TG and Fb and between µT and NFC.

In Table 4.9 Spearman's correlation coefficients of the variables that correlated with one another within the group of white women are given. The following positive correlations were found: between LH & FSH, between ALB & TP, TC correlated with TG, Fb & BMI, between TG and both Fb & BMI, between RBC & Hb, between Fb and both NFC & BMI and between NFC and BMI. Negative correlations occurred between E2 & RBC, between Fb & µT and between µT & NFC.

CHAPTER 5

DISCUSSION, CONCLUSIONS & RECOMMENDATIONS

5.1 INTRODUCTION & LIMITATIONS OF THE STUDY

Although the discussion in this Chapter will focus on specific variables, variables will also be considered together in their totality in order that a holistic approach be used to extrapolate the findings of this study to the main objectives. The major problem associated with the interpretation of the results are hidden within the fact that the word "socio-economic status" could be very controversial, as no quantitative value could be ascribed directly to it other than the years and percentage lifetime spent living in an urban area. This also indicates that personal interpretation could play a major role in the discussion, and may lead to unwanted speculation. The writer of this dissertation will guard against this.

The literature (Markowe **et al.**, 1985; Brunner **et al.**, 1999) describes that socio-economic background is considered as a major factor leading to either metabolic abnormalities or protective properties related to specific factors within the environment of the individual. The aim of this study was to investigate the possible effect of different levels of socio-economic status on fibrin network architecture in three groups of women - less urbanized black, urban black and white. The rationale behind this was mainly ascribed to literature describing the effect of socio-economic status on fibrinogen concentration. It is described that any factor having an effect on fibrinogen concentration (Møller & Kristensen, 1991), should have a direct effect on fibrin network architecture. Results from this study could be used to determine the degree to which this theory can be proven in practice. The value of this work lies in the fact that it may open new perspectives for

biomedical technology as scientific method of investigation, as well as within the normal day-to-day clinical practice used as a diagnostic tool.

In order to evaluate the true effect of socio-economic status on fibrin network architecture, a study of epidemiological nature including large numbers of subjects would be preferred. This study can be classified as a preliminary study opening doorways to future investigations.

Studies investigating the health of women in developing countries have largely been focused on maternal nutrition. Little attention has been paid to the well-being of women and their health status, including the effect of nutrition, physical performance and social and economic activities (Goldberg, 1997). Some developing countries are experiencing an epidemiological transition, with a move from infectious diseases and undernutrition to chronic diseases such as hypertension and diabetes as the most important cause of morbidity and mortality (Walker, 1997). The variation between rural and urban areas and among social classes will affect the nutritional issues of most relevance to women. The main issues for more affluent women in these countries or for the majority of women in countries in transition are probably similar to those of women in developed countries.

South Africa can be regarded as a developing country, but differs from other developing countries in the sense that large parts of the country, as well as certain population groups, are technologically highly developed. At present rapid urbanization from rural areas where traditional ethnic lifestyles previously persisted, are now taking place. The detrimental effects of urbanization on dietary patterns, nutrient intakes and nutritional status (Popkin, 1994), lifestyle changes (Yach **et al.**, 1990), risk of stroke (Seedat **et al.**, 1982), diabetes mellitus (O'Dea **et al.**, 1990; Levitt, 1997), coronary heart disease risk factors (Steyn **et al.**, 1991; Slabber **et al.**, 1996) and others have

been described in South African as well as in international literature. The low prevalence, on the other hand, of colon (Walker, 1996) and breast cancer among African populations (Walker & Burger, 1997), as well as the rarity of hip fractures in sub-Saharan Africans (Schnitzler **et al.**, 1996) can possibly also be ascribed to lifestyle. Iron deficiency, which has been rare in traditional African women, has also increased during the last decades (Patel **et al.**, 1992). On the other hand, the beneficial effects of urbanization such as an increased life expectancy, lower incidence of undernutrition, decreased infant mortality rates and increased quality of life have also been described (Jiménez, 1993; Shi, 1993).

In the Free State Province it has been shown that urbanization has already taken place on a large scale, and that little difference exists in the disease risk prevalence of urbanized and so-called semi-urbanized and rural populations (Mollentze **et al.**, 1995).

In this study, a group of women was selected according to strict inclusion criteria. One of the most important criteria, having a very significant effect on the results, stated that all volunteers should be apparently healthy. This was also confirmed by a medical examination performed by a registered general practitioner. It also implicates that all detrimental effects of socio-economic status were not allowed to follow their normal route within the subjects. Consequently, the effect of factors such as HIV status, the prevalence of tuberculosis, smoking habits, alcohol intake etc., which could be considered as part of socio-economic status, were excluded from this study. From this it can be reasoned that the true effect of socio-economic status is not reflected within the results of this study. This is, however, not true. It is rather a limitation with reason.

It was considered as important to use diseases of lifestyle as an exclusion factor in order to prevent skewing of results due to associated expected high levels of plasma fibrinogen. Plasma fibrinogen is an acute phase protein (Dippel, 1992). Thus, any metabolic abnormality evoking an acute phase response would cause a natural increase in plasma fibrinogen concentration (Resch & Ernst, 1994). This will directly have an influence on the fibrin network architecture variables and would result in leading of the conclusions made from them. A good example of this would be, for instance, individuals living with HIV/AIDS. Patients living with HIV/AIDS have abnormal coagulation profiles, which are associated with severe Rouloux or red cell aggregation, elevated erythrocyte sedimentation rate and elevated plasma viscosity (Lowe, 1993). Not only HIV/AIDS, but also a variety of other metabolic abnormalities associated with lifestyle, have very specific and profound effects on coagulation profiles of individuals. These abnormalities include, for instance, diabetes mellitus (**Ganda et al.**, 1992), tuberculosis, obesity (Vorster et al., 1989; Lip, 1995), hypertension (Kannel , 2000), hypertriglyceridaemia (Halle **et al.**, 1996), etc., all of which were excluded in this study. In this way, the influence of socio-economic status as determined by years spent living in an urban area on fibrin network architecture could be evaluated.

5.2 METABOLIC VARIABLES

It was evident that there were no major unexpected differences between the metabolic variables of the three different subject groups. All the subjects within the three groups were apparently healthy individuals, without any previously diagnosed disease states, such as hypercholesterolaemia, glucose intolerance, high blood pressure, etc. It is commonly believed that a more prudent lifestyle should be associated with a "healthy" metabolic profile. In this case, prudent is defined by low stress levels, healthy food intake (such as adequate dietary fibre, vitamin and mineral intake), adequate physical

exercise, etc. The question may be asked if any of the subject groups used in this study could be classified as "prudent lifestyle" individuals, also in South Africa "urbanization" does not necessarily mean better socio-economic circumstances. This is most probably not the case. It can be reasoned that a prudent lifestyle is more of an idealistic end-point.

Most variables that differed significantly between the different subject groups could not only be ascribed to the difference in socio-economic background, but may also be ascribed to genetic differences between the two groups of races used as subjects within this study. A variety of studies (Folsom **et al.**, 1993; Bao **et al.**, 1993) have shown that ethnic differences exist in some metabolic parameters. The Department of Chemical Pathology at the UFS found that there is a significant difference in the total protein levels and sub-protein levels of the black and white population of the Free State Province (unpublished data). This study in the early 1980's concluded that the TP levels of the South African black population were lower than that of the white population. Subjects in this study were also apparently healthy. In the current study exactly the opposite was found. A variety of questions may now be asked, including the question related to an overall change in socio-economic status as undergone by the black population from 1980 to 2001, and its overall effect on the health status of these individuals. Can urbanization and its associated effect on diet, lifestyle, activity levels, etc. contribute to these major changes? This question may be answered in another study.

One major difference between the different subject groups was found to be associated with serum hormonal levels, however, these differences were of no clinical significance. Both the mean LH and mean FSH levels of the white women were lower than that of the two black groups. The mean LH level of all three the groups were normal, while the mean FSH level of the two black

groups were elevated. Although the mean estradiol values of all three the groups were normal, there was a 45% difference between the mean values of the urbanized and less urbanized black women. However, this difference was of no clinical significance. The mean progesterone level of the two black groups were slightly lower than that of the white group and were also slightly below the normal ranges for day 21-30 of the menstrual cycle. A variety of socio-economic factors may play an important role regarding this aspect. Factors such as the number of pregnancies (Yin et al., 1998), the use of birth control agents (Folsom **et al.**, 1993; Lip, 1995), hormone replacement (Gebara **et al.**, 1998) etc. are all factors that are partially determined by a blend of social and cultural factors which should in return have a direct influence on the metabolic markers in blood. It is not possible to speculate on the specific factors such as religion and cultural beliefs within each culture that may influence these markers.

The next issue addresses the relevance of these minor differences and changes in metabolic parameters that took place over time, to the "change" in health status of these individuals. A quantitative survey using information from public health systems would have to be performed in order to assess if a true change in health status of the different population groups investigated in this study exists. Would a change in one metabolic variable such as TP have a significant impact on the health status of the individual? It can be speculated that the observed differences hint only for a tendency to change, and that these changes have not yet taken place completely. It is unknown what time such changes will need to take place and if these changes are expected.

5.3 HAEMATOLOGICAL VARIABLES

However unsuspected, there were no major differences between haematological variables "listed" as lifestyle sensitive. Haematological variables are sometimes difficult and intriguing to interpret. The main problem with haematological studies could mainly be ascribed to the fact that when the results are put into perspective, there are always other variables excluded from the study which may have been worth analyzing for interpretation purposes. The most limiting factor when deciding on a variable pool is most probably financial constraints. Haematological parameters are extremely expensive and usually labor intensive. For this reason, all results from haematological studies should be interpreted with utmost care. Also, these results should be interpreted taking the methodological techniques into consideration, i.e. immunological methods giving exact values, physiological or enzymatic methods giving derived values, etc.

In this study there were no major statistical differences in the haematological variables of the three different study groups. However, it is important to discuss the tendency of each group to differ from each other.

The haemoglobin values of both the urban and less urbanized black women were lower than that of the white group. However, this is of no clinical significance. The differences between the haematological values for different races have been well described (Cross **et al.**, 1983). In this study, the group of less urbanized black women had fibrinogen values higher than those of the white women, but lower than those of the urban black women. This difference between fibrinogen levels of the black and white women was expected (Folsom **et al.**, 1993). In correlation with this study, James **et al.** (2000) reported an increase in the plasma fibrinogen levels with urbanization. The urban black females had the highest plasma fibrinogen levels. It is also

interesting to note that the concentration falls above the upper limit set for plasma fibrinogen (4.0 g/L). The clinical significance of this is in question. It is expected that a similar plasma fibrinogen level that seems high for one group of subjects, may not be high for another group of subjects. It also seems that the risk for development of any disease associated with raised plasma fibrinogen may not depend on the concentration of the circulating protein alone, but also some other factors within the direct surroundings of the protein. This is why fibrin network architecture is such a good method describing the qualitative effect of the direct surroundings on the fibrinogen molecule and the resulting clotting process the molecule undergoes to form the final fibrin product. At this stage the information leading to this "hypothesis" is not proven yet, but results from previous studies (Veldman **et al.**, 1999) leads the way to this school of thought.

Results from this study indicated that between the three different groups of subjects, the group of white women had the healthiest network structures related to the mass-to-length ratio of the fibrin fibres, also associated with the fastest rate of network lysis (Gabriel **et al.**, 1992). This relationship between mass-to-length ratio should be expected due to the fact that lysis rate mostly depends on the surface area of the fibrin fibres available for cleavage to the fibrinolytic enzymes (Gabriel **et al.**, 1991). Fibrin, with its ordered structure appears to exert its rate-enhancing effect by presenting its binding-sites for interaction with tPA and plasminogen, thus concentrating and correctly orienting these two reactants on its surface and inducing conformational changes which lead to higher catalytic efficiencies (Nieuwenhuizen, 1994). The compaction of the fibrin networks of the white and urban black women was very close and was higher than that of the less urbanized black group. This also indicates that the white and urbanized groups have healthier fibrin network structures. These "healthier" networks are rendered less ridged and

more porous, which leaves the structures more plastic and deformable, and therefore the structures are believed to be less atherogenic.

5.4 CONCLUSION

The results of this study can be very useful for future references dealing with the effect of socio-economic factors on human metabolism and its different sub-divisions. This study originated from published data (Brunner **et al.**, 1999; Møller & Kristensen, 1991) which suggests that socio-economic status has a direct influence on plasma fibrinogen levels in human subjects. This study implies that the metabolic abnormalities associated with different levels of socio-economic status are not necessarily associated with socio-economic class itself, but rather with the associated factors related to the different levels of socio-economic status, such as the prevalence of tuberculosis, HIV, diabetes mellitus, hypercholesterolaemia, etc., which were exclusion criteria in this study. These diseases of lifestyle are all abnormalities that are more frequent within specific socio-economic groups of the population (Brunner et al., 1999; Marmot **et al.**, 1991). The study showed that there is a specific tendency towards differences in the metabolic variables, especially the hematological variables and associated fibrin network architecture variables. However, this "tendency" is not of statistical or clinical significance. Also, the study group was very small and this may contribute to the non-significance of the results.

In conclusion, the results from this study indicate that a small difference in the fibrin network architecture of individuals from different socio-economic background does exist. However, it also seems that if the individual is relieved from the burden associated with specific socio-economic backgrounds, we eventually seem all to inherit the same health status.

5.5 RECOMMENDATIONS

The results from this study clearly underline the need for further investigation. Prospective epidemiological trials would be helpful to evaluate the true effect of socio-economic variables and associated conditions on metabolic risk factors. A cohort design would be the method of preference. A much larger group of subjects should also be included in the study. Two questions should clearly be distinguished:

- What is the effect of a change in socio-economic status on the health of an individual, i.e. movement of an individual from a rural to an urbanized background, and
- What is the static/baseline effect between different levels of socio-economic status on the health of different individuals, i.e. people born in different levels of socio-economic status and growing up within that specific surrounding?

Major factors influencing general public health related to socio-economic status that need urgent attention include the role of peer pressure, advertising, government health regulations on consumer products, school feeding programmes and general nutrition education, as well as the knowledge of the general public concerning the products they consume. It will not be easy to evaluate the specific role of these factors on individual health. However, without this information it would not be possible to distinguish between different levels of socio-economic background and its effect on the health of the individuals living in these societies.

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APPENDIX A

BIOCHEMICAL VARIABLE UNITS AND NORMAL RANGES

VARIABLE	UNITS	NORMAL RANGE
BMI	kg/m^2	18.5 – 25
E_2	pmol/L	121 – 551
LH	IU/L	0.5 – 17
FSH	IU/L	1 – 9
Prog	nmol/L	10 – 89
GLC	mmol/L	3.6 – 5.6
ALB	g/L	35 – 52
TP	g/L	60 – 82
TC	mmol/L	4.1 – 6.2
TG	mmol/L	0.40 – 2.26
RBC	10^6/µL	4.2 – 5.2
Hb	g/dL	11.5 – 16.5
Fb	g/L	1.5 – 4.0
CFN	%	Not established
µT	$Da/cm \times 10^{12}$	Not established
NFC	g/L	Not established

APPENDIX B

FIBRINOGEN AND FIBRIN NETWORK STUDIES ON PRE-MENOPAUSAL WOMEN

SOCIO-ECONOMIC STATUS AND PERSONAL INFORMATION

Name:_____

Respondent number: ☐☐☐ 1-3

Birth date. ☐☐☐☐☐☐☐☐ 4-11

Interview date.

☐☐☐☐☐☐☐☐12-19

Address:_____

_____

_____

Telephone number:_____

1. Do you drink alcoholic beverages?

   Yes (1) No (2) ☐ 20

2. How often do you usually drink alcohol? ☐ 21
   1. Every day.
   2. 5-6 Days per week.
   3. 3-4 days per week.
   4. 1-2 days per week.
   5. Weekends.
   6. Less than once a week.

3. On a weekday when you do drink alcohol, how many drinks do you usually have per day?

1. Beer (bottles/cans)	☐☐ 22-23
2. Brandy/Whisky (tots)	☐☐ 24-25
3. Vodka/Gin (tots)	☐☐ 26-27
4. Sherry/Sweet wine (glasses)	☐☐ 28-29
5. Table wine (glasses)	☐☐ 30-31
6. Other, please specify_____	☐☐ 32-33

4. On a weekend day when you drink alcohol, how many drinks do you usually have?

1. Beer (bottles/cans)	☐☐ 34-35
1. Brandy/Whisky (tots)	☐☐ 36-37
2. Vodka/Gin (tots)	☐☐ 38-39
3. Sherry/Sweet wine (glasses)	☐☐ 40-41
4. Table wine (glasses)	☐☐ 42-43
5. Other, please specify_____	☐☐ 44-45

5. Is there a family history of chronic disease?

Yes (1) No (2) ☐ 46

6. If yes, please specify

1. Diabetes	☐ 47
2. Heart disease	☐ 48
3. High or low blood pressure	☐ 49
4. TB	☐ 50
5. Other, please specify_____	☐ 51

7. Weight (kg) ☐☐☐.☐ 52-55

8. Length (m) ☐.☐☐ 56-58

9. How long (years) have you been living in an urban area like Mangaung or Bloemfontein? ☐☐ 59-60

10. Do you smoke? Yes (1) No (2) ☐ 61

11. What is your highest level of education? ☐ 62
    1. None
    2. Primary School
    3. Std 6-8
    4. Std 9-10
    5. Tertiary Education

12. Employment status. ☐ 63
    1. Housewife by choice
    2. Unemployed
    3. Self employed
    4. Full time wage/salary earner
    5. Other, please specify
    6. Don't know

13. How many days per week do you work? ☐ 64

14. How long per day do you work (min)? ☐☐☐ 65-67

15. Do you play sport? Yes(1) No(2) ☐ 68

16. If yes, which type of sport? _____ ☐☐ 69-70

17. How long do you practice per week (min)? ☐☐☐ 71-73

18. Do you have leisure time? Yes(1) No(2) ☐ 74

19. If yes, do you ☐ 75
    1. Do sitting activities
    2. Do you walk
    3. Do you do standing activities

20. Do you follow any special diet?

Yes(1) No(2) ☐ 76

21. If yes, please specify ☐ 77

1. Diabetic
2. Slimming
3. Allergies
4. Other, please specify_____

22. Do you take any Vitamin supplements?

Yes(1) No(2) ☐ 78

23. Indicate which of the following best describes the eating pattern you usually follow. ☐ 79

1. More than 3 meals per day with eating between meals.
2. 3 Meals per day with eating between meals.
3. 3 Meals per day with no eating between meals.
4. 2 Meals per day with eating between meals.
5. 2 Meals per day with no eating between meals.
6. 1 Meal per day with eating between meals.
7. 1 Meal per day with no eating between meals.
8. Nibble the whole day, no specific meals.
9. Other, please specify_____

APPENDIX C

CONSENT FORM (Black women : Mangaung)

The association between levels of socio-economic status and fibrin network architecture in women aged between 35 and 44 years

Declaration by or on behalf of the participant:

Respondent number ☐☐☐

I, the undersigned, ...

[ID.....]

.....(address)

A confirm that:

1. I have been asked to participate in the research survey: Nutritional Health of Women (25-44 years) in Mangaung, 2000. The Technikon Free State and University of the Orange Free State will carry out this study. I also understand that if my results of the above mentioned study meets certain criteria, my blood will also be used to take part in another study carried out by the Technikon Free State namely: The association between levels of socio-economic status and fibrin network architecture in women aged between 35 and 44 years.

2. It has been explained to me that:

2.1 The purpose of the first research survey is to collect information on usual food intake, activity level, attitude towards health, risk for developing illnesses related to eating habits and lifestyle of women in the ages 25 to 45 years in Mangaung. The information collected will be used to determine nutritional problems and to develop solutions for these problems. The purpose of the second research survey is to collect information on my hormonal status, to determine may socio-economical status, and to determine the influence of this on fibrinogen concentration and on fibrin networks, risk for developing illnesses related to the

altered fibrinogen concentration and the changes in fibrin networks. The information collected will be used to determine the relation between socio-economical status and the risk for cardiovascular heart disease and possible preventative measures for higher risk of CHD.

2.2 In order to collect this information for these two studies I have been told that I will be asked a number of questions regarding:

- general background information;
- the types and amounts of foods I eat and how often I eat these foods;
- how active I am every day;
- my attitude towards leanness and fatness;
- general knowledge on my menstrual cycle, contraception, pregnancy and breast cancer.

2.3 I also understand that a medical doctor will perform a free medical examination and that blood samples will be drawn by a registered nurse. One of these blood samples will include a test for HIV-AIDS. I also agree to be weighed and measured. I will not eat or drink anything after 10:00 of the evening preceding the research day, I will be given some tea and sandwiches after I have been weighed, measured and the blood have been collected. I will bring a list of the medication that I usually use with me on the research day.

2.4 I have been told that this information will be collected from over 500 women in Mangaung and on 30 female staff members of the Technikon Free State and that these questions will only be asked once. The measurements and blood samples will also be taken once only.

2.5 I have been told that it will not take more than one day to collect the information. I understand that on this day I will be measured, weight, under go a medical examination, and answer questions in order to complete five questionnaires. There will also be blood taken from me, but no more than 20 ml.

3 I have been told that the measurements will not cause any harm to me in any way.

4 It was also explained to me that by participating in the research survey I will help other women in the country.

5. It was also explained to me that the information will be kept confidential but that it will be used anonymously (subject numbers will be assigned to each participant) for making known the findings to other scientists.

6. I understand that I will have no direct access to the results of the survey but I can contact the researcher who will inform me of the findings. It was also explained to me that if I want my HIV results, I will only be able to get them from the medical doctor who did the medical investigation, she will also assist me with any questions I might have regarding the result of the HIV test.

7. It was also clearly explained to me that I could refuse to participate in this research survey. If I refuse, it will not be held against me in any way.

8. The information in this consent form was explained to me by .....(name of interviewer) in .....(language) 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.

9. No pressure was applied on me to take part in this research survey.

10. Finally, after completion of my participation in this research survey I will receive a payment of R40. I will be responsible for my own transport home.

B I hereby agree voluntarily to take part in this research survey.

Signed/confirmed at ..... on 2000

.....	.....
Signature or hand mark of	Signature or hand mark of
Participant	Witness

APPENDIX D

CONSENT FORM (Staff: Technikon Free State)

The association between levels of socio-economic status and fibrin network architecture in women aged between 35 and 44 years.

Declaration by or on behalf of the participant:

Respondent number ☐☐☐

I, the undersigned, ...

[ID.....]

.....(address)

A confirm that:

1. I have been asked to participate in the research survey carried out by the Technikon Free State namely: The association between levels of socio-economic status and fibrin network architecture in women aged between 35 and 44 years.

2. It has been explained to me that:

2.1 The purpose of the research survey is to collect information on my hormonal status, on my socio-economic status, and to determine the influence of this on fibrinogen concentration and on fibrin networks, risk for developing illnesses related to the altered fibrinogen concentration and the changes in fibrin networks. The information collected will be used to determine the relation between socio-economic status and the risk for cardiovascular heart disease and possible preventative measures for higher risk of CHD.

2.2. In order to collect this I have been told that I will be asked a number of questions regarding:

- general background information;
- how active I am every day;
- my attitude towards leanness and fatness;

- general knowledge on my menstrual cycle, contraception, pregnancy and breast cancer.

2.3 I also understand that a medical doctor will perform a free medical examination and that blood samples will be drawn by a registered nurse. One of these blood samples will include a test for HIV-AIDS. I also agree to be weighed and measured. I will not eat or drink anything after 10:00 of the evening preceding the research day, I will be given some tea and sandwiches after I have been weighed, measured and the blood have been collected. I will bring a list of the medication that I usually use with me on the research day.

2.4 I have been told that this information will be collected from over 500 women in Mangaung and on 30 female staff members of the Technikon Free State and that these questions will only be asked once. The measurements and blood samples will also be taken once only.

3. I have been told that it will not take more than one day to collect the information. I understand that on this day I will be measured, weighed and answer a number of questions to complete the questionnaires. I also know that blood will be taken from me, but no more than 20 ml. I have been told that the measurements will not cause any harm to me in any way.

4 It was also explained to me that by participating in the research survey I will help other women in the country.

5. It was also explained to me that the information will be kept confidential but that it will be used anonymously (subject numbers will be assigned to each participant) for making known the findings to other scientists.

6. I understand that I will have no direct access to the results of the survey but I can contact the researcher who will inform me of the findings. It was also explained to me that if I want my HIV results, I will only be able to get them from the medical doctor who did the medical investigation, she will also assist me with any questions I might have regarding the result of the HIV test.

7. It was also clearly explained to me that I could refuse to participate in this research survey. If I refuse, it will not be held against me in any way.

8. The information in this consent form was explained to me by .....(name of interviewer) in .....(language) 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.

9. No pressure was applied on me to take part in this research survey.

B I hereby agree voluntarily to take part in this research survey.

Signed/confirmed at ..... on 2000

.....	.....
Signature or hand mark of Participant	Signature or hand mark of Witness

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