THE EFFECT OF DIETARY VITAMIN A FORTIFICATION ON PLASMA FIBRINOGEN LEVELS OF YOUNG FEMALES

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DEDICATED TO

My loving and supportive family – Eben, Marko, Amé and my parents

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ABSRACT

Epidemiological studies indicated that vitamin A status is associated with plasma fibrinogen levels. It has been shown that Africans have high plasma fibrinogen levels compared to Europeans, probably contributing to their high risk for stroke. In this study the hypothesis that increased intakes of vitamin A will lower plasma fibrinogen levels, has been examined. The study formed part of a clinical intervention trial under controlled conditions to examine the effects of vitamin A fortified sugar on plasma fibrinogen of African females aged 13-25 years.

The methodology included questionnaires determining demographic profile, food consumption patterns and compliance to fortified sugar consumption. Measurements included anthropometric measurements and double baseline biochemical measurements on 95 subjects in the randomly selected sample population. The sample population was randomly divided into an experimental (n=46) group consuming fortified sugar (80 IU vitamin A per gram sugar), and a control group (n=49) consuming non-fortified sugar. Measurements were repeated after 4, 8 and 12 weeks of sugar consumption. Plasma fibrinogen levels were determined by the Dade Behring - Multifibren® U method. The method is a modification of the Clauss method.

The baseline measurements of plasma fibrinogen showed that 5.1% of respondents had a fibrinogen level of < 2.71 g/l, 83.8% a fibrinogen level 2.72 – 3.30 g/l and 11.1% of respondents had a fibrinogen level > 3.30g/l.

Baseline measurements of serum vitamin A indicated that 12% of respondents had a level of <30 µg/dl, 33.7 % a level of 30-40µg/dl, 38.6% a level of 40-50µg/dl and 15.7% of the respondents had a serum vitamin A level of >50µg/dl.

During the experimental period, the subjects consumed approximately 60g sugar per day, providing an additional 4800 IU or 1446 µg RE vitamin A per day to the experimental group.

The results indicated that mean plasma fibrinogen levels of the experimental subjects were significantly lower than the baseline results (2.81 g/L) after 4 weeks (2.64 g/L) and 8 weeks (2.65 g/L). However, levels increased and returned to baseline levels at week 12 (2.84 g/L). The experimental subjects showed an increase in body weight at week 12, and it is speculated that this increase could have been responsible for the increase in plasma fibrinogen.

In conclusion the study confirmed that by the intake of fortified sugar providing additional vitamin A in the diet of these African women, the plasma fibrinogen levels were significantly decreased at 4 and 8 weeks. It is further recommended that the influence of vitamin A on fibrinogen should be examined in subjects with raised fibrinogen and decreased vitamin A levels.

This work has been presented.....*see Annexure 12.*

Key words: Fibrinogen, vitamin A fortification

OPSOMMING

Epidemiologiese studies toon aan dat vitamien A status geassosieer word met plasmafibrinogeenvlakke. Dit is aangetoon dat swart Suid-Afrikaanse bevolkingsgroepe 'n hoër plasmafibrinogeenvlak in vergeleke met Europeërs het, wat 'n moontlike bydraende faktor tot die hoë risiko vir beroertes in die populasie kan wees. In hierdie studie word die hipotese dat 'n toename in die inname van vitamien A, plasmafibrinogeen verlaag, ondersoek. Hierdie studie vorm deel van 'n kliniese ingreepstudie in gekontroleerde toestande, om die effek van vitamin A gefortifiseerde suiker op plasmafibrinogeen van Suid-Afrikaanse swart vroue, 13-25 jaar oud, te ondersoek.

Die metodologie sluit vraelyste in om die demografiese profiel, suikerverbruik en aanvaarbaarheid te bepaal. Meetinstrumente sluit in 'n dubbele basislyn antropometriese en biochemiese bepalings op 95 proefpersone in 'n ewekansige steekproef populasie. Proefpersone is verdeel in 'n eksperimentele groep (n=46) wat gefortifiseerde suiker gebruik het (80 IU vitamien A per gram suiker), en 'n kontrole groep (n=49) wat plasebo suiker gebruik het. Bepalings is op 4, 8 en 12 weke van verbruik herhaal. Plasmavlakke is deur 'n Dade Behring - Multifibren® U metode bepaal. Die metode is 'n aangepaste Clauss metode.

Die resultate van basislynbepalings van plasmafibrinogeenvlakke was as volg: 5.1% van die proefpersone het 'n fibrinogeenvlak minder as 2.71 g/l gehad, 83.8% van die proefpersone 'n fibrinogeenvlak tussen 2.72 en 3.30 g/l en 11.1% van die proefpersone 'n plasmafibrinogeenvlak van meer as 3.30g/l.

Basislynbepalings van serum vitamin A vlakke het getoon dat 12% van die respondente 'n vlak van meer as 30 µg/dl gehad het, 33.7 % 'n vlak van tussen 30 en 40µg/dl, 38.6% 'n vlak van tussen 40 en 50µg/dl en 15.7% van die respondente 'n serum vitamin A vlak van meer as 50µg/dl.

Gedurende die proefperiodes het die populasie gemiddeld 60g suiker per dag gebruik. Dit het 'n addisionele 4800 IU of 1446 µg RE vitamin A aan die eksperimentele groep voorsien.

Die resultate het getoon dat die gemiddelde plasmafibrinogeenvlakke van die eksperimentele groep betekenisvol verminder het vanaf die basislynresultate (2.81 g/L). Na 4 weke van die intervensie was dit 2.64 g/L en 8 weke 2.65 g/L. Waardes het egter weer toegeneem tot op basislynvlak tydens week 12 (2.84 g/L). Die kontrolegroep se resultate toon toename in gewig tydens week 12. Daar word gespekuleer dat die toename in gewig moontlik verantwoordelik was vir die toename in plasmafibrinogeenvlakke.

As resultaat van die studie word tot die gevolgtrekking gekom dat die inname van gefortifiseerde suiker wat addisionele vitamien A verskaf het in die dieet van die proefpersone, plasmafibrinogeenwaardes betekenisvol verminder het tydens weke 4 en 8.

Die aanbeveling word gemaak dat toekomstige studies die effek oor 'n langer periode, met proefpersone wat 'n hoë plasmafibrinogeenvlak en 'n lae vitamien A status het,behoort te ondersoek.

Hierdie resultate is reeds aangebied sien:.....annexure 12

Sluitelwoorde: Fibrinogeen, vitamien A fortifisering

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List of abbreviations

10^9/L	10^9 cells per liter
Å	Armstrong
BMI	Body Mass Index
°C	Degree Celsius
Ca^{2+}	Calcium
CARDIA	Coronary Artery Risk Development in Young Adults.
CHD	Coronary heart disease
CHE	Christian Higher Education
Cn	Cyanide
CRP	C-reactive protein
CVD	Cardiovascular disease
Da	Dalton
EDTA	Ethylenediaminetetracetic acid
Etc.	*Et cetera*
E.g.	Example
FAO	Food and Agricultural Organization of the United Nations
FDP	Fibrinogen Degradation Products
Fe^{2+}	Iron
g/l	Gram per liter
GIT	Gastrointestinal tract
Hb	Haemoglobin
Hct	Haematocrit
HDL	High density lipoprotein
HMW	High molecular weight
HPLC	High-performance Liquid Chromatography
IL	Interleukin
IVAG	International Vitamin A Consultative Group
IU	International Unit
K	Potassium

kDa	Kilodalton
LDL	Low density lipoprotein
LMW	Low molecular weight
Lp	Lipoprotein
Lp(a)	Lipoprotein a
MCV	Mean cell volume (red cell)
MI	Myocardial infarction
Min.	Minute
mmol/l	millimoles per liter
Nm.	Nanometer
PAI	Plasminogen Activator Inhibitors
p-lip	Phospholipid
Plt	Platelet count
QffQ	Quantified food frequency questionnaire
RBC	Red blood cell count
RDA	Recommended daily allowance
RE	Retinol Equivalents
SA	South Africa
SD	Standard deviation
SHHS	Scottish heart health study
SMC	Smooth muscle cell
SPSS	Statistical Package for Social Sciences
THUSA	Transition and Health during Urbanisation in Southern Africa
tPA	Tissue –type plasminogen activator
USAID	United States Agency for International Development
UV	Ultraviolet
VA	Vitamin A
VAD	Vitamin A deficiency
VITA	Vitamin A Initiative
WBC	White blood cell count

WHO	World Health Organisation
α	*Alpha*
β	*Beta*
γ	*Gamma*
μ	*Micro*

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Chapter 1

The problem and its setting

1.1 Introduction

The clear association between high fibrinogen levels and the risk of cardiovascular disease (CVD) is of increasing practical relevance as well as scientific interest. The relationship was first reported in preliminary results from the Northwick Park heart study in 1980 (Meade *et al.*, 1980:1050).

Numerous other prospective studies have almost without exception confirmed a strong and independent effect of raised plasma fibrinogen in both the onset and progression of ischaemic heart disease (IHD), stroke and lower extremity arterial disease. As evidence accumulates to implicate fibrinogen as a risk factor for CVD, it becomes important to characterise the levels and correlates of fibrinogen in diverse populations. Knowledge of the correlates of fibrinogen can contribute to disentangle the independent contribution of elevated fibrinogen concentration to CVD (Folsom, 1995:21).

Changes in coronary heart disease (CHD) have been linked with dietary changes during war years and have been advanced as evidence supporting the diet-lipid-CHD hypothesis. It seems unlikely, however, that diet could have such a rapid effect in modifying mechanisms that lead to atherosclerosis. Furthermore, a number of prospective studies have shown relationships between diet and CHD mortality even after adjusting for serum cholesterol levels. It seems likely that mechanisms other than those that involve lipid infiltration could link diet and heart disease. In particular, the relationship between nutritional variables and haemostatic factors (Rogers *et al.*, 1988:197) is thought to contribute to this link.

1.2 Motivation

In the face of the present HIV/AIDS epidemic in many countries, it is unlikely that their fragile economies will be able to cope with a major epidemic of CVD, unless emphasis is shifted from secondary treatment to primary prevention of risk (James *et al.*, 2000:384). Due to changes in the political dispensation, the African population of South Africa is under a rapid urbanisation process (ANON, 1998:200). The increase in hypertension and stroke rates of urban Africans have been well described (Bradshaw *et al.*, 1999:40), but less is known about the influence of diet and lifestyle on plasma fibrinogen levels (James *et al.*, 2000:384), one of the major risk factors for stroke. For prevention of stroke, it therefore seems important to examine factors that influence plasma fibrinogen levels of Africans.

1.3 Problem statement

Murray and Lopez (1996:350) point out that in 1990, IHD was the number one cause of death worldwide with cerebrovascular disease (CVA) in second place. They estimated that these two would remain first and second leading causes of death in the year 2020, mainly because of increased prevalence in developing countries.

Steyn (1992:20) indicated that chronic diseases of lifestyle and their risk factors have major impact on disease patterns in South Africa. It is suspected that as infectious diseases are controlled in developing sectors, chronic diseases of lifestyle will become more common. These chronic diseases include CHD, stroke, hypertension, type 2 diabetes mellitus and some cancers.

Table 1 ***Leading causes of death in rural areas of South Africa****

RANK	AGE GROUP		
	35-54 years	**55-74 years**	**≥ 75 years**
1	Assault *10%*	CVA *11%*	IHD *11 %*
2	CVA *8%*	IHD *9.2%*	CVA *6%*
3	Motor accidents *7%*	Pulmonary tuberculosis *9%*	Pulmonary tuberculosis *4%*
4	AIDS *6%*	Genito-urinary cancer *3.3%*	Diarrhea *3%*
5	IHD *5%*	Motor accidents *2.9%*	Gastro-intestinal cancer *2%*

* In the rural areas of South Africa, the Agincourt study from 1992 to1995 the leading causes of death by selected age groups are as shown in ***table 1.*** Adapted from Fritz (1997:585); The Argincourt study, (1992-1995).

If one accepts the hypothesis that elevated plasma fibrinogen levels are causally linked to atherogenesis and to its thromboembolic complications, lowering fibrinogen levels should retard the atherosclerotic process and reduce cardiovascular events, which is of considerable clinical and public health interest (Markowe *et al.*, 1985:1313).

There is some but not conclusive evidence that low vitamin A status may be associated with raised plasma fibrinogen (reviewed by Vorster *et al.*, 1997(b):683). It is also known that South African children have a problem of vitamin A deficiency as indicated by the SAVACG study results (SAVACG, 1995:335). A meta-analysis of available literature showed that black South African adolescents and adults generally have lower than recommended intakes of vitamin A (Vorster *et al.*, 1997(b):683).

Therefore, Africans with low vitamin A status, high fibrinogen levels (Vorster *et al.*, 1998:170) and high stroke incidence and prevalence (reviewed by James *et al.*, 2000:390) should be an appropriate group to examine the hypothesis that increased intake of vitamin A will lower plasma fibrinogen levels.

1.4 Purpose of the study

1.4.1 Objectives

The major objective of this project was to examine the effects of sugar fortified with vitamin A in young, black South African females aged 13 to 25 years in a placebo-controlled clinical trial on plasma fibrinogen levels.

1.4.2 Subsidiary objectives

The subsidiary objectives were to establish in the same sample population, the following:

a) What is the vitamin A status of the young African females in the Vaal Triangle?

b) What is the fibrinogen status of the young African females in the Vaal Triangle?

c) Is there a correlation between vitamin A status and plasma fibrinogen levels?

d) Is there a correlation between plasma fibrinogen levels and white cell count?

e) Is there a correlation between plasma fibrinogen levels and red cell parameters (Red cell count (RBC), haemoglobin (Hb), haematocrit (Hct), and mean cell volume (MCV)?

f) Is there a correlation between plasma fibrinogen levels and iron status parameters (iron, ferritin, transferrin, and soluble transferrin receptors)?

g) Is there a correlation between plasma fibrinogen levels and BMI?

1.5 Research methodology

1.5.1 Literature study

A literature study was done using sources at the Vaal Triangle Technikon library as well as the Ferdinand Postma Library at the Potchefstroom University.

The following areas were included in the literature study:

- Vitamin A deficiency: globally and in South Africa
- Food fortification with vitamin A
- Fibrinogen structure
- Fibrinogen physiology
- Fibrinogen pathophysiology
- Epidemiological studies on fibrinogen status: globally and in South Africa
- Interaction between vitamin A and fibrinogen

1.5.2 Empirical research

A double blinded, "placebo"-controlled trial for 12 weeks in young African women, aged 13-25 years was conducted, with sugar fortified with vitamin A as intervention and plasma fibrinogen as outcome.

Validated questionnaires were used to determine demographic profiles, food consumption patterns and compliance of fortified sugar consumption or placebo sugar during the trial. Anthropometry and double baseline biochemical measurements were done on 95 randomly selected subjects. Measurements were repeated after 4, 8 and 12 weeks of sugar consumption.

Anthropometry measurements included:

- Weight / Height and waist hip circumferences to determine the body mass index (BMI) and W:H ratio

Biochemical measurements included:

- Serum vitamin A levels (10 ml clotted blood, light protected).
- Plasma fibrinogen levels (5ml citrated blood (1part sodium citrate (0.11 mol/l) with 9 parts venous blood)).
- 5ml Blood in an EDTA tube (purple lid) for full blood count
- 10 ml blood in a silicone-coated tube for preparation of serum for the analysis of iron, ferritin, transferrin and soluble transferrin receptor (sTrF).

1.5.3 Subjects:

A random sample of 100 African women volunteers aged 13-25 was selected using the following sampling procedures: The Vaal Triangle population was divided into groups, consisting of the different towns in the Vaal Triangle. The names of the suburbs per town were thrown in a hat and drawn. Thereafter the names of all the schools, tertiary institutions and clinics in the selected suburbs were thrown into a hat. One school or tertiary institution and one clinic per suburb were drawn from the hat to form part of the sample population.

The sample population of 100 was then randomly divided into an experimental (n=46) group consuming fortified sugar (80 IU vitamin A per gram sugar), and a control group (n=49) consuming non-fortified sugar.

1.6 Contribution of author to the study

This project was a team research project. Dr. Wilna Oldewage-Theron co-ordinated and administrated the project as project leader. This study was also an under-study of Dr. Wilna Oldewage-Theron's Ph.D. study. Ms. Emsie Dicks studied the compliance and consumer acceptability of the fortified sugar. Ms. Mosa Selepe did the nutritional data gathering and analyses. Mr. Jose van Rensburg assisted in blood analysis.

The author's contributions to the study were:

- Standardising laboratory infrastructure (Apparatus placements, reagent purchasing, and apparatus calibration).
- Training of the nursing sister for fieldwork.
- Management of the laboratory test procedures and quality control program.
- Co-ordinate consigns of the vitamin A samples.

1.7 General outline of the thesis

Chapter 1: Problem statement and its settings

This chapter includes a brief discussion covering the motivation of the study, the problem statement, research objectives and aims as well as research methodology.

Chapter 2: Literature review

In chapter two an analysis of the literature regarding fibrinogen, vitamin A, fortification, and possible interactions between vitamin A status and plasma fibrinogen was made.

Chapter 3: Methodology

In this chapter the study design, experimental subjects and different methods used in this study are discussed.

Chapter 4: Results and discussion

In chapter four the results are given and compared to findings of other studies.

Chapter 5: Conclusions and recommendations

The results of the study are interpreted in chapter 5. Conclusions are drawn and recommendations regarding the intervention of fortified vitamin A on plasma fibrinogen levels of the sample population made. The need for further research is indicated.

Chapter 2

Literature review

2.1 Introduction

Smith (1995:11) reported that Rokitansky (1852) described the atheromatous process and suggested that the deposit is a product derived from the blood and for the most part from fibrin of the arterial blood. In 1946 Duguid demonstrated that fibrin existed both within, and on the surface of apparently intact fibrous plaques. Meticulous histological studies demonstrated thrombotic occlusion, frequently associated with plaque rupture, in most necropsies with myocardial infarction. In 1952 Gilchrist and Tulloch, studying patients with acute MI in the Edinburgh Royal Infirmary, reported for the first time elevated plasma fibrinogen levels in patients with arterial disease.

Since the discovery of the importance of micronutrients, intensive research has provided an appropriate perspective regarding some aspects of their essentiality as well as their role in relation to the prevention of disease. It is realised today that micronutrients not only protect against deficiency diseases, but are also involved in the prevention of some chronic, degenerative diseases. However the relationship between micronutrient status and plasma fibrinogen, as well as the consequences of raised fibrinogen levels have not been studied in detail.

2.2 Fibrinogen

2.2.1 Historical background

An understanding of the fibrinogen molecule, its structure, synthesis and function is necessary in order to analyse its independent contribution to CVD, as well as to improve CVD risk assessment and intervention.

Blombäck (2001:1) in a keynote address at the 16th International Fibrinogen Workshop gave a historical review on fibrinogen research. He stated that the observation of the coagulation of blood could be traced back to early historical times. In about 300 BC Aristotle declared that blood clotting was similar to freezing of water; blood clot when energy from the heart dissipated. It was associated with death. Galen, the teacher of humoral pathology, described the phenomenon that in many states of disease, clotted blood had a whitish cap on top of red cells. Galen thought it was due to an excessive and dangerous secretion of one of the four humors, the phlegm. It was only in the 17th century that Malpighi described a clot as a white fibrous substance obtained from a "red cell clot" after washing with water. In 1770 Hewson showed that coagulation takes place in plasma. Deni de Commercy proposed in 1859 that there existed a precursor of fibrin that he called fibrinogen. Fibrinogen transforms into fibrous fibrin by a process called denaturation. Alexander Schmidt indicated that a "thrombic" enzyme was involved in fibrin formation. Olof Hammersten prepared a highly purified form of fibrinogen from horse plasma through precipitation with salt. Hammersten's preparation did not clot spontaneously but required the addition of a 'thrombotic enzyme". Then came a lull in fibrinogen research. In the 1940s and 1950s numerous measurements of electrophoretic mobility, sedimentation and diffusion constants, osmotic pressure, intrinsic viscosity, flow birefringence and light scattering were made on fibrinogen, especially by American investigators. From different physicochemical measurements, a picture of the fibrinogen molecule as a prolate ellipsoid emerged. This model had a length of 500-700 Å and axial ratio of 5-20 Å. Molecular weight varied considerably but the most reliable was considered to be 330-340 kDa.

By the end of 1970 the major architectural features as well as the complete covalent structure of fibrinogen, was known. Recent crystallographic studies in the laboratories of Davie, Doolittle, and Cohen have not only confirmed the early interpretations of fibrinogen structure and function, but have also given exciting additional information at the atomic level.

The role of fibrin as a structural element in pathological thrombi had been evident since Virchow's time. Reports on the interaction of fibrinogen or fibrin with cellular elements started to emerge in the 1980s. Epidemiological studies have shown that an increase in plasma fibrinogen levels is a risk factor for ischaemic heart disease. High fibrinogen levels cause tight network structures during clotting. These structures are elastic, with thin fiber strands, and therefore brittle. Such structures are believed to be thrombogenic. Low fibrinogen levels give rise to plastic structures that deform in flow, considered as nonthrombogenic.

A new fibrinogen analogue, with a molecular weight of 420 kDa, has been discovered. This interesting protein is fully clottable. However, it is present in very low concentrations (1-4% of total fibrinogen) and its role in physiology is far from enigmatic.

2.2.2 Structure of Fibrinogen

Human fibrinogen is a large glycoprotein encoded on chromosome 4, with a mass of approximately 340 000 Da. It is composed of three pairs of non-identical polypeptide chains (α (alpha), ß (beta), γ (gamma)) joined together by disulfide bonds (Lip, 1995:155). The major characteristic is a slightly bent and twisted long tri-nodular shape. It has a half-life of 3-4 days (Resch & Ernst, 1994:170).

Fibrinogen molecules are elongated 45-nm structures consisting of two outer D domains; each connected by a coiled-coil segment to a central E domain (*see figures 1 and 2)*. It has six polypeptide chains of three types: two Aα, two Bβ and two γ chains, which are joined together within its N-terminal E domain by 5 symmetrical disulfide bridges, one pair at position Aα28, two pairs between Aα36 and Bβ65, and a third set between γ8 and γ9 positions (Mosesson *et al.*, 2001:13). Biochemical and immunological studies have shown that the central globule (fragment E) comprises the aminoterminal ends of all six polypeptide chains, whereas the distal globules (fragments D) are composed of the more

carboxyl-terminal regions of the chains. The Aα chain consists of 610-amino acids, the Bβ chain 164-amino acids and the major γA 411-amino acids (Mosesson, *et al.*, 2001:13). From a review of relevant literature, Iacoviello *et al.* (1998:270) concluded that polymorphism in coagulation factor genes contribute to the variability in plasma levels of clotting factors within their range of distribution. It was also mentioned that the polymorphism contributes to the risk of CVD by influencing a person's response to environmental risk factors.

Most of the body's fibrinogen is found in plasma. It exists as a population of slightly different molecules (Blombäck, 1996:30). The major fraction of ± 50% is high molecular weight fibrinogen (HMW, 345 KD), followed by ± 40% low molecular weight fibrinogen (LMW, 300 KD). The remaining fractions are LMW fibrinogen (280 kD) and fibrinogen 420. Fibrinogen 420 (1-4% of total fibrinogen), contains two extended α chain isoforms (αE) (Grieninger *et al.*, 1997:2610).

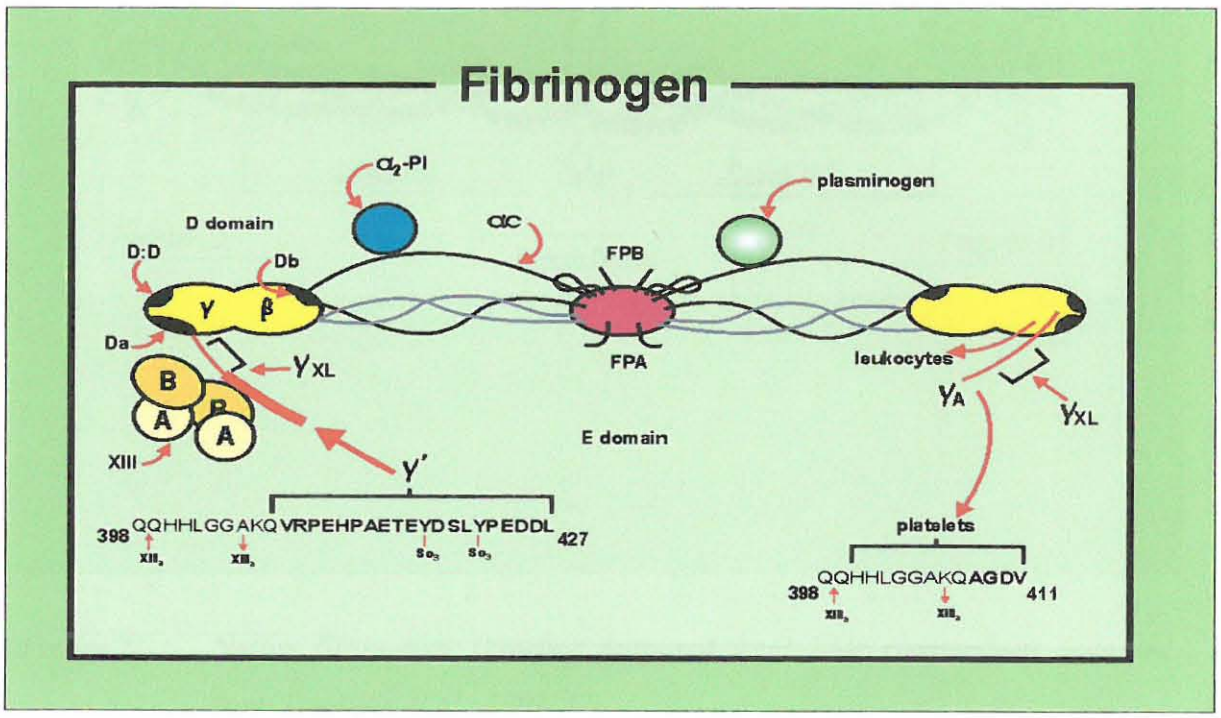

Figure 1 ***Schematic diagram of fibrinogen showing the major structural domains, the association sites that participate in fibrin polymerization and crosslinking, and other molecular and cellular binding interactions (adapted from Mosseson et al., 2001:12)***

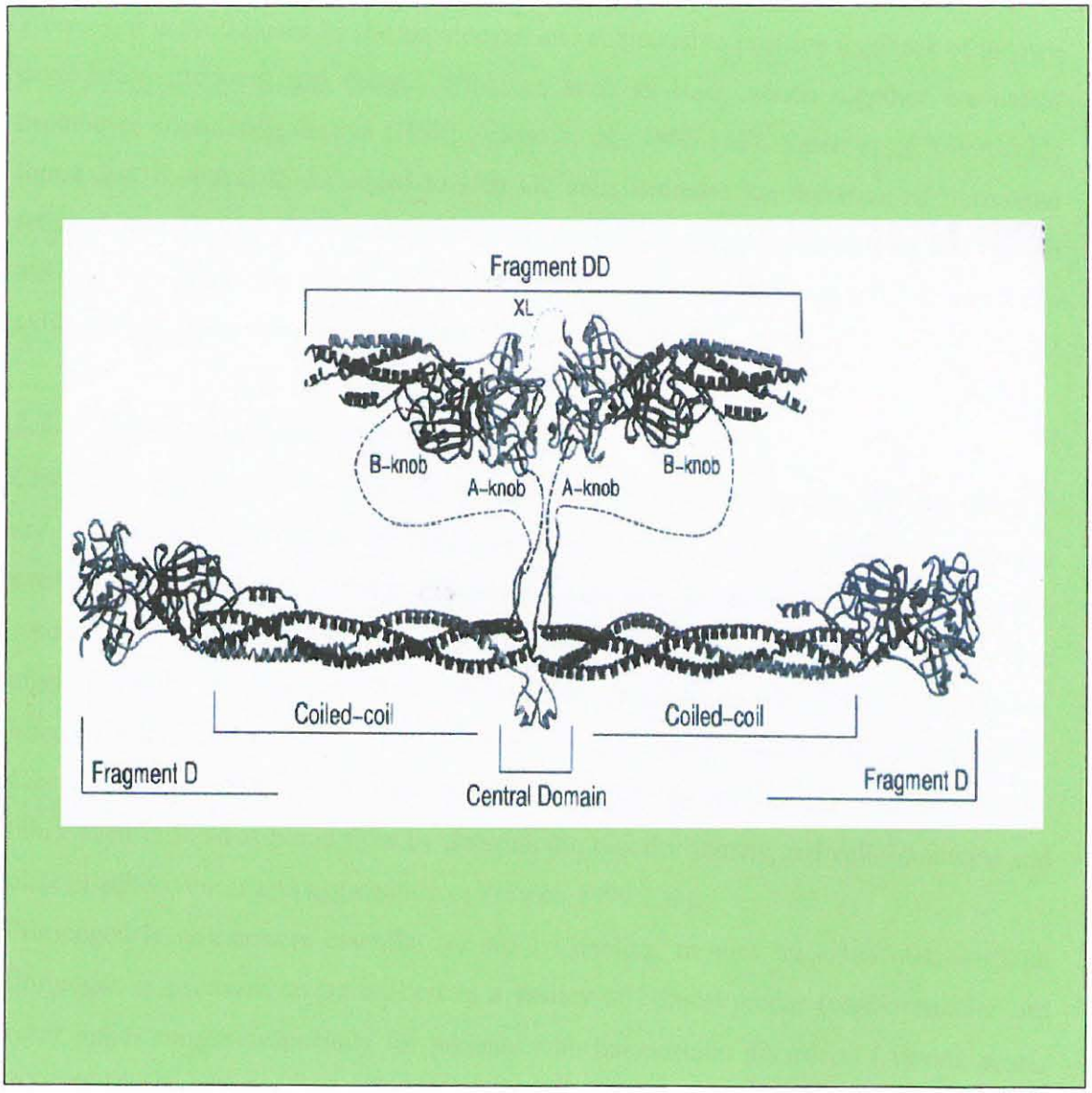

Figure 2 ***Native fibrinogen showing proposed knob-hole connections (adapted from Doolittle et al., 2001:37)***

2.2.3 Production of fibrinogen

Fibrinogen is synthesised by the hepatocyte, and subjected to positive feedback of its own degradation products and certain cytokines such as IL-6, which together are called hepatocyte stimulating factors (HSF) (Vasse *et al.*, 1996:332). Vasse *et al.* (1996:332) found that IL-4 and IL-13 added to Hep G2 cells decrease the secretion of fibrinogen released into the Hep G2 conditioned medium. This effect was related to a decrease in mRNA expression for fibrinogen. The effect of IL-10 was less intense and only evidenced by a decrease in expression of fibrinogen mRNA.

2.2.4 Function of fibrinogen

Circulating fibrinogen comprises a heterogeneous family of closely related molecules that are soluble and which share the ability to form a fibrin gel via a series of molecular events (Nieuwenhuizen, 1995:6). Fibrinogen is the key protein of the blood coagulation cascade *(see figure 3)*. The first process involves the formation of a temporary and unstable clot by the platelets. Thereafter, platelets are equipped with fibrinogen receptors. Finally fibrinogen stabilises the clot in order to stop bleeding. (Resch & Ernst, 1994:170).

Circulating plasma fibrinogen, the coagulation and fibrinolytic systems:

Fibrinogen influences blood flow by determining blood viscosity, red cell, leukocyte and platelet adhesiveness and aggregability (Vorster, 1999:139).

Fibrinogen is furthermore essential for wound healing, to such an extent that artificial fibrinogen is produced to be utilised in a variety of clinical arenas (cardiovascular and other major surgery, especially for patients with haemostatic disorders) (Amrani *et al.*, 2001:566).

Fibrinogen is also an important acute phase reactant. Levels can rise 2-20 fold with acute stress, such as infection surgery, etc. (Folsom, 1995:22).

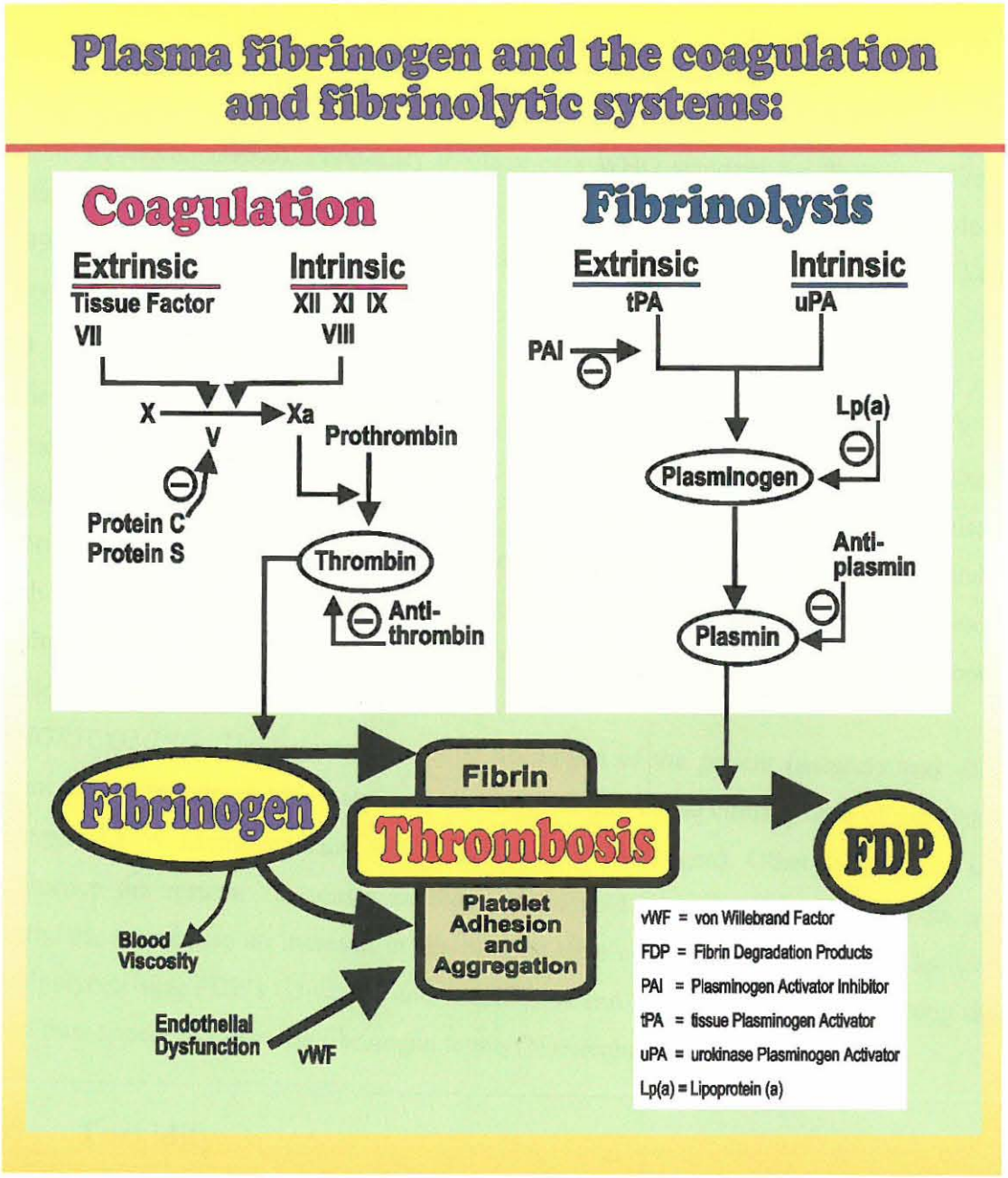

Figure 3 *The role of plasma fibrinogen in the coagulation and fibrinolytic system*

(Adapted from Lip, 1995:155)

2.2.5 Determination of fibrinogen

Several methods are available, but the molecular heterogenecity of fibrinogen (and other plasma factors) can influence the results of these assays to different extents (Niewenhuizen, 1995:6). Previously the lack of a WHO standard for fibrinogen limited inter-laboratory comparisons, because of differences in absolute values (Meade, 1997:14), but an international fibrinogen standard is now available (Gaffney & Wong, 1992:431)

a) Clotting Rate

The most frequently used method is that of Von Clauss (Von Clauss, 1957:231-237): The time between the addition on thrombin to plasma and clotting is recorded. The excess amount of thrombin chosen ensures that the clotting time depends on the plasma fibrinogen levels. A calibration curve is usually compiled by a series of diluted standard values. Plasma is obtained when anticoagulant is added during collection of blood in order to prevent coagulation. After separation the 55% fluid portion on top of the blood is called plasma (Brown, 1988:1)

SHORTCOMING: The heterogeneities of fibrinogen of the patient (subject) may differ from the calibration material, and may cause variability in the clotting time of the plasma sample (LMW fibrinogen clots slower than HMW fibrinogen). Other factors may also influence the results. Increases in blood sialic acid content, such as found in liver cirrhosis, may cause an increase in the clotting time of the sample. Increased levels of antipolymerising FDP's (D-dimer, and fragment X and Y), will prolong the clotting time and thus appear to lower the fibrinogen levels (Niewenhuizen, 1995:7).

b) Turbidity

This method is based on the change in turbidity when fibrinogen clots, detectable at a wavelength of 300nm (Ellis & Stransky, 1961:480).

SHORTCOMING: Any factor that will influence the structure and, therefore also the turbidity, will influence results (HMW/LMW/LMW' ratio). The concentration of calcium ions present in the sample may also play a very important role (Niewenhuizen, 1995:8).

c) Precipitation

Fibrinogen can be precipitated from plasma by heating at 56°C, or by adding salts such as sodium sulphite.

SHORTCOMING: A non-specific method that can be influenced by other plasma proteins (Niewenhuizen, 1995:8).

d) Immunological methods

Polyclonal antibodies are used to detect fibrinogen in assays such as radial immuno diffusion and Laurell rocket immunoelectrophoresis.

SHORTCOMING: These methods are not specific for fibrinogen but also detect fibrinogen derivatives such as soluble fibrin and FDPs.

DEVELOPMENTS: Enzyme immunoassay methods have recently been developed that are specific for the total HMW and LMW fibrinogen molecules. These methods do not detect soluble fibrin, LMW' and FDPs. Time-to-result duration is only one hour (Niewenhuizen, 1995:8).

e) Clottable protein

Thrombin is added to a plasma sample, which is then allowed to clot exhaustively. The clotted proteins are collected and washed extensively. The mass of the clot is assayed, directly by weighing or indirectly by dissolving in urea and the optical density (OD) measured at 279nm. In another version, the OD can be taken at 315 nm to compensate for contaminants such as entrapped lipids.

SHORTCOMING: The heterogeneities seem to have no or little effect on this procedure. This procedure is however, time-consuming and laborious (Niewenhuizen, 1995:7).

2.2.6 Fibrinogen and cardiovascular diseases: Pathophysiology of fibrinogen

Evidence exists that fibrinogen is involved in early local atherosclerotic changes of the vessel wall. Experimental de-endothelialised arterial segments indicated that fibrinogen is integrated directly into the vascular lesion, where it is converted into fibrin and fibrinogen degradation products and promotes the formation of thrombi in a dose-dependant manner (Resch & Ernst, 1994:171). The same authors reviewed the evidence that a dose-dependant stimulating effect seems to exist on:

- The migration of smooth muscle cells
- The proliferation at the site of the lesion
- The permeability for proteins which leads to accumulation of low-density lipoproteins
- The enhancement of the adhesives of white blood cells, which is pathologically relevant in microcirculation

Monocyte activation could play a central role in atherogenetic pathogenesis by inducing:

- The expression of tissue factor and plasminogen activators responsible for fibrin and fibrin degradation products formation which are mitogenic for smooth muscle cells (Vasse *et al.*, 1996:332).
- The production of cytokines (IL-6, Oncostatin M (OSM), Leukaemia inhibiting factor (LIF)), stimulating the fibrinogen production by the hepatocyte with different efficiency (Vasse *et al.*, 1996:332). These cytokines are also responsible for vessel injury, whereas cytokines such as IL-4, IL-10, IL-13, which protect vessels against injury, are capable of antagonising *(in vitro)* the increase in fibrinogen induced by inflammatory factors (Vasse *et al.*, 1996:335).

Mechanisms through which increased plasma fibrinogen levels promote ischaemic events (*see figure 4):*

- *Atherogenic:*

Fibrinogen may infiltrate the arterial wall where it can be converted to fibrin. Within the arterial wall, fibrin binds to low density lipoprotein and is converted into degradation products which stimulate smooth muscle cell proliferation as well as the uptake of lipids by macrophages (Smith, 1995:12).

- *Determinants of whole-blood and plasma viscosity:*

The total peripheral resistance is composed of a vascular and a viscous component (Resch & Ernst, 1994:170).

Fibrinogen is an important determinant of plasma viscosity and red cell aggregation and hence, of whole blood viscosity (Lowe & Rumley, 1999:92) due to its slightly bent and twisted long tri-nodular characteristic (Resch & Ernst, 1994:170). Raised levels of elongated high molecular weight fibrinogen increase viscosity which, in the presence of peripheral arterial narrowing, is associated with the risk of intermittent claudication, reduced perfusion pressure and reduced microcirculatory blood flow. Therefore, elevated plasma fibrinogen may promote atherogenesis, thrombogenesis and ischaemia, through its rheological effects (Lowe *et al.*, 1993:1920).

- *Increased platelet aggregation and thrombus formation:*

Increased plasma fibrinogen levels may be thrombogenic, by promoting platelet aggregation, as well as, fibrin formation.

- *Fibrin thrombus structure, deformability and lysability:*

High plasma fibrinogen levels are associated with an increased tendency to form an increased size of fibrin thrombi. Alteration of the structure of the fibrin clot reduces the lysasibility of the fibrin thrombi (Ernst & Resch, 1993:960).

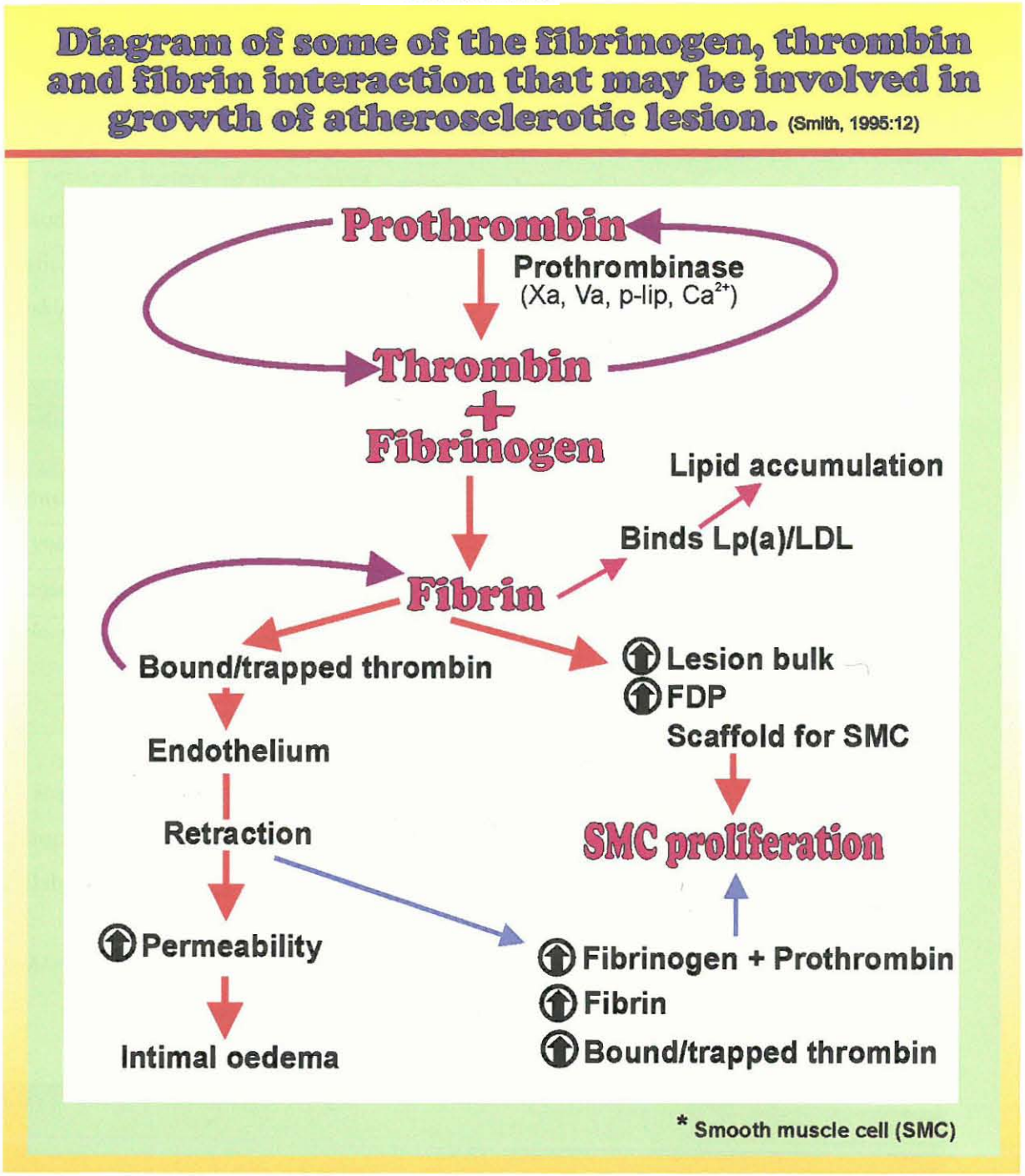

Figure 4: *Diagram of some of the fibrinogen, thrombin and fibrin interactions that may be involved in growth of atherosclerotic lesions (adapted from* Smith, 1995:12)

In the Scottish Heart Health Study (SHHS), persons with a family history of heart disease or personal history of high blood pressure, diabetes, stroke, or presence of intermittent claudication all had higher plasma fibrinogen concentrations than those without as indicated in ***Table 2*** (Lee *et al.*, 1993:338).

Table 2: ***Evidence from SHHS of correlation between CHD and increased plasma fibrinogen levels***

History	Plasma fibrinogen level (g/L) and standard deviation	
	Male	Female
None	2.27(0.01) (n=3367)	2.34(0.01) (n=3096)
Myocardial infarction	2.51(0.02) (n=248)	2.63(0.04) (n=72)
Angina	2.45(0.02) (n=394)	2.50(0.02) (n=398)

Values Differ significant at $p<0.001$

SHHS: Scottish Heart Health Study

CHD: Coronary heart disease

In assessing the importance of fibrinogen, viscosity, and white blood cell count as risk factors for ischaemic heart disease results were obtained in the Caerphilly and Speedwell collaborative Heart studies are indicated in ***Table 3*** (Yarnell *et al.*, 1991:838).

Table 3: ***Caerphilly and Speedwell collaborative Heart studies – the importance of fibrinogen, viscosity, and white blood cell count as risk factors for ischaemic heart disease (IHD)***

Test	Incidence		Age and area –standardized difference	
	No major IHD (n=4408)	Major IHD (n=233)	Mean	95%CI
Fibrinogen *(g/l)*	3.66±0.82	4.09±0.92	0.38	0.28-0.49
Viscosity *(cp)*	1.688±0.096	1.735±0.099	0.045	0.032-0.057
WBC *(10^9/l)*	7.02±2.01	7.86±2.22	0.84	0.57-1.10

Sanchez-Bayle *et al.* (1993:325) studied the correlation between plasma fibrinogen concentration in Spanish children and adolescents, and their family history for stroke and IHD. Children with a family history were found to have a significantly ($p<0.05$) higher plasma fibrinogen than the group without a family history.

Meta-analyses of prospective studies indicated that individuals in the upper third of the plasma fibrinogen distribution have about twice the risk of ischaemic heart disease or stroke compared to individuals in the lower third. This increased relative risk is similar in both sexes, and in individuals with or without baseline evidence of CVD. From the above evidence it seems that fibrinogen adds to risk factors of major cardiovascular predictive such as smoking, blood pressure and serum cholesterol (Lowe & Rumley, 1999:92). Therefore measurements of plasma fibrinogen could improve the assessment of CVD risk.

In the FINRISK haemostasis study fibrinogen and Lp (a) were associated with prevalent CHD in a Finnish population (Salomaa *et al.*, 1994:1293).
The 20-year follow up of the Framingham Heart Study revealed that fibrinogen had a powerful effect on initial cardiovascular events in men and in women, but that it influenced recurrent events only in men. This study also showed that the magnitude of risk diminished with advancing age in women (but not in men) and that fibrinogen was a stronger risk factor in men than in women (Kannel *et al.*, 1996:91). The SHHS showed that fibrinogen was a strong predictor of fatal, nonfatal, new or recurrent CHD, and of death of unspecified cause in both men and women (Woodward *et al.*, 1998:60).

A number of clinical trials indicated that in both men and women, a plasma fibrinogen level of 2.8 to 3.00 g/l may already be associated with a modest trend towards an increased CHD risk (Becker *et al.*, 1996:145).

2.27 Factors influencing fibrinogen levels

a) Fibrinogen and socioeconomic conditions

The Whitehall study of British civil servants (1967) showed a steep inverse association between social class, as assessed by grade of employment, and mortality from a wide range of diseases. The Whitehall II study investigated (between 1985 and 1988) the degree and causes of social morbidity in a new cohort of civil servants. An inverse association between employment grade and prevalence of angina, ischaemia and symptoms of chronic bronchitis was found, which persisted over a period of 20 years (Marmot *et al.*, 1991:1387). Adult plasma fibrinogen concentration was inversely associated with childhood environmental factors (adult height, father's social class, and educational participation) (Brunner *et al.* 1996:1008). Lower socioeconomic status (as shown by employment grade) was associated with higher fibrinogen concentrations. These findings are believed to be a marker of the biological pathways that mediate the inverse socioeconomic gradient in coronary disease (Brunner *et al.* 1996:1008). The Kuopio Ischaemic Heart Disease Risk Factor Study confirmed observations of an inverse association between plasma fibrinogen concentration and socioeconomic conditions, by utilising five socioeconomic indices (lifetime occupation, education, income, ownership of material possessions, and childhood socioeconomic status) in middle-aged Finnish men (Wilson *et al.* 1993:292). Markowe *et al.* (1985:13130) showed that between grades of employment significant differences in plasma fibrinogen concentration existed but found no difference in clotting factor II, VII, VIII, and X.

The causes of higher plasma fibrinogen levels in lower socio-economic strata are not clear. It could be related to environmental factors such as the smoking habits, nutritional status and/or low-grade infections

b) Seasonal changes

Higher plasma fibrinogen levels occur in winter compared to levels in the summer (Meade, 1995:33). Variation in fibrinogen levels can be 5-20% higher in winter than in

summer. There are speculations that higher fibrinogen levels in winter may reflect either increased body temperature or an acute-phase response to respiratory infections. It has also been speculated that the increased death rate from ischaemic vascular disease in winter is due to increased plasma fibrinogen levels (Folsom, 1995:24).

Van der Bom *et al.* (1994:18) indicated that in the Rotterdam study a 0.32g/l higher fibrinogen level (adjusted for age, gender and smoking) occurred in winter than in summer. The difference was greater in subjects 75 years and older (0.42g/l) than in those aged 55-74 years (0.28 g/l). Platelet count and body mass index were also higher in winter. Surprisingly, leucocyte count was lower in the winter than in summer (possibly due to a higher incidence of viral infections).

Crawford *et al.*, (2000:23) also found that significant seasonal rhythms were present for fibrinogen (amplitude 0.08g/l, peak May/June, p=0.05).

c) Diet

There exists some controversy in the literature about the effect of diet on plasma fibrinogen levels. Meade (1997:13) is of the opinion that fairly extensive evidence exists that fat, carbohydrate and fibre do not influence fibrinogen levels, at any rate in the short term. Equivocal evidence exists on the lowering effect of fish oil on plasma fibrinogen levels. Lee *et al.* (1990:915) reported that in the Scottish Heart Health Study, one of the first population based reports, that high fish consumption, especially of white fish, was weakly associated with elevated fibrinogen levels. From numerous study results it can however been concluded that although fish oil may inhibit platelet aggregability and lower some coagulation factors, it may also impair the fibrinolytic potential by lowering tPA and raising PAI-1 (Vorster *et al.*, 1997:677).

Rogers *et al.* (1988:199) indicated that total daily fibre intake was negatively associated with fibrinogen levels.

Cepelak *et al.* (1991) indicated that a low-energy diet could improve the fibrinolytic profile of patients with a deep-vein thrombosis with up to 50%.

Comparing two groups of Africans, it was found that the group who had a higher fat intake had a higher fibrinogen level than the group with lower fat intake; this group also had a higher fibre and lower cholesterol intake (Vorster *et al.*, 1997:674).

Blaberg *et al.* (1995:240) showed that a diet rich in stearic acid compared with one rich in myristic plus lauric acids increased plasma fibrinogen levels.

Very few studies have reported effects of vitamins on coagulation. It has, however, been reported that in healthy elderly women who took micronutrient supplements, higher levels of serum vitamin A, retinol binding protein, pyridoxal and pyridoxal phosphate were associated with lower plasma fibrinogen levels (Kruger *et al.*, 1994:112). The MONICA study has also shown that low plasma retinol levels were associated with higher fibrinogen levels and impaired fibrinolytic activity (reviewed by Vorster *et al.*, 1997:678). Delport (1999:14) also found that risk of CHD associated with vitamin A deficiency was notably higher compared to that associated with the deficiency of other anti-oxidative vitamins.

Increased intake of vitamin E results in decreased fibrinogen levels. When vitamin E is added to polyunsaturated fatty acids (PUFA's) it also results in decreased fibrinogen levels. The monounsaturated oleic acid, and the saturated palmitic acid decreased fibrinogen secretion, both alone and in combination with vitamin E. Vitamin E may have these effects by preventing the oxidation of PUFA's (Vorster *et al.*, 1997:678).

There is evidence that specific food or substances may also have an effect on other haemostasic factors. Strongly flavoured foods such as onions, chilies, and spices, as well as green tea may increase fibrinolytic activity (Vorster *et al.*, 1997:678).

The THUSA study of Africans in the Northwest province of South Africa (James et al., 2000:392), showed that subjects with the highest intakes of dietary fibre, plant proteins, vitamin E, and iron and with the highest P/S ratio; and with the lowest intake of *trans* fatty acids and animal protein had the lowest plasma fibrinogen. These results suggested that a low nutritional status could be associated with high plasma fibrinogen. James *et al.* (2000:386) found significant differences per fibrinogen quartile of some biochemical variables indicative of nutritional status. In both men and women, small but significant increases in serum calcium and magnesium were associated with a rise in plasma fibrinogen. Similarly, higher total serum protein and globulin levels were associated with higher fibrinogen, whereas significant decrease in vitamin E and vitamin B6 were associated with increased plasma fibrinogen levels. A decrease in serum iron and percentage saturation of transferrin was also associated with higher fibrinogen levels.

The associations between high plasma fibrinogen and low iron status (James et al., 2000:392) further support the hypothesis that under- nutrition is associated with high fibrinogen levels.

d) Physical activity

Strenuous exercise is associated with lower plasma fibrinogen levels. A difference of 15% in the risk of IHD, compared to sedentary lifestyle, has been reported. These results were derived from the Caerphilly Prospective Heart Study, SHHS and Gothenburg study (reviewed by Lip, 1995:160).

During the SHHS, Lee *et al.* (1990:915) indicated that the inactive in leisure group had higher fibrinogen levels. Females who claimed to be inactive at work had higher values than the average or active groups. Individuals indicating that they had reduced their level of physical activity over the previous year also had elevated fibrinogen levels.

A study of 51-year-old men living in the county of Copenhagen, showed that there was a clearly significant univariate and multivariate association between physical inactivity and fibrinogen level (Møller & Kristensen, 1991:344). The THUSA study of Africans in the Northwest province of South Africa, found that subjects who reported high physical activity (regular exercise and/or sport, in addition to walking long distances) had significantly lower fibrinogen levels than the moderately active and inactive subjects (James *et al.*, 2000:385). Results from the Northern Ireland Health and Activity survey supported that physical fitness (measured by using VO_2 max) has beneficial effect on the fibrinogen levels (MacAuley *et al.*, 1996:260).

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 the blood. However, less is known about the acute effect of physical activity on plasma fibrinogen levels.

e) Smoking

The Framingham study found that age-adjusted fibrinogen values were significantly higher in cigarette smokers than in nonsmokers (*see table 4 and 5)*. Fibrinogen increased

with the amount of cigarettes smoked in each sex. Ex-smokers had values as low as non-smokers did; suggesting a direct but reversible effects (Kannel *et al.*, 1987:1007).

Table 4 Fibrinogen by cigarette habit: subjects 47-79 years of age[#].

Cigarettes/day	Male (mg/dl)[Ω]	Female (mg/dl)[=]
None	278	292
< 1pack	281	296
> 1pack	301	306

[Ω] *p<0.001*

[=] *p<0.05*

[#] Data from the Framminghan study (Kannel *et al.*, 1987:1007)

Table 5: Age adjusted means of fibrinogen by sex and smoking status[#]

Smoking status	Male (mg/dl)	Female (mg/dl)
Nonsmokers	275(n=108)	293(n=371)
Former smokers	282(n=109)	290(n=115)
Current smokers	296(n=336)	302(n=275)

[Ω] *Differences significant at p<0.001 for men and p<0.07 for women*

[#] Data from the Framminghan study (Kannel *et al.*, 1987:1007)

A switch from cigarette to cigar smoking is inexplicably associated with a large increase in plasma fibrinogen, which is in keeping with the observation that cigar smokers are at an increased risk of IHD (Lip, 1995:160).

Lip (1995:165) reported numerous study results in a literature review, which showed the positive relationship between smoking and plasma fibrinogen levels:

- The WHO Co-operative trial (15 000 men) of clofibrate found the highest fibrinogen levels amongst smokers.
- Results of the Copenhagen cross-sectional population study, (439 men aged 51 years) indicated that one of the strongest independent associations with plasma fibrinogen was smoking habit.

- A Swedish study of a cohort of 788 healthy men aged 54 years indicated that plasma fibrinogen positively correlated with cigarette smoking.
- The Northwick Park Heart Study found the lifetime duration of smoking was a determinant of initial plasma fibrinogen levels.

There are various possible mechanisms that explain the effect of smoking on the plasma fibrinogen levels (Lip, 1995:160):

- Smoking may lead to endothelial damage, resulting in the stimulation of the coagulation system and increased production and release of coagulation factors.
- Smoking may directly lead to an increase in the concentration of clotting factors, resulting in a tendency to thrombosis and the formation of mural microthrombi on otherwise damaged endothelium.
- Activation of lung macrophages by smoking, which can produce hepatocyte-stimunlating factor (IL-6), which increases fibrinogen synthesis and secretion.

Results from the MONICA study (Eliasson *et al.*, 1994:15) indicated that although plasma cotinine concentration (the main metabolite of nicotine) were higher in snuff users than in smokers, snuff users had fibrinogen levels lower than smokers, equivalent to non-smokers. The conclusion was drawn that the detrimental effect of smoking on fibrinogen levels is not mediated by nicotine. A small study of nicotine gum corroborates these conclusions (Johnston *et al.*, 1984:103).

f) Adult height

Brunner *et al.* (1996:1009) indicated that taller men ($p<0.05$) and women ($p<0.005$) had lower fibrinogen concentrations than shorter men and women. This observation is intriguing and may be related to socio-economic class and nutritional status in childhood. Shorter men and women may be a consequence of chronic malnutrition (stunting) as children.

g) Metabolic syndrome

Fibrinogen concentration shows a direct association with features of the metabolic syndrome, including raised fasting insulin concentration, high waist hip ratio, raised serum triglycerides, hypertension and low high-density lipoprotein cholesterol (Brunner *et al.*, 1996:1011). In the Framingham Study and SHHS diabetic subjects had a higher plasma fibrinogen concentration than non-diabetic subjects (reviewed by Lip, 1995:159). The relationship between fibrinogen and the features of the metabolic syndrome, suggest a common aetiology.

h) Furnace dust exposure

Long-term inhalation of particles retained in the lungs will create chronic low-grade inflammation in association with an increase in plasma fibrinogen.

This confirms the general hypothesis regarding inhaled particle exposure (ferromanganese / silicomanganese of furnace workers and manganese-exposed Moroccan miners) and the occurrence of IHD (Sjögren, 1998:236).

i) Stress

A cross-sectional study of a occupationally homogeneous group of healthy middle-aged males in middle-management positions indicated that chronic work stress in terms of high effort and low reward is associated with significantly increased relative risks of having abnormal levels of LDL-cholesterol and plasma fibrinogen (Siegrist *et al.*, 1997:147,154).

j) Gender

Comparing men and women, fibrinogen concentration is substantially higher in premenopausal women than in men (Brunner *et al.*, 1993:197). The exceptions are 13-14-year –old Jewish children, the white South African 17-20 year olds, and two groups in the French study (4-14 and 40-50 year olds) found in studies reviewed by Vorster (1999:139).

A study determining the plasma fibrinogen concentration in Spanish children and adolescents showed higher levels of fibrinogen in girls than in boys, with a more marked but reversed difference than in adults (Sanchez-Bayle *et al.*, 1993:325).

k) Age

In women, fibrinogen shows no relation with age before menopause, but rises with age after menopause. The interaction of menopause status and age is such that the difference in fibrinogen associated with menopause is close to zero at 45, rising to about 10% at the age of 55. This finding presumably reflects time elapsed since the menopause, rather than chronological age (Brunner *et al.*, 1993:197). In men and women fibrinogen increases about 0.1-0.2 g/l per decade during adulthood (Folsom, 1995:21). It is not clear if these increases in fibrinogen with age are related to overall health status or to age *per se.*

l) Obesity and body mass index (BMI)

In obese patients (BMI >30 Kg/m^2) the plasma viscosity and plasma fibrinogen levels are significantly increased compared to healthy subjects (BMI <25 Kg/m^2) (Lip, 1995:160). Bao *et al.* (1993:324) demonstrated in the Bogalusa Heart Study a consistent increase in fibrinogen levels with ponderal index and skinfolds in children. Møller & Kristensen (1991:344) found a strong univariate association between the waist-to-hip ratio and the fibrinogen levels in a study of 439 51-year-old men.

In the THUSA study of Africans in the Northwest province of South Africa, BMI showed a significant positive correlation with plasma fibrinogen level($r=0.25$; $p=0.000$) (James *et al.*, 2000:385).

Folsom *et al.* (1991:200) developed two models to predict changes in fibrinogen for a 5-kg/m^2 increase in BMI. The first model showed a 0.85 g/l increase in men and a 1.23g/l increase in women, and the second a 0.6 g/l increase in men and a 0.98 g/l increase in women.

It was therefore assumed that the influence of BMI on fibrinogen levels was greater in women. These findings are supported by the results from the second WHO MONICA Augsburg Survey (Korobot *et al.*, 1992:785). In contrast, Iso et al. (1996:1152) found a positive association between BMI and fibrinogen in white and Japanese men, but not in

Japanese women. Little is known about the effect of weight loss and fibrinogen levels. Vorster *et al.* (1997:120) indicated that an increase in plasma fibrinogen levels during rapid weight loss may be related to the increase in free fatty acids. However, slow weight loss is associated with a decrease in fibrinogen levels.

m) Alcohol consumption

A decrease in plasma fibrinogen of approximately 0.78% per 10g alcohol consumed has been noted (Lip, 1995:160).

Lee *et al.* (1990:915) reported that drinkers had lower fibrinogen levels than non-drinkers for both sexes according to the SHHS study. Among males, there was no dose-response, but females that claimed to have consumed low quantities of alcohol during the previous week had much higher fibrinogen levels than those consuming larger amounts.

A prediction based on results from the ARIC study and the use of two different models, showed that an ethanol intake of 100g per week was associated with 0.22 g/l and 0.09g/l lower plasma fibrinogen levels in men and 1.29g/l and 0.92g/l lower levels in women (Folsom *et al.*, 1991: 200).

n) Hormones

In the CARDIA study the use of oral contraceptives by women was associated with higher fibrinogen levels than in nonusers (Folsom *et al.*, 1993:1030). In the SHHS it was found, however, that women who had been taking oral contraceptives had lower plasma fibrinogen levels compared to the women who had no history of taking oral contraceptives (Lip, 1995:159). Karpanon *et al.*, (1992:354) showed a decrease in plasma fibrinogen levels from the ovulatory phase to the follicular phase in hypertensive women (from 2.92 to 2.72 g/l) and an increase in normotensive controls (from 2.22 to 2.47 g/l; p, 0.0008). Brunner *et al.* (1993:197) found that fibrinogen levels of post-menopausal women are higher than the fibrinogen levels of pre-menopausal women. Pregnancy is sometimes labeled as a "physiological model of hyperfibrinogenaemia", but is accompanied by a compensatory rise in fibrinolytic potential (Yin *et al.*, 1998:235). It seems that the effect of female sex hormones on fibrinogen levels has been studied in some detail. Very little about the effects of other hormones is available.

o) Hyperlipidaemia

The Leigh Study indicated that the incidence of myocardial infarction (MI) was six times greater in subjects with raised plasma fibrinogen (>3.5 g/l) and raised cholesterol levels (> 6.2mmol/l), when compared to those with low fibrinogen and low cholesterol levels (Lip, 1995:160). In the Bogalusa Heart Study, a positive association of plasma fibrinogen with VLDL cholesterol and LDL cholesterol and a negative association with HDL cholesterol were found (Bao *et al.*, 1993:324). It is also believed that higher plasma fibrinogen level for familial hypercholestrolaemic female patients than in male patients (Jerling *et al.*, 1997:95).

Results from the THUSA study of Africans in the Northwest province of South Africa, showed that a small but significant positive correlation's exist between serum cholesterol, LDL cholesterol and, in women, triglycerides with plasma fibrinogen (James *et al.*, 2000:391).

p) Chronic inflammation

Fibrinogen is an acute phase protein: Elevated plasma fibrinogen levels have been indicated in the presence of an even bland chronic inflammation, which is the most common concept for the pathophysiology of atherosclerosis (Resch & Ernst, 1994:171). Persons with rheumatic disease have elevated fibrinogen levels due to the acute phase reaction (Folsom, 1995:22). Chronic inflammatory gingival and periodontal infection is also associated with MI, increased plasma fibrinogen and white cell counts (reviewed by Lip, 1995:161).

q) Genetic factors

Several recent studies support the notion of genetic influence on plasma fibrinogen levels, but also reflect controversies:

- A small but significant increase in fibrinogen level was associated with the rare cutting sites of haeIII and HindIII, BcII and TaqI. At all polymorhic sites homozygosity for frequent alleles was associated with about 0.2g/l lower plasma fibrinogen levels than the heterozogosity at the respective sites (Vasse *et al.*, 1996:332).

- Thomas *et al.* (1996:186) indicated an interaction between smoking, age and the allele frequency of the G/A-455 polymorphism of the Bβ fibrinogen gene and plasma levels.
- Carter *et al.* (1996:1266) reported that a G/A-455 polymorphism is related to the development of CHD in diabetic patients, without influencing the plasma fibrinogen levels.
- Van der Bom *et al.* (1998:623) found that subjects with one or two A alleles had significantly higher fibrinogen levels than those with the G/G genotype; this polymorphism did not influence the MI risk.
- In contrast, Yu *et al.* (1996:156) showed that the G/G genotype was an independent predictor of MI in a co-dominant manner.
- Kessler *et al.* (1997:2882) found that the A/A genotype was more common in patients with large vessel disease. A similar pattern has been reported by Iacoviello *et al.* (1998:265) for the BcII polymorphism.

It seems that individuals with the rearest genotype (B2/B2) have a 15-20% higher fibrinogen level than those with B1/B1 genotype, and that this polymorphism could be associated with peripheral arterial disease, the severity of CHD, and familial MI. There are indications that the effects of this polymorphism are smaller in women than in men (Iacoviello *et al.*, 1998:266).

r) Birth weight

Low birth weight is associated with higher fibrinogen levels in adults. It is hypothesized that intra-uterine growth retardation may contribute to the determination of an individual's fibrinogen concentration in later life (Barker, 1993:105). It is believed that the effect of infant's weight on fibrinogen is only significant in men (Henry *et al.*, 1997:556). However, data on this phenomenon is scarce. In the light of childhood malnutrition and later risk of chronic diseases, more research is needed in this area.

s) Ethnicity

Across populations, plasma fibrinogen concentration seems to co-vary with the population level of IHD. Fibrinogen levels are low among Asians, intermediate among English, Gzechs and North American whites, highest among Finns, Scots, African Americans and some American Indian tribes. Gambians who are presumably at low risk of CVD, have been found to have high fibrinogen levels, a finding attributed to parasitic infection (Folsom, 1995:21).

Table 6: Sex, race, and age-specific mean plasma fibrinogen levels (mg/dl) and percentage of participants with fibrinogen levels >350mg/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

[#] Data from the CARDIA study, 1990-1991 (Folsom *et al.*, 1993:1027).

As Table 6 shows, mean fibrinogen levels were higher ($p<0.001$) in those aged 30 years or more than in those aged less than 30 years, in women than in men, and in blacks than in whites (Folsom *et al.*, 1993:1027).

In the Bogalusa Heart Study fibrinogen values 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.*, 1993:324). The authors suggested an increase earlier or to a greater extent for black females, which resulted in the highest adulthood levels seen in black females. Iso *et al.* (1989:930) indicated that regardless of smoking status, fibrinogen level in Caucasians was higher than in urban or rural Japanese.

t) Blood pressure

In the Bogalusa Heart Study, (Bao *et al.*, 1993:324) a positive association of fibrinogen levels with blood pressure in children was found. A number of other studies reported significant positive correlation's of plasma fibrinogen levels with systolic and diastolic

blood pressure, but only in women (reviewed by Vorster *et al.*, 1998:174; Ishikawa *et al.*, 1997:891).

u) Microalbuminuria

A number of studies have reported that microalbuminuria, in both normal and diabetic individuals, is associated with CVD. It was reported that those with microalbuminuria had higher levels of plasma fibrinogen (Folsom, 1995:24).

Table 7: Summary of factors associated with plasma fibrinogen concentration

Positive association		No association	Negative association	
Strong	**Modest**		**Modest**	**Strong**
• Age • Female gender • Cigarette smoking • Inflammatory condition • Stress • Obesity • Diabetes • Menopause • Oral contraceptives • Leucocyte count	• Winter • Ethnicity • Blood pressure • Lipoproteins • Homocysteine • Serum insulin • Microalbuminuria	• Dietary fat • Nicotine gum	• Alcohol intake • Physical activity • Fish oil • HDL cholesterol	• Social class • Education level • Estrogen replacement • Birth weight • Ethnicity

As table 7 shows, the association between the factors influencing plasma fibrinogen levels varies from strong to modest. Association can either be positive or negative.

2.2.8 Fibrinogen status: normal ranges

Fibrinogen plasma levels of 1.5-4.5 g/l are considered normal. This concentration far exceeds the minimum concentration of 0.5-1 g/l necessary for normal haemostasis. The upper limit of 4.5 g/l is also considered normal but refers to fibrinogen as an acute phase protein. In the absence of an acute inflammatory disease, levels exceeding roughly 3 g/l have shown to be associated with an increased risk for cardiovascular events (Resch & Ernst, 1994:170 and Lip, 1995:155). As a response to stimuli such as acute inflammatory

processes, plasma fibrinogen levels may easily double within days and usually normalise almost as fast when the pathological stimulus is no longer present (Resch & Ernst, 1994:170).

The plasma fibrinogen levels (g/l) of HIV negative African men and women stratified for age and level of urbanisation found in the THUSA study are indicated in *Table8.* Age showed a significantly positive (p=0.001) correlation with plasma fibrinogen of men (r=0.14) and women (r=0.11) (James *et al.*, 2000:386).

Table 8: The plasma fibrinogen levels (g/l) of HIV negative African men and women stratified for age and level of urbanisation found in the THUSA study.[#]

Gender and age group		Level of urbanisation											
Men	***n*=**	**S1**		**S2**		**S3**		**S4**		**S5**		**TOTAL**	
		Mean	**SD**	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**
15-25	169	2.56	0.53	3.14	0.69	3.01	1.19	2.97	1.19	3.14	0.80	2.95[a,b,c]	0.92
25-35	148	2.74	0.84	3.44	0.62	3.25	1.58	2.91	1.15	3.31	0.94	3.11[d]	1.10
35-45	111	2.91	0.93	3.63	0.77	3.70	1.23	2.96	0.90	3.64	1.03	3.24[a,e]	1.01
45-55	112	3.28	1.16	3.76	0.79	3.13	1.39	2.95	1.01	3.19	0.83	3.23[b]	1.09
55-65	75	3.38	1.21	3.83	0.47	3.33	1.17	4.03	2.02	2.69	1.67	3.55[c,d,e]	1.37
65+	39	2.87	1.07	5.20	3.92	4.52	1.38	3.17	0.84	-	-	3.21	1.32
Women													
15-25	171	2.91	0.66	3.42	0.78	3.78	1.78	3.80	1.55	3.64	0.88	3.24[a]	1.16
25-35	226	3.11	0.91	3.58	0.78	3.92	1.35	3.67	1.03	3.90	1.05	3.38[b]	1.10
35-45	201	3.38	0.87	3.76	0.85	3.69	1.25	3.84	1.04	3.97	0.81	3.54	1.01
45-55	157	3.39	0.91	3.79	0.87	3.87	1.32	3.93	1.47	3.57	1.34	3.49	1.15
55-65	93	3.76	0.92	4.01	1.19	3.61	1.05	3.96	1.27	3.89	-	3.71[a,b]	1.23
65+	37	3.29	0.8	3.24	0.37	3.06	0.18	3.85	1.36	-	-	3.37	1.22

Abbreviations: S1=rural; S2=farms; S3=informal housing areas (squatter camps); S4=urban (middle class); S5=urban professional.

[a,b,c,d,e] *The same symbol differs significantly (p<0.05) within gender between age groups*

[#] James *et al.*, (2000:386) reported values obtained from the THUSA study.

Results obtained in the BRISK study in the Cape Penisula, South Africa, are given in *Table 9.* Mean fibrinogen of men and women were higher than published data for Europeans but slightly lower than values for African Americans. Women aged 45-54 years had the highest level (3.13 ± 0.89g/l) and men aged 15-24 years had the lowest (2.13 ± 0.88 g/l). Fifteen per cent of men and twelve per cent of the women had a level greater than one SD of the mean for their age group (Vorster *et al.*, 1998:171).

Table 9: Plasma fibrinogen values obtained in the BRISK study[#]

Men Age	n	Mean	SD
15-24	120	2.31	0.88
25-34	84	2.42	0.88
35-44	68	2.58	1.03
45-54	45	2.72	1.04
55-64	35	2.63	0.59
Women			
15-25	151	2.62	0.88
25-35	116	2.78	0.77
35-45	85	2.70	0.82
45-55	54	3.13	0.89
55-65	41	3.07	0.99

[#] A study of Africans in the Cape Peninsula South Africa (Vorster *et al.*, 1998:171).

2.2.9 Modifying fibrinogen levels

If one accepts the hypothesis that elevated plasma fibrinogen levels are causally linked to atherogenesis and to its thromboembolic complications, lowering the fibrinogen levels should retard the artherosclerotic process and reduce cardiovascular events (Koenig, 1995:56). As discussed above plasma fibrinogen levels are regulated by an interesting and complex interplay between environmental and genetic factors (De Maat, 2001:509). Factors proven to have a lowering effect on plasma fibrinogen are:

- Fibric acid derivatives such as bezafibrate (but not gemfibrozil) lower plasma fibrinogen levels up to 20% or more. These agents also modify lipid levels in a

beneficial direction. Agents lowering fibrinogen levels selectively are under development (Meade, 1995:33).

- Dietary supplementation of healthy young men and women with fish oil capsules, by using olive oil as a placebo, lowered plasma fibrinogen in women who had relatively high baseline values and not in men, who had lower baseline values (Oosthuizen *et al.*, 1994:560).

Therefore, it seems that optimal nutritional status (or balanced diet); long term weight loss, not smoking and physical activity are the lifestyle interventions that would lower plasma fibrinogen. These interventions will also have beneficial effects on other CHD and CVD risk factors.

2.3 Vitamin A

2.3.1 Characteristics of vitamin A

Vitamin A, with the common name retinol, occurs in nature mainly as fatty acid esters. They are polyunsaturated, making them susceptible to destruction by oxidation. Vitamin A is generally organised in two groups:

- *Retinoids* – exist as retinol, retinyl esters, retinal and retinoic acid (preformed vitamin A) (*see figure 5)*
- *Carotenoids* – include α, β, γ, carotene and cryptoxanthin. B-carotene are the most important form (*see figure 5).*

(Reviewed by: Weigley *et al.*, 1997:168; Williams, 1993:171; Bohman *et al.*, 1982:251)

2.3.2 Function of vitamin A

Vitamin A is essential to the numerous metabolic processes of the human body (Bohman *et al.*, 1998:251).

a) Growth

Vitamin A plays an essential role during cell differentiation. Therefore, vitamin A is involved in reproduction, growth, bone and tooth development, synthesis and maintenance of healthy epithelia, maintenance of the skin and mucous membranes

(Klemm & Ross, 1999:45; Department of Health, 1998:5; Stoltzfus & Klemm, 1997:58; Weigley *et al.*, 1997:168).

b) Immunological functions

Vitamin A is essential in the functioning of the immunological system. It has been proven that the immune reaction of children with vitamin A deficiency (VAD) could be enhanced by means of improving their vitamin A status. Which leads to a reduction in prevalence of illness from infectious diseases and in mortality (Department of Health, 1998:5; Stoltzfus & Klemm, 1997:58; Sommer, 1997:4).

c) Vision

The most commonly recognised function of vitamin A is its role in vision. Rhodopsin, a protein containing vitamin A, is the eye pigment sensitive to light. When light shines on rhodopsin, it splits into opsin and retinal. Some vitamin A is lost in this process. In the dark, opsin and retinal rapidly recombine (provided there is enough vitamin A). Insufficient vitamin A leads to slow regeneration and the eye cannot adapt to changes in light (condition called "night blindness") (Klemm & Ross, 1999:44; Weigley *et al.*, 1997:169).

d) Antioxidant

An antioxidant is a natural or synthetic compound that is itself oxidised readily, thus preventing other compounds from being oxidised. Vitamin A and β-carotene are dietary antioxidants, thought to play a major role in preventing oxidative damage in body cells. Other oxidants include Vitamin D and E (Weigley *et al.*, 1997:164-1.65)

2.3.3 Dietary sources of vitamin A

Several sources (Weigley *et al.*, 1997:169; Department of Health, 1998:7; Mutare city Health Department, 1997:3) mention that pre-formed vitamin A is only contained in animal foods: egg yolks, liver and kidneys, dairy products such as butter, margarine, yellow cheese, whole-milk cheeses, cream, whole milk, and cod liver oil. The precursors

of vitamin A, α- and β-carotene and other carotenoids are mainly present in green leafy and red vegetables and in dark yellow and orange fruits and vegetables.

2.3.4 Metabolism of vitamin A

Retinol is freed in the gastrointestinal tract, transported across the intestinal cells and estrified to retinyl esters (Neal, 1998:4). Vitamin A, either as pre-formed retinol and its esters or as pro-vitamin A carotenoids, is absorbed in the upper part of the small intestine. In chylomicrons it passes through the lymphatics into the systemic circulation, where it is bound to lipoprotein. Vitamin A is stored in the liver and on request retinyl esters in the liver are hydrolised to free retinol (Neal, 1998:4). When vitamin A is released from the liver it binds to specific proteins, retinol-binding protein (RBP) and transthyretin (TTR) (Weigley *et al.*, 1997:170; Williams, 1993:173).

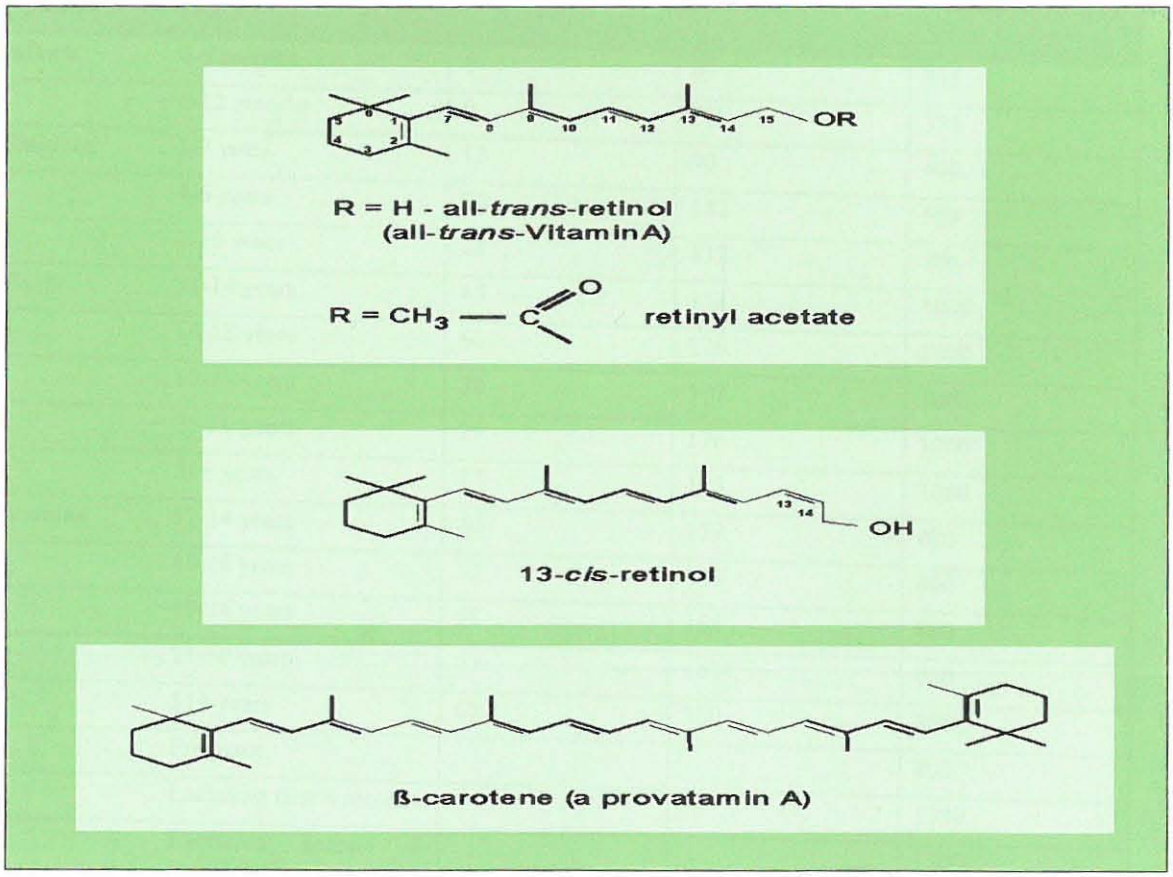

Figure 5: ***Vitamin A molecular composition adapted from*** **Bohman *et al.*, 1982:251.**

2.3.5 Dietary vitamin A requirements

The amount of vitamin A in food is commonly expressed in International Units (IU) or retinol equivalents (RE) (Weigley *et al.*, 1997:168; Williams, 1993:179 Department of Health, 1998:10). RE is a unit that was developed to standardise the measurements of available vitamin A activity of different vitamin A sources:

1RE = 3.33IU vitamin A activity from retinol

1RE = 1µg retinol or 6 µg β-carotene.

Table 10 Recommended daily allowance for vitamin A

Category	(Age)	Weight (kg)	Height (cm)	Vitamin A (RE)
Infants	0-6 months	6	60	375
	6-12 months	9	71	375
Children	1-3 years	13	90	400
	4-6 years	20	112	500
	7-10 years	28	132	700
Males	11-14 years	45	157	1000
	15-18 years	66	176	1000
	19-24 years	72	177	1000
	25-50 years	79	176	1000
	51+ years	77	173	1000
Females	11-14 years	46	157	800
	15-18 years	55	163	800
	19-24 years	58	164	800
	25-50 years	63	163	800
	51+ years	65	160	800
	Pregnant			800
	Lactating first 6 months			1300
	Lactating second 6 months			1200

***As described by* Weigley *et al.*, 1997:168-169; Mahan & Escot-Stump, 2000:70**

2.3.6 Vitamin A Status

Vitamin A status, on the basis of serum vitamin A concentration, was classified by the World Health Organisation (WHO) as follows:

Plasma retinol	:	Description of status
Less than 10 µg/dL	:	VAD
10-19,9 µg/mL	:	low (marginal vitamin A status)
20-29,9 µg/dL	:	adequate status
More than 30 µg/dL	:	normal, well-nourished status

(UNICEF & WHO, 1994:15).

2.3.7 Vitamin A deficiency

Vitamin A is a powerful child survival tool, reducing child mortality by 23-24 (Malanick, 1999:1; Humphrey, 1998:S2). VAD as a public health problem occurs within an ambience of ecological, economical and social deprivations in the macroenvironment in which populations are found, as well as the microenvironment where families live. The individual environment and malnutrition can take a variety of forms that contribute to each other, such as deficiencies of micronutrients that include iodine, iron and vitamin A, and protein energy malnutrition (Bellamy, 1998:1; WHO, 1995:5).

Globally, vitamin A is a major problem in 75 countries. In developing countries, 125-190 million children of preschool age are at risk of VAD. Five to 10 million of these children will develop xerophtalmia, and every year about 500 000 of these children will lose their sight. These conditions can lead to 1-2.5 million child deaths annually (Bellamy, 1998:2; Humphrey, 1998:S2; Department of Health, 1998:3)

In 1994 the SAVACG study determined that one in three children, under the age of six years had a marginal vitamin A status. Only one percent of children had a serum retinol level of higher than 50µg/dL. The prevalence of VAD in children was found to be higher in rural areas (38%) compared to urban areas (25%) (Labadarios, 1999:5; Department of Health, 1998:3; SAVACG, 1995:4; Department of Health, 1999:12).

In addition to young children in South Africa, it is suspected that adolescents and especially women of child bearing age may also be vitamin A deficient. In a meta-

analysis of dietary intakes, Vorster *et al.* (1997:109) showed that urban black females aged 16-24 years, vitamin A intake were 56.5% of RDA.

2.3.8 Intervention strategies

Elimination of VAD as a public health problem should be a principle element of child- and maternal survival programmes (Sommer, 1997:3). It is important that any recommended strategy should be implemented in conjunction with other public health measures, such as oral rehydration programmess, control of intestinal parasitic disease, prevention and treatment of infections and promotion of breast feeding (SAVACG, 1995:19, 144; Department of Health, 1998:17).

A multi-sectorial alliance, called the vitamin A initiative (VITA), was combined by the USAID, comprising of private sector, non-governmental organisations, private voluntary organisation and civic organisations (Malanick, 1999:1). VITA launched a multi strategy plan in order to reduce VAD. Strategies included supplementation, fortification and diversification of food (Malanick, 1999:1).

a) Supplementation

Vitamin A supplementation is a low-cost, reliable and effective way of combating VAD, and can be rapidly implemented as a programme strategy on a national scale (USAID, 1993:8; Sommer, 1998:3).

Based on the SAVACG report (1995:8-10), a supplementation programme for South Africa was recommended:

- A national vitamin A capsule distribution programme. Distribution to be started in provinces at highest risks, for a period of three years, for all children 6-71 months of age;
- All children arriving at health centers presenting with malnutrition, measles or diarrhea, should receive a high dose vitamin A supplementation;
- Children under the age of 2 years should be the targets of nutritional intervention and their mothers for nutritional education.

b) Fortification

Food fortification, more than any other technology available today, offers an affordable and immediate opportunity to improve lives and accelerates socioeconomic development. Food fortification has been responsible for eradicating most of the vitamin and mineral deficiencies in developed countries (Anon., 1997a:12).

Table 11: Tested strategies for vitamin A food fortification

Country	Food fortification program	Date
Denmark	VA fortified margarine	1918
Canada	Milk fortified with vitamin A	1945
Guatemala	Vitamin A fortified sugar	1970
USA	Vitamin A and Vitamin D to fluid milk	1984
Philippines	Exploring of vitamin A fortified monosodium glutamate – not implemented	1985
Bangladesh	VA fortified wheat	1986
Philippines	VA fortified wheat	1993
Andes of Peru	Fortified school breakfast	1993
Thailand	Instant noodles fortified with vitamin A, iron and iodine	1994
Mexico	Chocolate powder drink fortified with vitamin A, iron and iodine	1994
Indonesia	Vitamin A fortified rice	1994-1996
Japan	Rice with vitamin A and B-vitamins	1999

Adapted from Lofti, 1997:7; Murphy, 1996:69

c) Food diversification

Food diversification is a long-term intervention strategy that includes approaches to change behavior in order to improve consumption of vitamin A rich foods. This can be achieved through communication, social marketing and nutrition education, home food provisioning through activities such as home gardening, and technological developments regarding food and nutrient preservation.

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d) Breast-feeding

Breast-feeding is known to substantially reduce the risk of VAD, a protective effect, which can extend to the third year of life (Department of Health, 1998:17; SAVACG, 1995:19, 144).

2.4 Fortification of foods with vitamin A

Fortification could be described as the addition of nutrients at levels higher than those found or never found in original food (Weigley *et al.*, 1997:29). Another definition describes fortification as the addition of one or more nutrients to food, whether or not it is normally contained in that specific food, in order to prevent or correct a demonstrated deficiency (Brady *et al.*, 1996:12).

For a programme to be effective it is necessary that the fortified food (fortification vehicle) must reach the target population (Fitzgerald, 1997:1). To ensure that the most vulnerable members of the population benefit from the fortification process, a staple food must be used as vehicle (Anon., 1997a:12).

At the International vitamin A Consultative Group (IVACG) meeting 1996, it was reported that the average sugar consumption per person per day in SA is 100g (Nalubola & Nestel, 1999:100). The THUSA study reported an average of 35-60 g per adult per day in the North West Province of SA (Macintyre, 1998:258). Studies indicated that 93% of the families in SA consume sugar (Hendricks, 1999:4). It can therefore be assumed that sugar is a suitable vehicle for fortification with vitamin A, in the combating of VAD.

2.5 Hypothesis of this study

The hypothesis tested in this study states that the consumption of vitamin A fortified sugar by a randomly selected group of young (13-25 year old) African girls, a group known to have a raised plasma fibrinogen level, will improve vitamin A nutritional status and decrease risk for future stroke or CVD by decreasing plasma fibrinogen levels, regardless of their initial vitamin A status.

The theoretical basis and known scientific evidence, on which this hypothesis rests, will be discussed briefly.

2.5.1 Vitamin A and fibrinogen

The relationship between nutrition and plasma coagulation factors, especially fibrinogen, is far from clear. In *Table 12* the results of a few studies which examined these relationships are summarised.

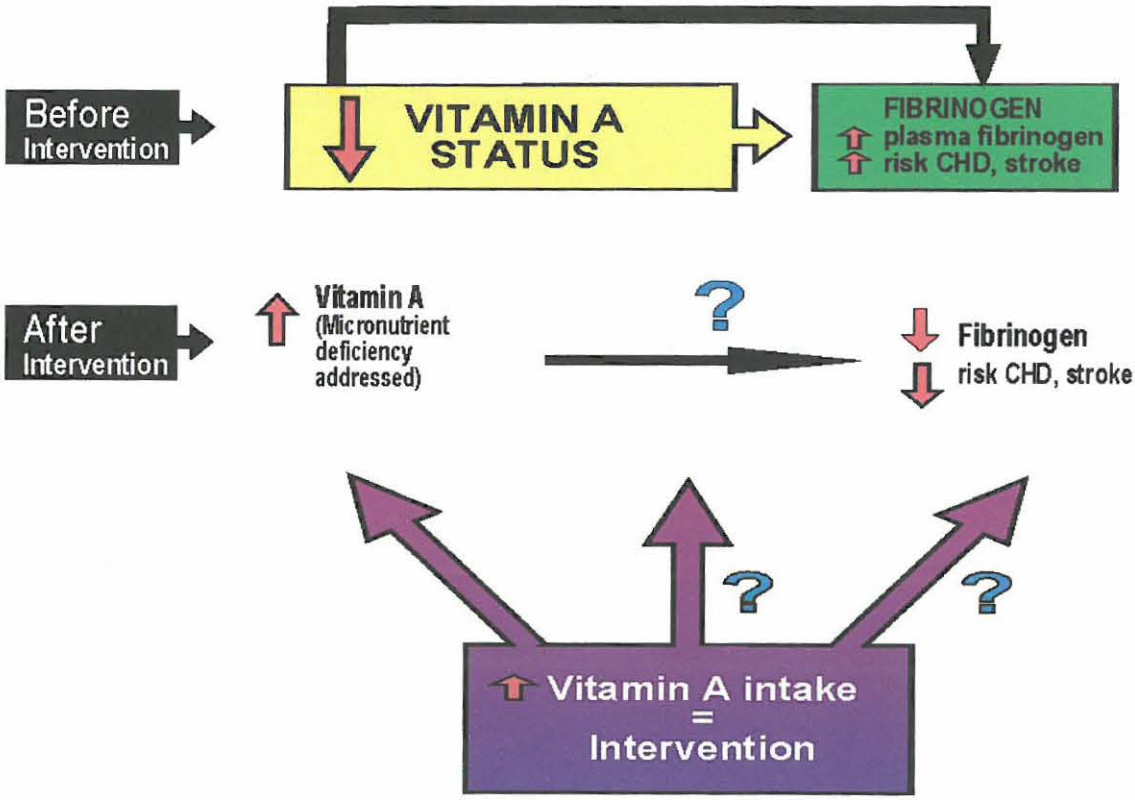
It was mentioned in 2.2.7(s) that there is evidence that black South Africans have higher plasma fibrinogen levels than comparable subjects from the other population groups. The causes of these higher levels are not known, but it has been speculated that the high plasma fibrinogen contributes to the high stroke rate in South African blacks (Vorster *et al.*, 1998:169). It is therefore important to examine if vitamin A supplementation of the diet of black South Africans may have beneficial effects on their plasma fibrinogen – based on the suggestions in the literature that there may be an interaction between vitamin A status and levels of fibrinogen.

Table 12: Relationship between vitamin A and coagulation factors as proved by research studies

Researchers	Sample population	Findings	Reference
Van Giezen, Boon, Jansen & Bouma (1993)	Rats	Retinoic acid increased fibrinolysis by selectively increasing tPA	Van Giezen *et al.*, 1993:384.
Van Bennekum, Emeis, Kooistra & Hendricks (1993)	Vitamin A deficient rats	Retinoic acid increased fibrinolysis	Van Bennekum *et al.*, 1993:R936.
Kruger, Vorster, Venter & Viljoen (1994)	Healthy elderly women	Subjects with higher levels of vitamin A and SRBP had significantly lower fibrinogen levels with simultaneous intake of micronutrient supplements.	Kruger *et al.*, 1994:113.
Eliasson, Asplund, Evrin, Huhtasaari & Johansson (1995)	MONICA study – humans	High plasma retinol levels were associated with lower plasma fibrinogen levels, but also with low tPA and high PAI-I levels, thus with impaired fibrinolytic activity	Eliasson *et al.*, 1995:90.
Hankey, Rumley, Ha, Lowe & Lean (1996)	Patients with angina	Positive associations between plasma retinol concentration standardised for total plasma lipids and PAI-I activity and factor VII_c	Hankey *et al.*, 1996: 193.
James *et al.* (2000)	Africans in transition	Low nutritional status in men and obesity in women were associated with high plasma fibrinogen	James *et al.*, 2000:390

2.5.2 The hypothesis

The hypothesis developed for this study, namely that increased vitamin A intake through consumption of vitamin A fortified sugar, will decrease plasma fibrinogen levels of a group of black South African women, is based on the evidence that there is a possible interaction between vitamin A and plasma fibrinogen. As indicated in *figure 6,* individuals with a low vitamin A status will, due to the stressful or stannous influence of VAD and under nutrition on the body as a reactive condition, have an increased plasma fibrinogen level. Increased plasma fibrinogen levels increase the risk for cardiovascular disease.



Associations between vitamin A and fibrinogen status and suggested consequences of increased intakes of vitamin A

Figure 6 Hypothesis

CHAPTER 3

STUDY DESIGN AND METHODS

3.1 Introduction

If one accepts the hypothesis that elevated plasma fibrinogen levels are causally linked to atherogenesis and to its thromboembolic complications, lowering fibrinogen levels should retard the atherosclerotic process and reduce cardiovascular events, which is of considerable clinical and public health interest.

The THUSA project in the North West Province found that the average daily sugar consumption of adult Africans between 15-65 years was between 35 and 60 grams. Based on these findings, it was decided to use vitamin A fortified sugar in this project to examine relationships between vitamin A and plasma fibrinogen. The objective was to ensure that vitamin A needs were met in the target population, without resulting in excessive intakes. It was decided that 15 micrograms (µg) of vitamin A per gram sugar will both satisfy needs and remain below the maximum acceptable limit (OMNI, 1996: 1) if up to 100 g fortified sugar is consumed daily. Therefore, participating subjects in this study were requested to consume 60 g of sugar daily, providing 800 µg vitamin A.

This study form part of a clinical intervention trial that evaluated the fortification of sugar with vitamin A. Dr. Wilna Oldewage-Theron co-ordinated and administrated the project as project leader. This project formed part of Dr. Wilna Oldewage-Theron Ph.D. study. Emsie Dicks studied the compliance and consumer acceptability of the fortified sugar. Mosa Selepe did the nutritional data gathering and analyses. Jose van Rensburg assisted in blood analysis. The researcher of this thesis was responsible for standardising laboratory infrastructure, training the nursing sister for fieldwork, and managing the laboratory test procedures and quality control program.

The researcher also co-ordinated consigns of the vitamin A samples.

3.2 Ethical Considerations

Both the Ethics Committees of Potchefstroom University for Christian Higher Education (CHE) and the Vaal Triangle Technikon approved the study. The protocol was submitted in accordance with the existing policy for research in both institutions (Annexure 1). Written parental or guardian consent for drawing of blood for subjects younger than 21 years of age were obtained. All the subjects older than 21 years signed the voluntary informed consent form prior to the inclusion of the subject in the study. The consent form included information explaining the purpose of the study as well as the procedures to be followed during the study (Annexure 2).

3.3 Study design

Randomly selected subjects were "blinded" and received anonymous intervention. They knew that sugar was the intervention product, but did not know what it contained. The vitamin A fortified sugar was indistinguishable from the non-fortified sugar (placebo), and was packed in identical quantities of 5 kg purple bags. The sugar was issued to the participants every four weeks for a period of 12 weeks after double baseline measurements and blood samples were taken. The researchers who took measurements and analysed blood samples were also blinded to the intervention. The study was therefore a double blind, placebo-controlled parallel study.

See figure 7 for a schematic presentation of the study design

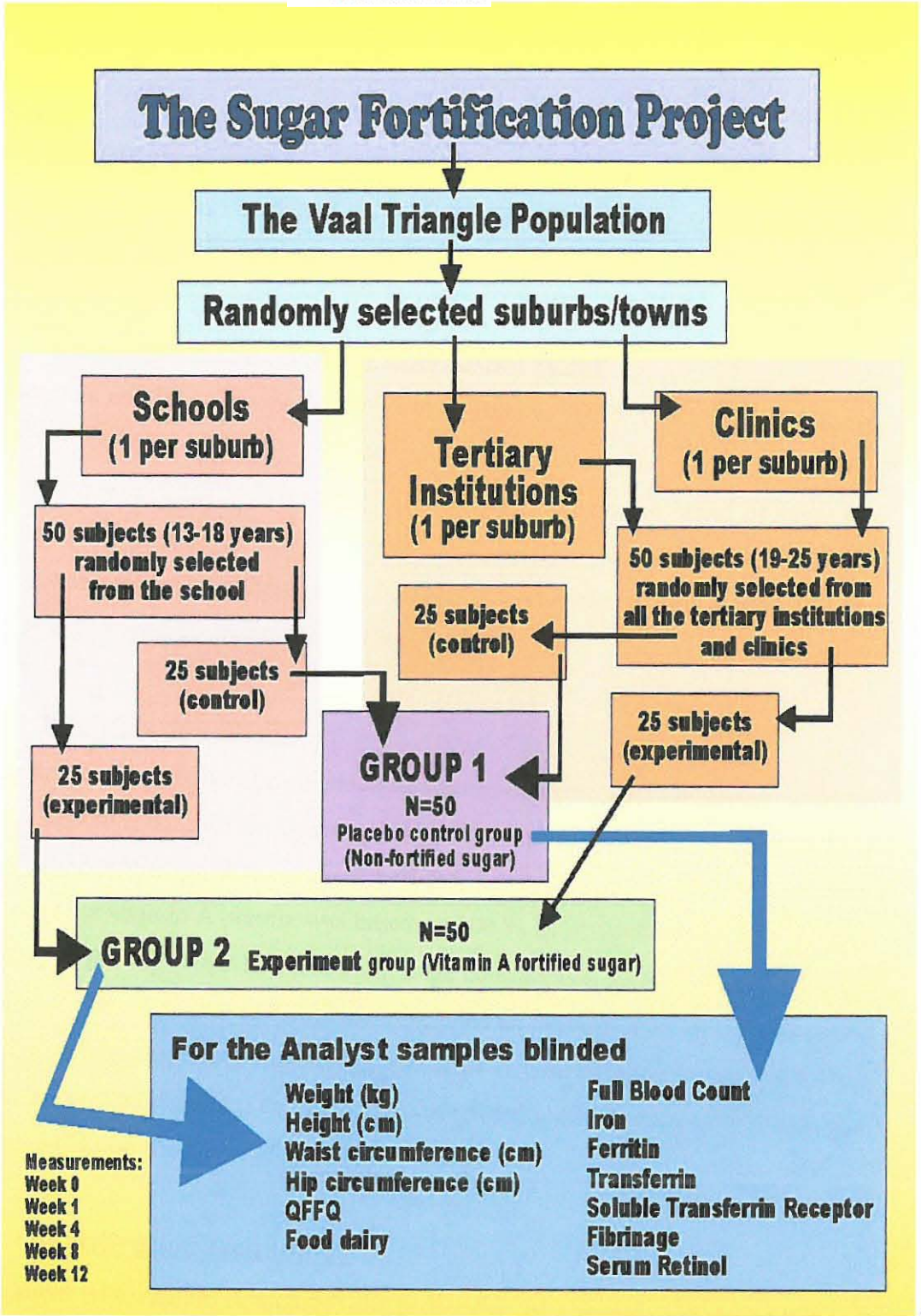


Figure 7 *Schematic diagram of the study design*

3.4 Fortification of sugar

Fortification of sugar as discussed in the thesis of Dr. W.H. Oldewage-Theron (Oldewage-Theron, 2001).

3.4.1 Procurement of vitamin A fortified sugar

Roche Vitamins and Fine Chemicals (Pty) Ltd in Isando, South Africa agreed to fortify the sugar with vitamin A during February and March 2000. The sugar used for this research project was Selati® white sugar (12,5kg bags). The same brand of sugar was used throughout the project for both the fortified and non-fortified product. The reason for using the same brand of sugar was to ensure uniformity in quality, crystal size and colour (also determined by crystal size) of the sugar.

3.4.2 Fortification levels

The concentration level of fortificant added to sugar is critical. The underlying objective was to have effective, but safe fortification. The information required to determine the level of vitamin A added to sugar for this project included:

Preparation of the vitamin A premix was based on 100 % RDA for females 13 to 25 years which is 800µg RE/day (4000 IU), and an average consumption level of 60g of sugar per day. The recommended level of fortification included an average of about 20 % to cancel possible loss due to processing and storage. Vitamin A concentration in the premix was 80000 IU/g sugar (24,0mg RE/g) and was mixed to refined sugar at a ratio of 1:1000 to give 80 IU/g sugar (24,0µg RE/g).

3.4.3 Fortification technology

Fortifying sugar with vitamin A included the preparation of a premix and the addition of premix to sugar (Arroyave & Dary, 1996: 13). This process is facilitated by diluting retinyl palmitate (form of vitamin A used in sugar fortification) (containing 75 000 µg/g (250 000 IU/g), in a small amount of sugar to form a premix (Anon., 1997:1; Arroyave & Dary, 1996: 6). A vegetable oil, low in peroxides and low in unsaturated fat, adheres the vitamin A

beadlet to the sugar crystal (Figure 6). An antioxidant (Ronoxan-A) blended from natural antioxidants, for example, ascorbyl palmitate (250 mg/g), DL-alpha tocopherol (50 mg/g) or lecithin (700 mg/g), prevents the oil from rancidness. The antioxidant also prevents the development of adverse sensory characteristics of sugar.

The vitamin A levels of the different batches of fortified sugar were analysed and verified by Roche Products (Pty.) Ltd. (Annexure 3).

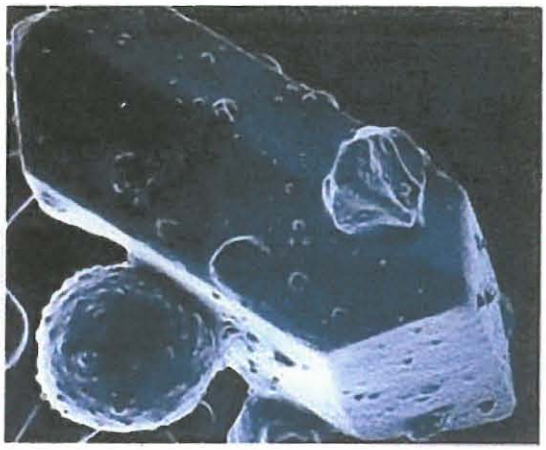


Figure 8 ***Electronmicrograph indicating vitamin A beadlets adhered to the sugar crystal (Anon., 1997:2; Mora & Dary, 1995:1)***

3.4.4 Packaging of fortified sugar

The fortified sugar was packed in plastic bags, where after it was placed into strong carton boxes. The label on the boxes included the manufacturing date and weight (25 kg) of each batch. The sugar was transported to the Vaal Triangle Technikon. The sugar was repacked two days before distribution every fortnight. Strong ultraviolet (UV) resistant purple coded plastic bags, each containing 5kg of sugar were used.

3.4.5 Instructions

Written instructions were compiled to ensure that the sugar would be used and stored correctly (Annexure 4). Every time the sugar was distributed, each subject received an information leaflet. The aim of the leaflet was to ensure that the subjects would use the sugar according to the provided instructions discussed in the nutrition education program and to encourage other household members to use the given sugar.

3.5 Training of fieldworkers and protocol of procedures

- Ten fieldworkers were recruited from the postgraduate Vaal Triangle Tehnikon students in Food and Nutrition and Food Service Management. All were Sotho speaking women. From the onset of this project, extensive training was incorporated. Both training for the initial implementation of the activities and refresher courses throughout the project were included. The fieldworkers received detailed instructions regarding anthropometric measurements and administering the qualitative food frequency questionnaire. Emphasis was placed on ensuring that the fieldworkers were aware of the objectives and importance of the project. In addition to the initial training sessions, fieldworker manuals were prepared and printed in English (Annexure 5). All fieldworkers throughout the clinical intervention trial used the instruction manuals. The purpose of the manual was to ensure standardisation and uniform procedures. (The manual and training was facilitated by W Oldewage-Theron).
- A qualified nursing sister was incorporated in the fieldworker's team. The nursing sister received a fieldworkers manual, was trained by the researcher in the expected sampling procedure as well as sample handling.
- The group of researchers developed a fieldwork administration form (Annexure 6). The form was utilised to check the attendance of each subject at each activity point (station) during the intervention trial. The activities were numerically numbered and were:

❖ Station 1: Recruitment of subjects and handing in of completed forms
❖ Station 2: Anthropometry
❖ Station 3: Handout and/or completion of the different questionnaires

- Station 4: Clinical signs and collection of blood samples
- Station 5: Snacks handed out
- Station 6: Nutrition education programme
- Station 7: Issuing of the sugar

3.6 Sampling strategy

3.6.1 Power calculations

Florey (1993:1882) indicated that power calculations are necessary in determining the likeness to detect an effect for a given sample size, effect size and level of significance. The variable used in the power calculation was plasma fibrinogen concentration because the main objective of this study was to examine the effect of dietary vitamin A fortification on fibrinogen levels. The sample population, however, varied from the calculated value to accommodate the calculated sample size for other parameters used in the study (ex. Soluble transferrin receptor n=88).

To calculate the sample size, the means of the continuously distributed variable, fibrinogen, the baseline difference between the means of the experimental- and placebo groups (d), the likely standard deviation (s) of the variable (same for both groups) and the selected values for significance (α) and power (β), were used.
For this study 95 % significance and 90 % power was chosen and the formula for sample size (n) was thus:

$$n = 2\,(1{,}96+1{,}28)^2\, s^2/d^2 \quad \text{where}$$

The sample size is for each group, experimental and placebo and the total number of subjects in the study must thus be 2n (Florey, 1993:1183-1184).

H_0 : Vitamin A fortified sugar (80 IU per gram) consumption will not decrease fibrinogen levels by 0,5 mg/L.

H_A : Vitamin A fortified sugar (80 IU per gram) consumption will decrease fibrinogen levels by 0,5 mg/L.

$$n = 2\,(1{,}96+1{,}28)^2\ 0{,}4^2/0{,}5^2$$
$$= 13$$

Based on the power calculation, it was decided to include 44 subjects per treatment group. A scaling-up factor was built into the sampling procedure to provide for dropouts. In the end 50 subjects per treatment group were selected to participate in the study.

Usually an experiment should be just long enough to allow for the effect of exposure change (Margetts & Nelson, 2000:427). The half-life of fibrinogen is 4 days; the trial could thus have been conducted over a short period of time. Due to the diversity of plasma fibrinogen and possible interactions of factors influencing the concentration, more useful data could be collected over a longer period of time and may thus increase the statistical power (Margetts & Nelson, 2000: 427). The time frame for this study was thus extended to a period of 12 weeks to compensate for the lower number of subjects.

3.6.2 Sampling procedure

The Vaal Triangle population was divided into groups, consisting of the different towns in the Vaal Triangle. The names of the suburbs per town were thrown in a hat and drawn. Thereafter the names of all the schools, tertiary institutions and clinics in the selected suburbs were thrown into a hat. One school or tertiary institution and one clinic per suburb were drawn from the hat to form part of the sample population.

The sample population of 100 was randomly divided into two groups of equal size. The one group consisted of 50 subjects forming the experimental group. The experimental group consumed vitamin A fortified sugar for a period of 12 weeks. The second group consisted of 50 subjects forming the control group who consumed non-fortified sugar for a period of 12 weeks.

3.7 Questionnaires

The verification, completion and interpretation of the questionnaires formed part of Dr. Wilna Oldewage-Theron Ph.D study (Oldewage-Theron, 2001). The questionnaires included:

- **Demographic questionnaire:** Included questions on age, home language, and the number of residents and rooms in the household. The residence setting and the responsible person for preparing meals in the household were also noted. Alcohol consumption and smoking habits were also questioned. The questionnaire also determined the prevalence of high blood pressure, diabetes mellitus, stroke and obesity in the family. Habitual sugar consumption patterns were also assessed using this questionnaire (Annexure 7).
- **Health and medication questionnaire:** The validated health questionnaire of the Gauteng Provincial Administration (GPA) was used to assess the general health of participating subjects (Annexure 8). A complementary medication questionnaire was drawn up and included questions on types brand names and dosages of medication taken regularly (Annexure 9).
- **Quantified food frequency questionnaire (QFFQ):** The validated QFFQ that was used in the THUSA study (MacIntyre, 1998:200) was used in this study to obtain qualitative, descriptive information about usual food consumption patterns, specifically those containing vitamin A and iron (Annexure 10).
- **Food diary:** The food diary served as reference measure for the QFFQ. The main aim was to determine whether the subjects consumed vitamin A-rich foods and also to determine sugar consumption patterns during the trial (Annexure 11).
- **Reproducibility:** Ten Vaal Triangle Technikon student volunteers were used in the reproducibility testing of all the questionnaires, except the QFFQ. Subjects completed the questionnaires after fieldworkers explained the purpose and content of each of the questionnaires. Repeated interviews took place for four consecutive weeks after the first interview. The subjects were randomly assigned to the fieldworkers and were not necessarily interviewed by the same fieldworkers. This was done to eliminate observer bias. All the completed questionnaires were statistically analysed to detect

variances for individual subjects. No consistent pattern of variances was reported. MacIntyre (1998:221) using 74 volunteers tested the relative validity of the QFFQ in the THUSA study. The reference measurements were a seven-day weighed records and an attempt was made to validate nitrogen intakes against nitrogen excretion in 24-hour urine collections. The reproducibility of the QFFQ was tested on a sub-sample of 125 volunteers from the THUSA study. The purpose was to obtain the same results when administered to the same subjects at different times. The QFFQ was completed by means of an interview at an interval of six to 12 weeks between repeat administrations. Reproducibility was tested for energy, macronutrients, cholesterol, calcium, iron, vitamin A and vitamin C (MacIntyre, 1998:221).

3.8 Anthropometry

Anthropometry is the measurement of body size, weight and proportions and is valuable in monitoring the effects of nutritional interventions for disease or malnutrition.

The WHO recommended height-for-age and BMI-for-age as anthropometric indicators for adolescents (WHO, 1995:271). For this reason weight, height, and weight-for- height, hip- and waist circumference were recorded during weeks 1, 4, 8 and 12 of the trial. These measurements are among the most fundamental and easily obtained anthropometric measurements. A graphical record was kept for each subject, and it allowed for the repeated measurements to be plotted on the reference percentiles.

All the subjects were weighed in light clothes without shoes on a portable electronic bathroom scale. Two measurements were made and were not to vary by more than 0,5 kg. Two measurements for height were made with no more than 0,5-cm variance. Height was measured with an upright stadiometer placed against a perpendicular wall at the clinics and tertiary institutions. Body mass index (BMI) was calculated using the formula weight (kg)/height (m^2).

3.9 Blood sample collection

Blood was drawn from the *vena cephalica* of seated subject after an 8-12 h fast using a 21-gauge butterfly needle with minimal use of tourniquets. A qualified nursing sister collected the blood samples from the subjects. Vacutainer blood collecting tubes were labeled in advance with the subject's trial number as well as the week number.

Blood was collected as follows:

- 5ml Blood in an EDTA tube (purple lid) (Vacutainer ™- lot no 9S001) for full blood counts (Hb, Hct, RBC, MCV, Plt and WBC).
- 10 ml Blood in a silicone-coated tube (Vacutainer ®- lot no 9Y122) for preparation of serum for the analysis of iron, ferritin, transferrin, sTrF.
- 10 ml Blood in a silicone-coated tube (Vacutainer ®- lot no 9Y122) for preparation of serum for the analysis of serum retinol. This tube was immediately (after collection) protected against UV light, by covering the tube with aluminum foil.
- 4.5 ml Blood in a tube containing 0.5 ml sodium citrate (0,11 mol/L) (Vacutainer ™- lot no 9W139) for the preparation of plasma to measure fibrinogen levels.

3.10 Sample handling

Blood was separated (centrifuged at 1.500Xg for 20min.) within 2 hours of blood collection. Separated plasma and serum was eliquoted in marked Eppendorf test tubes. Two qualified medical technologists continuously audited the separating procedure. Citrated plasma was stored at 15°C until assayed. Once plasma has been separated, fibrinogen concentration remains fairly stable for up to about 24 hours but may deteriorate unpredictably. Plasma can, however, be stored at low temperature for analysis (Meade, 1997:14). Serum for the analyses of iron, ferritin, tranferrin and soluble transferrin receptors were stored at -10°C. Serum for serum retinol was covered by aluminum foil and stored at -10°C until it was couriered to the SAIMR central laboratory in Johannesburg. The EDTA tubes were directly placed on a sample tube mixer for immediate analyses.

3.11 Laboratory test procedure

A double baseline measurement was done one week apart before commencing with the trial. The same biochemical measurements were repeated during the baseline measurements, 4th, 8th, and 12th week of the trial. A total of 500 blood samples were collected for the determination of serum retinol, full blood count, iron, ferritin, transferrin, sTrF, and plasma fibrinogen.

Table 13 A summary of methods used to determine serum variables

Variable	Method	Laboratory	Coefficient of variation*
Haematocrit (Hct)	Numeric integration	Vaal Triangle Technikon	1.97
Haemoglobin (Hb)	Cyanmethaemoglobin-colorimetric method	Vaal Triangle Technikon	1.52
Mean cell volume (MCV)	Impulse generating	Vaal Triangle Technikon	1.16
Red blood cell count (RBC)	Coulter counter; ABX $MICROS_{CT}$	Vaal Triangle Technikon	0.36
White blood cell count (WBC)	Coulter counter; ABX $MICROS_{CT}$	Vaal Triangle Technikon	1.66
Serum iron	Colorametric; Roche Unimate 5 Iron	Vaal Triangle Technikon	.024
Serum ferritin	Immunoturbidity; Roche Unimate 3 FERR	Vaal Triangle Technikon	0.02
Transferrin	Immunoturbidity; Roche Unimate 3 TRSF	Vaal Triangle Technikon	0.09
s-Transferrin receptor	Orion Diagnostica Immunoturbidity assay at 600 nm	Vaal Triangle Technikon	1.79
Plasma Fibrinogen	Modified Clauss Method	Vaal Triangle Technikon	0.56

* *Coefficient of variation:* $\frac{\text{standard deviation}}{\text{mean}} X\ 100\ \%$

a) Full blood count

Full blood count (RBC, WBC, Hb, Hct, MCV) was done on a haematology auto-analyser, the ABX MICROS$_{CT}$ within 6 hours of blood collection on a well mixed EDTA sample:

- The ***cell counting*** (RBC, WBC) principle is based on an impedance variation generated by the passage of cells through the calibrated micro-aperture. The sample is diluted in an electric diluent (current conductor). The dilution is pulled through the calibrated micro-aperture. Two electrodes are placed on each side of the aperture. An electric current passes through the electrodes continuously. When the cell passes through the aperture, electric resistance between the two electrodes increases proportionately with the cell volume. Two measuring chambers and detection circuits separately carry out the analysis of white blood cells and red blood cells. Each type of cell is analysed by the microprocessor.
- To determine the ***Hb***, venous EDTA blood is mixed with a reagent that contains potassium ferricyanide (K [Fe (Cn)]) and potassium cyanide [KCN]. The ferricyanide oxidises the iron in the haemoglobin, thereby changing haemoglobin to methaemoglobin. The methaemoglobin binds with the cyanide to form cyanmethaemoglobin. The cyanmethaemoglobin produces a colour measured by spectrophotometry, at a wavelength of 550 nm.
- ***MCV:*** The height of the impulse generated by the passage of a red cell through the micro-aperture is directly proportional to the volume of the analysed cell.
- The ***Hct*** is measured as a function of the numeric integration of the MCV.

$$Hct = \frac{MCV \times RCC}{10}$$

b) Serum iron

Serum iron was measured using the Roche Unimate 5 Iron (8X30 ml) (Cat. no. 07 5181 2). Colorimetric test with Ferrozine ® or ascorbic acid on a COBAZ MIRA autoanalyser (The Sceintific Group South Africa). Iron is released from transferrin by guanidine hydrochloride and reduced to Fe^{2+} by ascorbic acid. Bivalent iron forms a red coloured complex with Ferrozine. The colour intensity is directly related to the iron concentration and is measured photometrically at 560 nm.

c) Serum transferrin

The Roche unimate 3 TRSF (4X1X5 mL) (cat. No.07 3708 8) an immunoturbidimetric assay® was used to measure serum transferrin. The procedure was done on the COBAZ MIRA autoanalyser. Human transferrin forms a precipitate with a specific antiserum, which is determined turbidimetrically (measurement of cloudiness) at 340 nm (fixed time method).

d) Serum ferritin

The Roche Unimate 3 FERR (1X20 ml) (cat. no. 07 5182 0) a latex immunoturbidimetry was used to measure the serum ferritin levels. Human serum ferritin forms a precipitate with the suspended anti-ferritin coated latex particles. These results in an increase in turbidity that is determined turbidimetrically (measurement of cloudiness) at 600 nm (fixed time method).

e) Soluble tranferrin receptor (sTfR)

The IDeA® sTfR-IT (The Scientific Group cat no. 67968) assay is based on the detection of an immunoprecipitation between sTfR in the sample and sTfR-specific antibodies on latex particles. The assay is standardised against human serum sTfR. Measurements were performed on the COBAZ MIRA autoanalyser.

f) Serum retinol

The first valid method available for the determination of serum vitamin A levels was a bioassay. A spectrophotometric method and a photometric method were established later. Both the spectrophotometric and the photometric method are liable to interference, since some substances (mainly caratonoids, derivatives and decomposition products of vitamin A) may absorb light in the same ultraviolet region or give similar colors with the reagents used. Above mentioned methods are not specific for vitamin A and the reliability depends on the purification methods. High Performance Liquid Chromatography (HPLC) is a

more precise, accurate and faster procedure and currently the widest used method (Bohman *et al.*, 1982:251).

A chromsystem high performance liquid chromatography (HPLC) column (CS34100, Separations) for analysis of vitamin A in serum/plasma was used to determine of serum retinol levels. An internal standard is added to a serum sample. Proteins are precipitated and the retinol extracted into isopropanol. After centrifuging the sample, the organic layer is pipetted off and injected onto the column.

g) Plasma fibrinogen

Plasma fibrinogen levels were determined within 8 hours after collection of the sample by means of the Dade Behring - Multifibren® U method (a modification of the Clauss method). Citrated plasma is coagulated by adding a large excess of thrombin. The coagulation time depends on the fibrinogen content of the specimen: substances that inhibit thrombin do not affect this test. Plasma (100 µL) is added to a 37°C pre-warmed test tube, incubated for 60 seconds, whereafter 200 µL Multifibren U reagent, pre-warmed to 37°C, is added and the clotting time determined. Fibrinogen concentration (g/L) is obtained by using a reference curve prepared in the laboratory, using standard plasma (lot. no 519857)

The Multifibrin U composition consists of bovine thrombin (50 IU/ml), fibrin-aggregation retarding peptide (gly-pro-arg-pro-ala-amide, 0,15 g/L), calcium chloride (1,5g/L), hexadimethrine bromide (15 mg/L), polyethylene glycol 600 (0,8 g/L), sodium chloride (6,4 g/L), Tris (50 mmol/L), and bovine albumin (10 g/L).

h) Quality control

Proper quality control is an important aspect of any laboratory test procedure. The following quality control procedures were followed for each test:

- ***Fibrinogen:*** A reference curve was established using fibrinogen standards (Dade Behring fibrinogen standard, lot no. 519855). These standards were tested as samples. Values obtained were plotted on a

log-log format. Checking the values of control plasma (Dade Behring control plasma N (lot no. 502711) and P (lot no.512651)) after every 15 tests monitored accuracy of the procedure. Control values did not vary more than 2SD from the prescribed mean.

- ***Full blood count:*** EIGHTCHECK-3WP-N (lot no. SFN-100B), EIGHTCHECK-3WP-L (lot no. SFN-100B) and EIGHTCHECK-3WP-H (lot no. SFN-100B) were used as haematology control blood. EIGHTCHECK-3WP-N is for normal values, EIGHTCHECK-3WP-L is for low abnormal values and EIGHTCHECK-3WP-H is for the high abnormal values. Control blood contains stabilised human red blood cells, fixed mammalian white blood cells and platelet components in a medium containing preservatives.
- ***Iron, Transferrin:*** OLYMPUS control serum level 1(ODC0003 lot no. 014) and level 2(ODC0004 lot. no. 014) were used to monitor the accuracy and precision of automated analytical assays of ferritin and transferrin. It is a lyophilised serum of human origin. The concentration and activities of level 1 are in the normal range, and level 2 in the pathological range. Control serums were repeated after every 15 samples. Control values did not vary more than 2SD from the prescribed mean.
- ***Ferritin:*** A FERR/MYO T Control (Roche lot no. 0751863) is a lyophilised preparation of human ferritin and myoglobin used to monitor the accuracy and precision of assays of ferritin and myoglobulin. Assigned values were printed on each vial label. Control serum was repeated after every 15 samples. Control values did not vary more than 2SD from the prescribed mean.
- ***sTrF:*** IdeA sTfR control serum (The Scientific Group cat no. 67968) high and low were used and treated as a sample. Control values did not differ more than 2SD from the mean.

- ***Serum retinol:*** Vitamin A control serum, level 1 (normal) and level 2 (abnormal) (Separations cat no. CS0032) were used and treated as a sample. Control values did not differ more than 2SD from the mean.

3.12 Statistical analysis

The results of the blood analyses were computerised and statistically analysed by a qualified statistician using the SPSS for windows® programme version 6.0. Changes from baseline to the end of the 12-week study in the experimental group for all variables were compared with those in the control group by using the Levene's two-tailed test for equality of variances. Paired tests were done to compare for significant differences within groups. Differences were considered to be statistically significant if p< = 0,05. Pearson correlation coefficients were used to test for associations between biochemical- and haematological variables, as well as between dietary and biochemical- and haematological variables. A correlation was considered to be present if r => 0 with significance level P = 0,001, 0,05 or 0,1.

The data entry programmes had a number of quality control mechanisms, including validity checks, duplicate detection and verification procedures, written in SPSS. All programmes were introduced and standardisation exercises performed at the time of training the research assistant.

3.13 Researcher's contribution

Contributions made by the researcher were:

- Standardising laboratory infrastructure (Apparatus placements, reagent purchasing, and apparatus calibration).
- Training of the nursing sister for fieldwork.
- Manage the laboratory test procedures and quality control programme.
- Co-ordinate consigns of the vitamin A samples.

All the blood samples were prepared and handled by a registered medical technologist under controlled and standardised conditions. One of the most important attributes of the project was the importance placed on the quality of the data. The registered medical technologist monitored each round of data collection; the blood collection points were checked and laboratory quality control managed, as well as supervising the data collection. Detailed monitoring checklists were maintained to verify whether appropriate techniques were being employed for each point of the data collection. The laboratories involved in the analysis used standard techniques according to accreditation procedures.

3.14 Research team contribution

Contributions made by other research team members were:

- Dr. Wilna Oldewage-Theron co-ordinated and administrated the project as project leader. This study was also a under-study of Dr. Wilna Oldewage-Theron's PhD study
- Emsie Dicks studied the compliance and consumer acceptability of the fortified sugar.
- Mosa Selepe did the nutritional data gathering and analyses.
- Jose van Rensburg assisted in blood analysis.

CHAPTER 4

RESULTS

4.1 Introduction

The focus of this dissertation is on the effects of the intake of a vitamin A fortified product on plasma fibrinogen levels. However, to interpret the changes in plasma fibrinogen, changes in other measured variables as reported by Oldewage-Theron (2001) are also given.

Results reported in this chapter are: dietary patterns, food diary, changes in the vitamin A and plasma fibrinogen status. The correlation between vitamin A and other variables and the correlation of plasma fibrinogen level with relevant variables will also be indicated.

4.2 Dietary patterns of the sample population

4.2.1 QFFQ results

The QFFQ's were analysed to determine the food items most often consumed by the sample population, and are summarised in *Table 14*.

Table 14 Top 22 items consumed by the sample population (P = 0,05)

Food Item	Mean daily intake (gram per person)	Number of subjects with daily consumption
Tea, brewed	267,3	74
Coffee, brewed	177,9	60
Cold drink	160,1	75
Milk, whole, fresh	101,8	90
Fruit juice	93,95	40
Yoghurt, low fat, flavoured	81,85	59
Bread/Rolls	77,1	90
Maize porridge, cooked	63,6	90
Oats porridge, cooked	52,3	55
Malt beverages	50,1	32
Rice, white, cooked	45,7	88
Apple, raw	43,5	83
Potato chips (French fries)	42,8	58
Maltabella, uncooked	39,4	52
Breakfast cereals	34,6	50
Banana, raw	34,2	80
Macaroni/spaghetti, cooked	32,4	67
Sugar, white	31	90
Polony	30,1	80
Cabbage, cooked	28,1	10
Mageu	27,1	19
Mango, raw	26,7	71

Table 14 shows that the six top items by weight consumed by these subjects, were tea, coffee, cold drinks, milk, fruit juice and yoghurt, indicating a westernised-type diet. Maize, the staple food of Africans in SA, was 8th on the list, and mean daily intake of maize porridge was only 63,6 g. The daily mean consumption of sugar was 31 g. The mean sugar consumption over the 12 weeks increased, varying from 98,67 g per day in week 1 to 63,66 g per day during week 12. The QFFQ mean sugar intake, measured at baseline,

was reported to be 31 g per day. The subjects thus reported much higher intakes when completing the food diaries. A possible reason for the higher consumption could be that the sugar was given to the subjects for free and that this encouraged them to consume more sugar than usual. This was particularly true for the first month after they had received the sugar (mean daily intake of 98,67 g). This reported high intake decreased gradually until the end of the study.

In addition to fruit juice, three other fruits namely apple, banana and mango were under the top 22 foods consumed. Of these foods, mango would contribute substantially to vitamin A intake, containing 66 µg RE vitamin A and 395 β-carotene equivalents in total carotenoids per 100 g (Kruger, *et al*., 1998:52). It is noteworthy that unless fortified (malt beverages and breakfast cereals) no special rich source of iron (red meat, spinach) appeared in this list.

4.2.2 Food diary results

The nutrient analysis of the food diaries that the subjects kept to monitor intake of a selected group of foods rich in vitamin A and iron (see Annexure 11) is given in *table 15*. The vitamin A added to the sugar, which the experimental subjects consumed, is not included in the analysis. (*See table 15)*

Table 15 Analysis of food diaries: daily mean intakes from selected items

Nutrient and unit	Week 1 measurement	Week 4 measurement	Week 8 measurement	Week 12 measurement	RDA*/RNI **
Energy (kJ)	4785±2632	4296±2234	3922±1657	4227±1307	
Protein (g)	28,1 ± 21,1	29,92±18,4	30,18±17,7	32,11±18,6	56*
Fat (g)	58,01 ± 51,2	54,12±46,4	49,5±30,4	52,25±21,6	
Cholesterol (g)	498,9 ± 451,6	561,51±395,7	551,23±349,6	505,15±350,3	
Carbohydrates(g)	129,12 ± 55,4	105,54±45,5	93,41±33,6	102,07±34,7	
Sugar (g)	98,67±50,25	76,25±40,0	64,5±26,55	63,66±25,9	
Dietary fibre (g)	5,06 ± 3,8	5,60±4,7	5,42±3,7	7,61±3,7	
Calcium (mg)	536,89 ± 459,9	484,46±361,7	478,21±356,1	583,08±386,7	700**
Iron (mg)	4,83 ± 4,4	5,56±4,6	5,80±4,1	6,00±4,8	14,8**
Sodium (mg)	652,54 ± 498,0	647,66±395,1	631,83±354,3	767,46±349,0	1,6**
Vitamin A (µg	1068±560	1201±959	1220±843	1349±954	1000*/600**

Nutrient and unit	Week 1 measurement	Week 4 measurement	Week 8 measurement	Week 12 measurement	RDA*/RNI **
RE)					
Thiamin (mg)	0,33 ± 0,2	0,35±0,2	0,35±0,2	0,40±0,2	0,4 mg/4200 kJ **j
Riboflavin (mg)	1,28 ± 1,1	1,35±1,0	1,41±0,9	1,46±1,0	1,4*/1,1**
Niacin (mg)	2,88 ± 2,5	3,13±2,7	3,34±2,4	3,96±2,7	6,6 mg/4200 kJ **
Vitamin B6 (µg)	0,54 ± 0,4	0,59±.0,4	0,60±0,4	0,68±0,4	15 µg/g protein **
Folic acid (µg)	268,99 ± 321,2	315,95±315,4	353,78±338,4	333,21±375,5	100*/200**
Vitamin B12 (µg)	6,94 ± 8,2	7,93±8,6	8,87±8,5	8,09±9,2	1,6*/1,5**
Vitamin C (mg)	28,41 ± 26,2	31,45±29,1	34,19±27,2	41,37±32,5	45*/10-40**
Vitamin D (µg)	4,35 ± 3,7	5,35±3,5	4,77±2,9	4,56±3,0	
Vitamin E (mg)	6,92 ±4,2	7,81±4,4	7,46±3,8	8,62±3,7	3**

* The Recommended Daily Allowances (RDA'S) for South African women. The RDA is the daily dietary intake level that is sufficient to meet the nutrient requirements of nearly all (97-98 %) individuals in a given life-stage and gender group.

** Reference Nutrient Intake (RNI) for the women in the United Kingdom. These intake levels should satisfy 97 % of the daily nutrient requirements.

The iron provided by these specifically selected foods rich in iron was between 50 % and 65 % of daily requirements. Mean vitamin A intakes (without the fraction provided by the fortified sugar) was also relatively constant varying from 1068 to 1349 µg RE. This was substantially higher than the 600 - 1000 µg RE recommended. However, the standard deviations were large, illustrating the large variability in intakes between subjects. The mean sugar consumption over the 12 weeks increased, varying from 98,67g per day in week 1 to 63,66 g per day during week 12. The QFFQ mean sugar intake, measured at baseline, was reported to be 31 g per day.

4.3 Vitamin A status

4.3.1 Baseline Vitamin A results

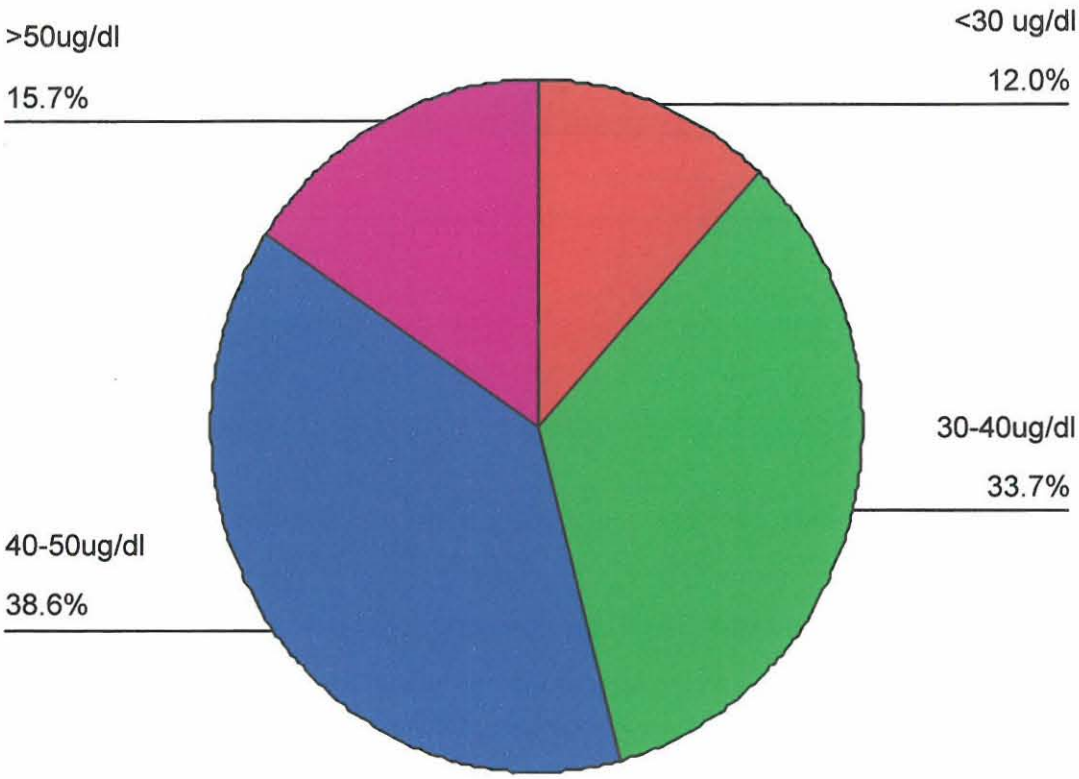


Figure 9 Baseline Vitamin A status

Baseline measurements of serum vitamin A indicated: 12% of respondents had a serum retinol of < 30 µg/dl, 33.7% a level of 30µg/dl -40µg/dl, 38.6% had a level of 40µg/dl - 50µg/dl and 15.7% of respondents had a serum retinol level of > 50µg/dl.

4.3.2 Changes in VA status during VA fortification period

Table 16 ***Comparison of individual serum retinol levels (µg/dL) at baseline and after 12 weeks*** (↑ increase, ↓ decrease, ↔ no change)

No	Experimental group		Change	No	Placebo group		Change
	Baseline (µg/dL)	Week 12 (µg/dL)	(> 5 g/dL)		Baseline (µg/dL)	Week 12 (µg/dL)	(> 5 g/dL)
4	41,7	47,8	↑	1	36,8	36,7	↔
5	57,2	57,0	↔	2	55,1	55,0	↔
7	45,5	59,0	↑	3	36,8	47,5	↑
10	49,9	72,0	↑	6	42,2	65,0	↑
16	37,6	37,4	↔	11	21,2	35,0	↑
18	41,3	66,8	↑	13	44,2	45,0	↔
22	40,8	69,7	↑	15	34,0	73,7	↑
23	40,2	64,3	↑	17	43,1	55,3	↑
25	56,5	64,2	↑	20	24,3	24,1	↔
27	49,8	67,0	↑	21	38,0	45,3	↑
28	45,2	48,0	↔	26	59,0	36,6	↓
30	105,5	105,5	↔	32	21,5	36,4	↑
31	25,5	26,1	↔	34	78,2	52,8	↓
41	107,1	71,0	↓	35	36,3	35,0	↔
44	29,4	34,7	↑	37	39,8	39,9	↔
50	47,2	48,1	↔	42	32,4	39,0	↑
52	59,9	61,3	↔	46	33,0	47,9	↑
53	57,3	61,4	↔	47	64,5	25,7	↓
54	44,1	50,3	↑	48	34,2	36,8	↔
56	63,3	69,3	↑	49	77,0	64,6	↓
58	31,3	31,4	↔	55	48,8	66,0	↑
60	24,8	30,3	↑	57	31,2	26,0	↓
62	60,8	47,1	↓	61	40,2	25,2	↓
63	45,1	26,8	↓	69	43,9	44,6	↔
64	45,0	36,9	↓	74	41,0	42,8	↔
65	35,0	43,4	↑	75	42,6	44,0	↔
66	65,0	71,0	↑	80	53,0	48,0	↔
67	29,0	30,2	↔	83	41,0	41,4	↔
68	33,7	41,2	↑	85	31,0	25,6	↓
70	37,2	17,9	↓	86	40,0	40,8	↔
71	43,8	38,3	↓	90	29,6	29,6	↔
72	43,6	55,0	↑	91	23,1	33,0	↑
73	43,3	36,0	↓	93	44,0	34,6	↓
76	24,9	43,7	↑	94	51,5	29,7	↓

No	Experimental group	Change	No	Placebo group	Change	68,9	↑
	Baseline (µg/dl)	Week 12 (µg/dl)	(> 5 g/dl)		Baseline (µg/dl)	Week 12 (µg/dl)	(> 5 g/dl)
79	66,0	74,7	↑	97	38,8	52,6	↑
81	51,0	43,4	↓	98	34,3	34,3	↔
82	44,0	53,3	↑	99	59,6	56,2	↔
84	47,0	54,0	↑	100	32,1	38,4	↑
87	38,5	52,1	↑				
89	35,6	47,7	↑				
92	35,5	52,8	↑				
↑	23	53,5 %		↑	15	37,5 %	
↓	11	25,6 %		↓	9	22,5 %	
↔	9	20,9 %		↔	16	40, 0 %	

4.4 Fibrinogen status

4.4.1 Baseline fibrinogen results

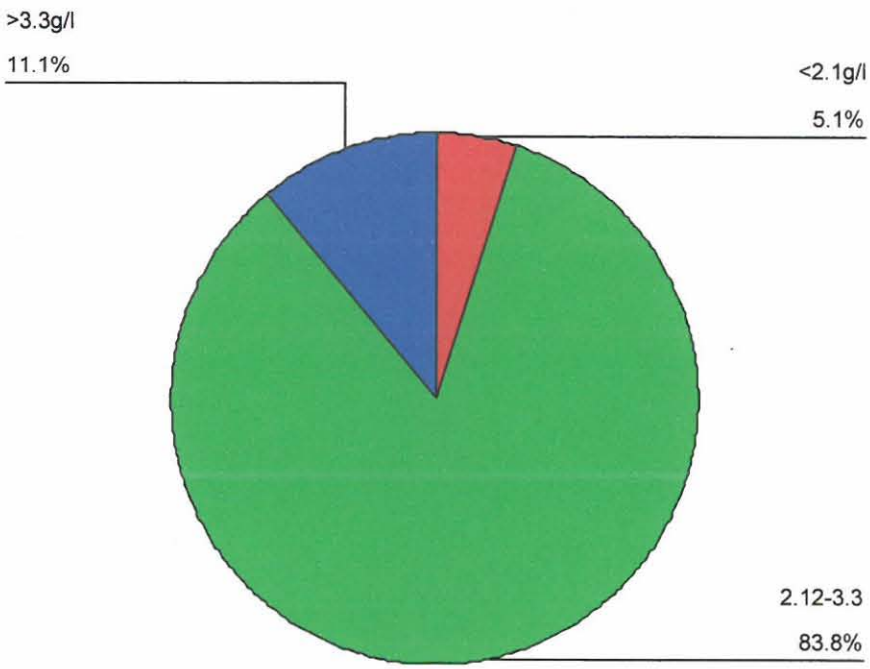


Figure 10 Baseline plasma fibrinogen status

There is some controversy in the literature about a "normal" plasma fibrinogen level (Vorster, 2000) and the level at which it starts to operate as a CHD risk factor. The Northwick Park Heart Study (Meade *et al.*, 1986) showed that one standard deviation increase (0,60 g/L) in fibrinogen was associated with an 84 % increase in CHD risk. The mean levels of fibrinogen (2,81 g/L) found in these women were similar to levels found by Venter *et al.* (1992) in Tswana-speaking black men and women of approximately 2,8 g/L. These were significantly higher than levels in white control subjects. It seems therefore that the subjects in this study had relatively high levels of plasma fibrinogen

4.4.2 Changes in fibrinogen status during VA fortification period

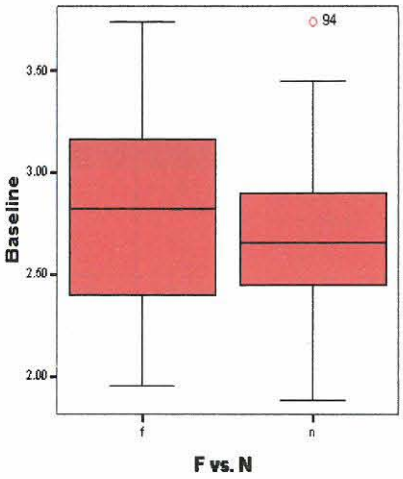


Figure 11

Baseline plasma fibrinogen results

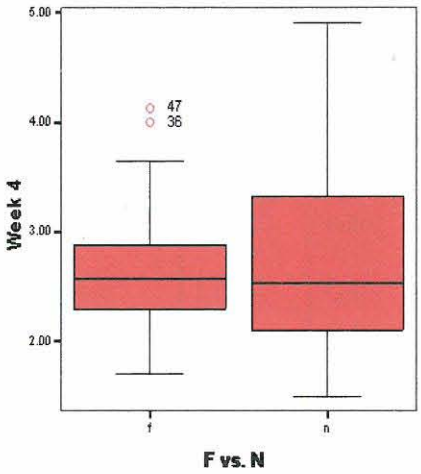


Figure 12

Week 4 plasma fibrinogen results

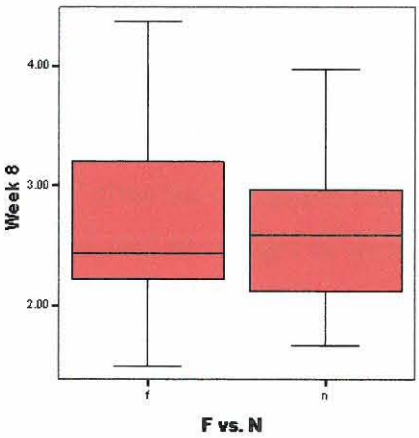


Figure 13

Week 8 plasma fibrinogen results

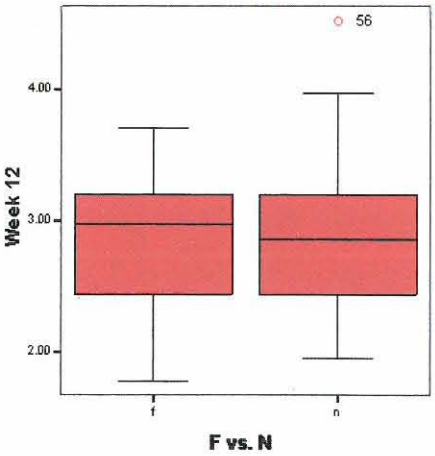


Figure 14

Week 12 plasma fibrinogen results

Table 17 Changes in plasma fibrinogen and related variables during VA fortification

		Experimental group (n = 44)				Placebo group (n = 40)			
Variable	Normal range and unit	Week 0/1	Week 4	Week 8	Week 12	Week 0/1	Week 4	Week 8	Week 12
Fibrinogen (mean) ± SD	2,72 – 3,30 g/L	2,82 [a,b] ±0,47	2,65 [a] ±0,52	2,67 [b] ±0,68	2,84 ±0,49	2,68 ±0,37	2,76 ±0,85	2,75 ±0,54	2,86 ±0,58
WCC (mean) ± SD	3,5-10,0 X $10^3/mm^3$ OR 3500-10000	4,96 [a,b] ±1,23	4,52 ±1,37	5,08 [a] ±1,35	4,98 [b] ±1,47	5,27 ±1,6	5,04 ±1,61	5,52 ±1,58	5,11 ±1,61
BMI (mean) ± SD	20 - 25 kg/m^2	22,0 [a] ±4,01	22,0 ±4,11	22,0 ±4,07	22,4 [a] ±3,83	22,1 ±3,13	22,1 ±3,06	22,2 ±3,16	22,4 ±3,03
Waist:Hip ratio (mean) ± SD	0,75	0,74 [a] ±0,05	0,74 ±0,05	0,73 [a] ±0,05	0,73 ±0,05	0,73 ±0,05	0,73 ±0,05	0,73 ±0,05	0,73 ±0,05
Serum retinol (mean) ± SD	≥ 30 µg/dL	47,0 [a,b] ± 17,20	43,90 ± 14,40	51,90 [a] ± 14,40	49,20 [b] ± 14,80	43,30 ± 14,70	43,30 ± 12,00	49,50 ± 14,00	47,60 ± 15,50

Means with the same symbol differs significantly within groups. The same subjects have been used throughout the trial.

Table 17 gives the changes in plasma fibrinogen observed during the 12-week intervention period, as well as changes in serum retinol, WBC and BMI. It is clear that no significant changes for any of these variables occurred in the placebo group. Despite only small, but statistically significant changes in serum retinol at 8 and 12 weeks, plasma fibrinogen decreased significantly after four and eight weeks in the experimental group, but returned to baseline values after 12 weeks. White blood cell counts were significantly higher in the experimental group after eight weeks while BMI was significantly higher in this group after 12 weeks compared to baseline.

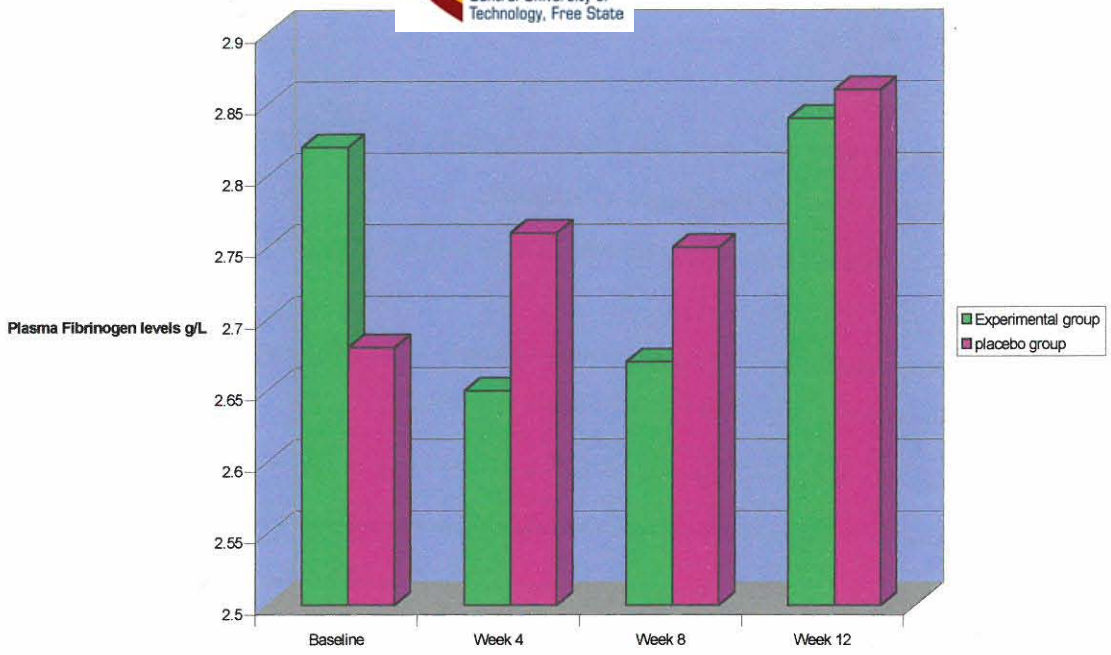


Figure 15 Summary of changes in plasma fibrinogen levels observed.

4.5 BMI status

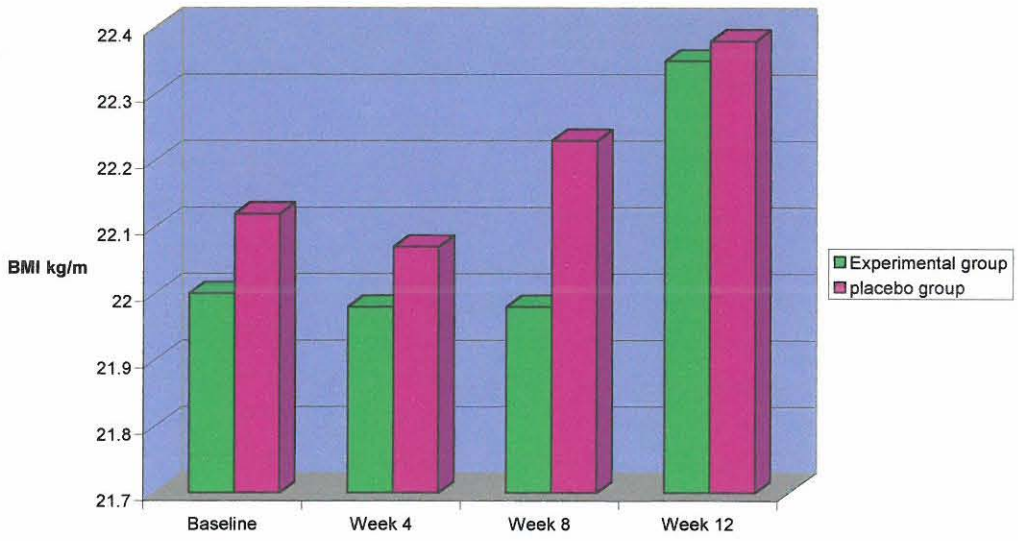


Figure 16 Summary of changes in BMI observed

Figure 16 gives the changes in BMI observed during the 12 weeks in the experimental and control group in the placebo group. A statistically significant (α=0.01) increase in BMI occurred during the 12 week intervention in both placebo and control groups.

Table 18 Summary of the BMI classification during the intervention period

Summary table of cut-off points for the variable: BMI

		Baseline	Week 4	Week 8	Week 12
Percentage of respondents <= 18.5 (Underweight)	F	15.22	15.22	13.04	11.11
	N	13.95	13.95	13.95	9.3
Percentage of respondents > 18.5 and <= 25 (Normal weight)	F	71.74	71.74	76.09	68.89
	N	74.42	74.42	72.09	74.42
Percentage of respondents > 25 and <=30 (Overweight)	F	8.7	8.7	6.52	15.56
	N	9.3	9.3	11.63	13.95
Percentage of respondents > 30 (Obese)	F	4.35	4.35	4.35	4.44
	N	2.33	2.33	2.33	2.33
	F: n =	46	46	46	45
	N: n =	43	43	43	43

F = Fortified group (experimental group)

N = Non-fortified (control group)

4.6 Correlation between serum retinol and other variables

Table 19 Correlations between serum retinol and other serum, blood and plasma variables and BMI as per Pearson correlation (two-tailed)

Variable Serum retinol with:	Baseline (n=100)		At end of study (week 12) (n = 83)	
	r	p	r	p
Red blood cell count	0,052	0,642	0,237*	0,031
Hb	0,295	0,007	0,322*	0,003
Hct	0,296	0,007	0,303*	0,005
Mean cell volume	0,288*	0,008	0,183	0,098
White blood count	0,201	0,068	0,009	0,933
Serum iron	0,413*	0,000	0,407*	0,000
Ferritin	0,316*	0,004	0,270*	0,014
Transferrin	-0,164	0,140	-0,116	0,014
S-transferrin receptor	-0,312*	0,004	0,205	0,063
Fibrinogen	0,092	0,408	0,067	0,547
Body mass index	0,195	0,079	0,402*	0,000

* Correlation is significant at the 0,05 level (two-tailed)

Table 19 gives the correlations between serum retinol and some of the other biochemical variables. At baseline serum retinol showed significant positive correlations with Hb, Hct, mean cell volume, serum iron and ferritin. No significant relationships with BMI, fibrinogen and transferrin were observed. However, a highly significant negative relationship with the serum transferrin receptor (r = -0,312; P = 0,004) was found at baseline.

At the end of the study of 12 weeks, during which half of the subjects consumed vitamin A fortified sugar, a significant relationship between serum retinol and red blood cell count emerged, as well as a significant negative correlation between transferrin and serum retinol. The other correlations observed at baseline were also apparent at 12 weeks, except that the correlation of serum retinol with the serum transferrin receptor was no longer significant (r = -0,025; P = 0,063).

4.7 Correlation between fibrinogen status and other variables

Table 20 ***Correlations between fibrinogen and all the other variables as per Pearson correlation (two-tailed)***

Variable	Baseline (n=100)		At end of study (week 12) (n= 83)	
	r	P	r	P
Serum retinol	0,092	0,408	0,067	0,547
Red blood cell count	0,075	0,499	-0,090	0,421
Hb	-0,021	0,853	0,124	0,263
Hct	0,008	0,946	0,119	0,285
Mean cell volume	-0,072	0,516	0,229*	0,037
White blood count	0,243*	0,022	0,113	0,293
Serum iron	-0,001	0,994	0,019	0,864
Ferritin	0,262*	0,017	0,053	0,633
Transferrin	-0,107	0,336	-0,217*	0,048
S-transferrin receptor	0,206	0,062	-0,086	0,442
Body mass index	0,232*	0,033	0,257*	0,018

* Correlation is significant at the 0,05 level (two-tailed)

Table 20 shows that in these subjects serum retinol were not related to plasma fibrinogen. Plasma fibrinogen showed significant positive correlations with the white blood cell count, ferritin and BMI. The relationship between plasma fibrinogen and the serum transferrin receptor was significant on a 6 % level (r = 0,206; P = 0,062) at baseline, but was not observed after 12 weeks, and was probably not reflecting a real association.

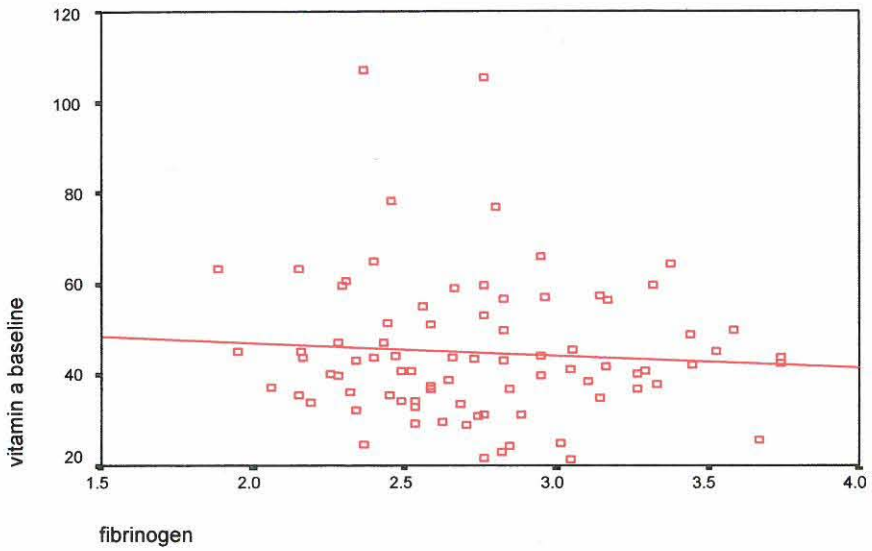


Figure 17 ***Scatterplot of the correlation between VA and plasma fibrinogen levels***

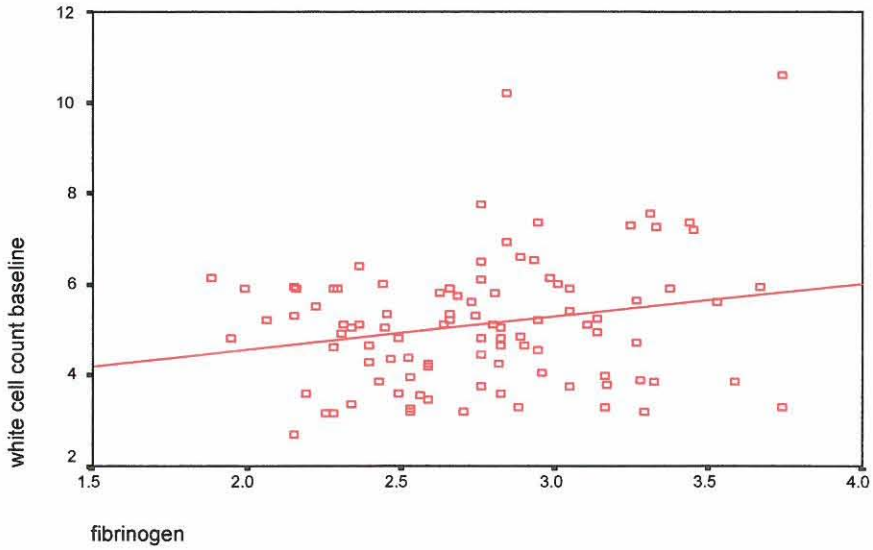


Figure 18 ***Scatterplot of the correlation between WCC and plasma fibrinogen levels***

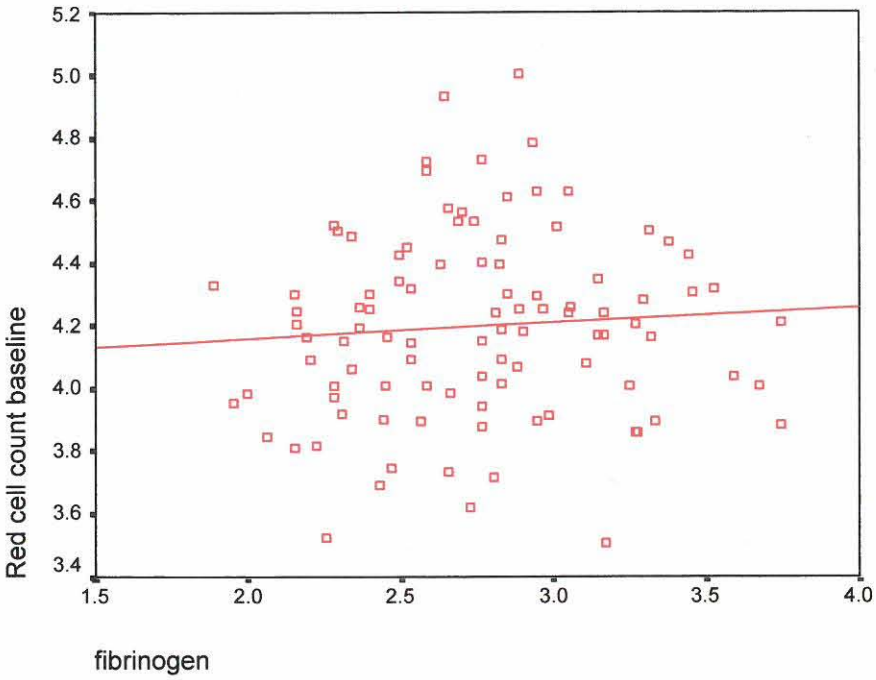


Figure 19 *Scatterplot of the correlation between RCC and plasma fibrinogen levels*

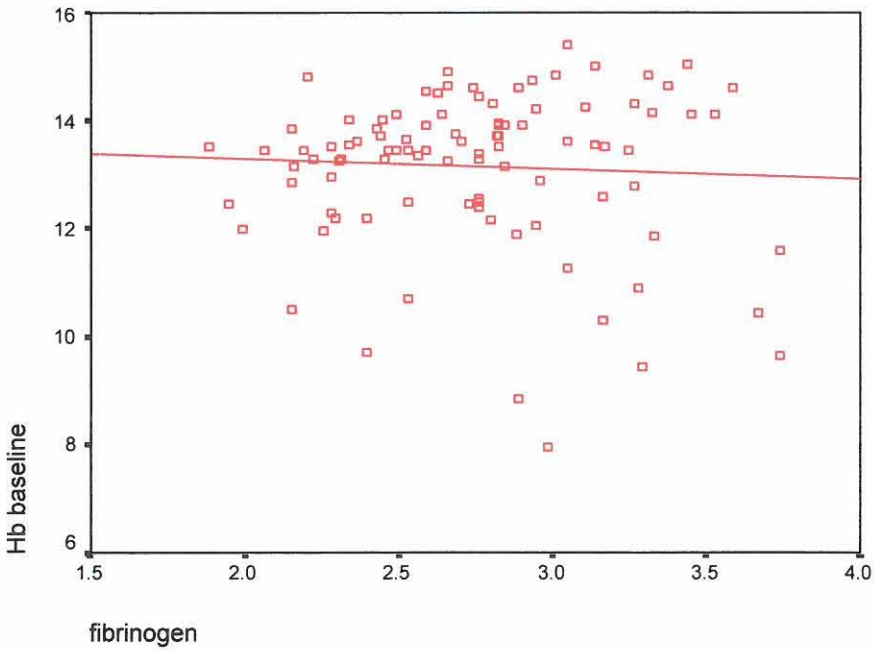


Figure 20 *Scatterplot of the correlation between Hb and plasma fibrinogen levels*

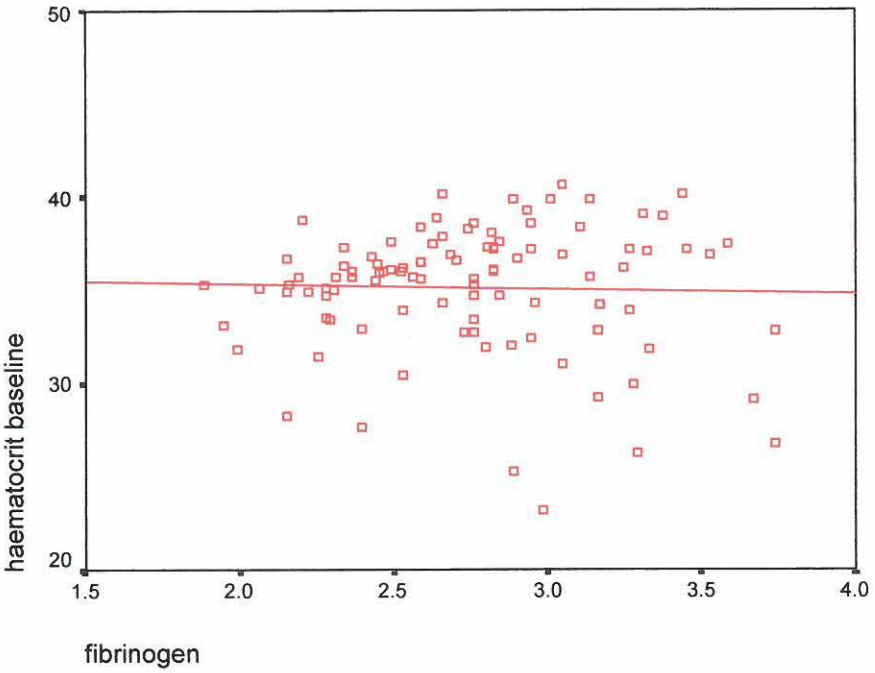


Figure 21 ***Scatterplot of the correlation between Hct and plasma fibrinogen levels***

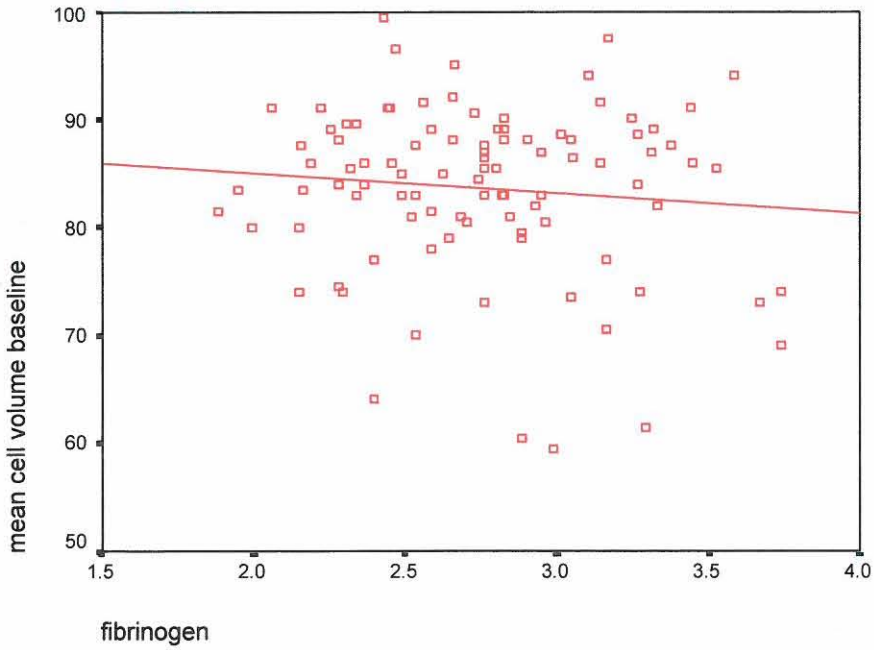


Figure 22 ***Scatterplot of the correlation between MCV and plasma fibrinogen levels***

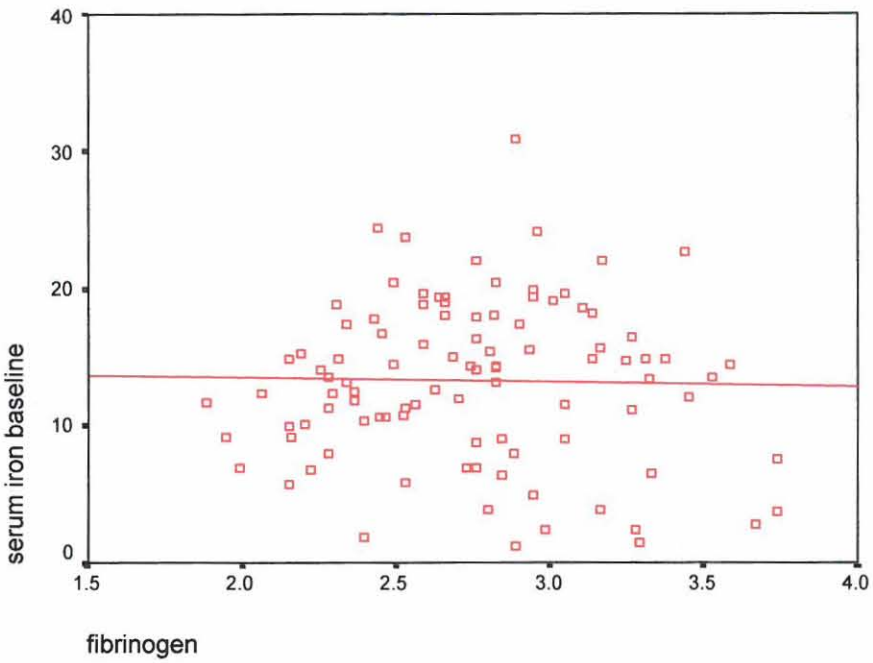


Figure 23 ***Scatterplot of the correlation between serum iron and plasma fibrinogen levels***

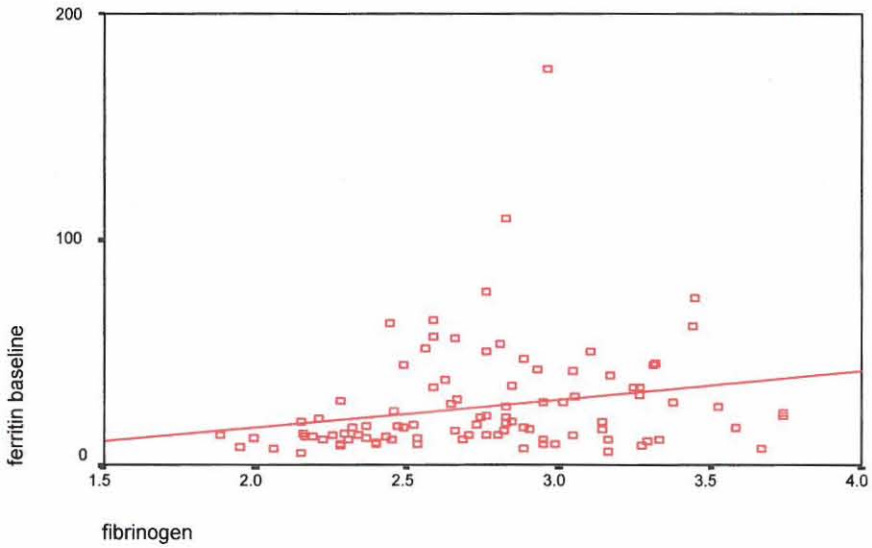


Figure 24 ***Scatterplot of the correlation between serum ferritin and plasma fibrinogen levels***

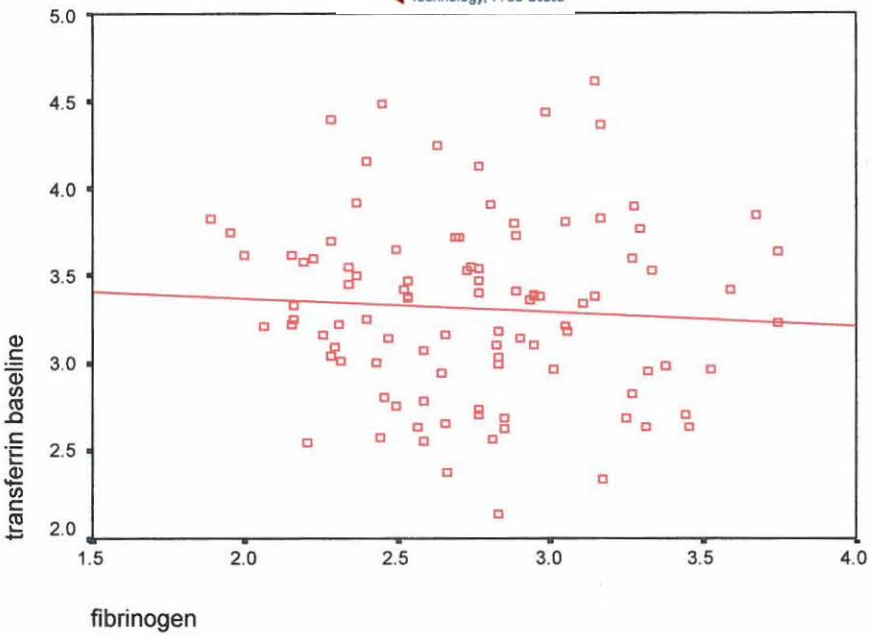


Figure 25 ***Scatterplot of the correlation between serum transferrin and plasma fibrinogen levels***

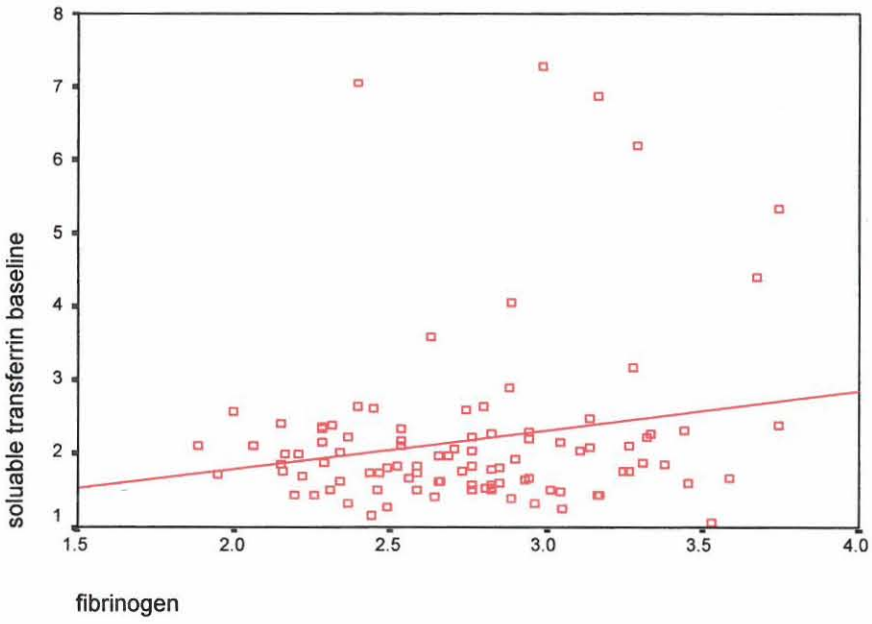


Figure 26 ***Scatterplot of the correlation between serum soluable transferrin receptor and plasma fibrinogen levels***

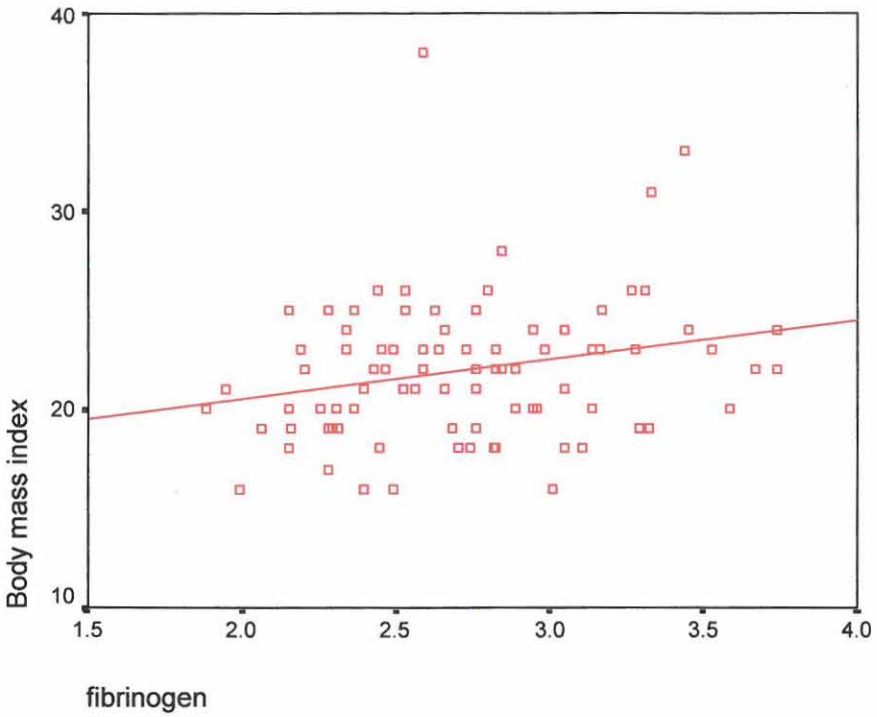


Figure 27 ***Scatterplot of the correlation between BMI and plasma fibrinogen levels***

4.8 Drop outs

At the end of the 12-week experimental period, 83 subjects completed the study protocol successfully and 17 % were lost to the study. A total of 40 subjects remained in the experimental group compared to 43 in the placebo group. The details of subjects who were lost to follow-up are compared to those of the subjects who completed the study in Table 21.

Table 21 ***Baseline characteristics of participants with those lost to follow-up and thus excluded****

Baseline measurement	Participants (n = 83) Mean ± SD	Drop-outs (n = 17) Mean ± SD
Body weight (kg)	56,20 ± 12,32 [a]	65,11 ± 8,21 [a]
Body mass index (kg/m^2)	21,86 ± 3,53 [b]	24,06 ± 2,46 [b]
Fibrinogen (g/L)	2,99 ± 0,51	2,70 ± 0,95
White blood cells (10^3/mm^3)	5,25 ± 1,56	4,74 ± 1,46
Temperature (°C)	36,80 ± 0,52	38.80 ± 0,35
Systolic blood pressure (mm Hg)	105,30 ± 11,10	103,50 ±13,20
Diastolic blood pressure (mm Hg)	69,50 ± 8,70	72,90 ± 8,49
Serum iron (µmol/L)	13,42 ± 6,50	13,69 ± 6,15
Transferrin (g/L)	3,55 ± 0,56	3,40 ± 0,55
Ferritin (ng/mL)	26,07 ± 24,32	24,47 ± 24,37
Haemoglobin (g/dL)	13,33 ± 1,50	13,45 ± 1,12
Haematocrit (%)	35,35 ± 3,48	35,60 ± 2,77
Mean cell volume (fl)	83,24 ± 7,63	85,18 ± 7,51
Red blood count (10^6/mm^3)	4,24 ± 0,29	4,13 ± 0,38
Serum retinol (µmol/L)	1,93 ± 1,02	1,53 ± 0,75

a, b : means with the same symbol differ significantly (P < 0,05)

* : Values are means with 95 % confidence interval. Seventeen subjects were lost to follow-up (Levene's test for equality of variances, P = 0,05).

The only statistically significant differences between the participants and the drop-outs were body weight and BMI. The drop-outs had a higher mean weight and BMI than the participants.

CHAPTER 5

COMBINED DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

5.1 Discussion

5.1.1 Introduction

The major objectives of this study were to examine the effects of the consumption of vitamin A fortified sugar on vitamin A status, and plasma fibrinogen of 13-25 year old black South African girls and women in a double blind placebo-controlled parallel designed clinical trial.

Increased plasma fibrinogen is a major risk factor for CHD (Folsom, 1995:21) and stroke (Krobot *et al.*, 1992:780). Fibrinogen is known to be increased in black South Africans (Vorster *et al.*, 1998:174) and the THUSA study (James *et al.*, 2000:392) suggested that these high levels were associated with undernutrition in men and overnutrition and obesity in women. The purpose of this study was to assess the relationship between plasma fibrinogen and to test the hypothesis that increased intakes of vitamin A will lower plasma fibrinogen.

Subsidiary objectives were to examine the correlation between plasma fibrinogen and WCC, red cell parameters, iron status and BMI.

The high prevalence of both undernutrition (especially of vitamins), and overnutrition and high incidence of CVD in SA (especially in black women), motivated this study.

5.1.2 Limitations of the study

- The first limitation of this study is probably the choice of subjects. Young black women volunteers were chosen, because they were thought to be the most vulnerable group regarding vitamin A and iron status, and also a group

with the potential of becoming obese and increased risk of CVD in later life. For the clinical intervention trial a group of vitamin A deficient subjects could have produced more definite results.

- The second limitation may lie in the exclusion of c-reactive protein (CRP) from the test panel. CRP gives a definite correlation between reactive condition and the influence on the plasma fibrinogen levels. The collection of the blood for the determination of week 12 was done in May. Therefore the seasonal change could have an influence on the results of week 12.
- The third limitation may be the fact that only serum retinol was measured to determine vitamin A status. Mahan and Escott-Stump (2000:70) mention that the serum retinol levels are maintained between 40 and 50 µg/dL in healthy adults with normal serum retinol levels, and in these subjects 91,7 % had normal serum retinol levels (≥ 30 µg/dL). Serum retinol binding protein (SRBP) should have been measured to provide additional information.
- Even though there were no statistical significance between the participants and the dropouts it could be considered as a limitation of this study.

5.1.3 Main findings

The salient findings of this study were:

- In the 83 subjects who completed the clinical intervention trial, 88 % had normal serum retinol levels (≥ 30 µg/dL). Of the experimental group, 23 (53,5 %) had an increase of more than 5 µg/dL serum retinol at week 12 compared to baseline, while 20,9 % showed no change and 25,6 % had lower levels. Comparative figures for the placebo group were 37,5 % that showed an increase, 40 % who had no change and 22,5 % that showed a decrease.
- This intervention study provides some evidence that increased intakes of vitamin A by subjects with acceptable vitamin A status resulted in small, but statistically significant decreases in plasma fibrinogen. These decreases were, however, not sustained, probably because of observed increases in BMI or due to seasonal changes. The experimental subjects showed a significantly higher BMI at week 12. Møller & Kristensen (1991:344) found a strong univariate association between the waist-to-hip ratio and the fibrinogen levels in a study

of 439 51-year-old men. Bao *et al* (1993:324) demonstrated in the Bogalusa Heart Study a consistent increase in fibrinogen levels with ponderal index and skinfolds in children. In the THUSA study of Africans in the Northwest province of South Africa, BMI showed a significant positive correlation with fibrinogen (r=0.25; p=0.000) (James *et al*, 2000:385). Folsom *et al* (1991:200) developed two models to predict changes in fibrinogen for a 5-kg/m^2 increase in BMI. The first model showed a 0.85 g/l increase in men and a 1.23g/l increase in women, and the second a 0.6 g/l increase in men and a 0.98 g/l increase in women. It seems reasonable to conclude that this increase in BMI has been responsible for the increase in plasma fibrinogen.

- Higher plasma fibrinogen levels occur in winter compared to levels in the summer. Variation in fibrinogen levels can be 5-20% higher in winter than in summer. Speculation that higher fibrinogen levels in winter may reflect either temperature or acute-phase response to respiratory infections. (Meade, 1995:33). Week 12 was done in early winter, and can be a possible explanation of the dramatic increase in the plasma fibrinogen levels of both the experimental and placebo group. Fibrinogen is an acute phase protein. The increased white blood cell count of the experimental group at weeks 8 and 12 could indicate some immune response with concomitant rises in plasma fibrinogen. The positive relationships between plasma fibrinogen and white blood cell count, as well as ferritin are not unexpected. White blood cell count can be expected to increase with infections, and both fibrinogen and ferritin are acute phase proteins, known to increase with infection. These results suggest that despite the inclusion criterion of "healthy", low-grade infections were present at baseline in these subjects.
- The relationship between plasma fibrinogen and serum ferritin (reflecting stored iron), and also observed in the BRISK study (Vorster *et al*., 1998:174), could possibly be because both are acute phase proteins – or there may be another mechanism involved. Excess iron is stored in the liver and fibrinogen is synthesised and secreted by the liver. However, to clarify these mechanisms, much more basic research is needed.

- Plasma fibrinogen showed significant positive correlations with white blood cell count, ferritin and BMI. The relationship between plasma fibrinogen and the serum transferrin receptor was significant on a 6 % level at baseline.

5.1.4 Possible mechanisms of vitamin A and fibrinogen interaction

Many demographic and environmental factors are known to affect fibrinogen levels. The effect of these environmental factors is largely through an effect on the acute phase reaction (Krobot *et al.*, 1992:780). It is also known that genetic factors determine the fibrinogen levels, and the response of fibrinogen levels to environmental factors. Estimates suggested 30-50% of the plasma fibrinogen level is genetically determined (Hamstein *et al.*, 1987:989).

The effect of dietary components on plasma fibrinogen levels is modest. Several components have been identified that have a modest effect on plasma fibrinogen levels. Only moderate alcohol consumption was found to have a consistent and clear fibrinogen lowering effect (De Maat, 2001:511).

These weak associations are surprising, since several dietary components affect mechanisms that regulate the fibrinogen synthesis. Dietary antioxidants would be expected to protect from processes brought about by oxidative damage, such as an upregulation of genes of inflammatory components (De Maat, 2001:511).

De Maat (2001:511) explained the fact that only minimal and often contradicting effects of dietary components on fibrinogen levels are observed, may be due to the study design.

The fact that fortified sugar was used in this study, as a supplier of vitamin A may be the reason for the unsustainablility of the down regulation of the plasma fibrinogen levels in this study. Supplied sugar and the dietary intervention as part of this study design, were possibly the reason for the increase in BMI, which also affected the plasma fibrinogen level.

5.2 Conclusions

There is some evidence that increased intakes of vitamin A by subjects with acceptable vitamin A status resulted in small, but statistically significant decreases in plasma fibrinogen. These decreases were, however, not sustained, probably because of observed increases in BMI and weight, as well as seasonal changes.

Although CHD prevalence is still low in black South Africans, it was suggested that high fibrinogen levels might be a major risk factor for stroke in this population group (Vorster *et al.*, 1998). It seems reasonable to conclude that fibrinogen levels were raised in these subjects, that they could therefore be lowered, and that they should be lowered to decrease risk for future stroke – even in these young subjects. This emphasises the need for lifestyle interventions to lower plasma fibrinogen concentrations.

5.3 Recommendations

5.3.1 Further research

The results of this study indicate that further research is needed addressing the following issues:

- The mechanism by which vitamin A and fibrinogen synthesis interact needs to be studied and determined in order to plan future intervention programmes.
- Future studies should examine the effect over a longer period of consumption.
- The influence of vitamin A on fibrinogen and its functionality (clottable protein, changes in turbidity, and immunological method specific for total High and low molecular weight fibrinogen (Niewenhuizen, 1995:8), should be examined in subjects with raised fibrinogen and low vitamin A status, often seen among African adults at risk for cardiovascular disease.
- Lifestyle intervention studies should be investigated among young South African women. The influence of lifestyle practices (like environmental pollution, diet and exercise) on plasma fibrinogen levels could be investigated.

5.4 Practical implications of the results

This research project provided the opportunity to determine the effect of vitamin A fortified sugar consumption on fibrinogen levels in a community and individuals at risk of VAD and CVD. It was the first time that an intervention study with vitamin A fortified sugar was conducted in South Africa.

The results of the clinical intervention trial provide information regarding the effect of fortified vitamin A on fibrinogen levels of healthy young females. The results could therefore have an impact on public health policy regarding the treatment of micronutrient, specifically vitamin A deficiencies. Programmes aimed at the prevention of micronutrient deficiency, rather than treatment, may help to prevent the rising health costs in the country.

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Annexure 1

Central University of Technology, Free State

POTCHEFSTROOMSE UNIVERSITEIT VIR CHRISTELIKE HOëR ONDERWYS

AANSOEK OM GOEDKEURING VIR EKSPERIMENTERING MET MENSE
(soos bygewerk in Augustus 1999)

VERTROULIK

(Volledig voltooide vorms **in vyfvoud** moet die sekretaris van die Etiekkomitee minstens een maand voor die aanvang van die eksperiment bereik. Projekte waar die navorser net bloedmonsters vir analise ontvang sonder dat hyself by die proefpersone betrokke is, moet steeds langs die gewone weg geregistreer word. Navorsers wat aan projekte deelneem wat by ander instansies se Etiekkomitees geregistreer is, moet hierdie komitee per brief van sodanige projekte in kennis stel en die komitee van 'n protokol voorsien.)

Hierdie vorm is ook op rekenaarskyf in WinWord- en MSWord-formaat of *via* die PUK-rekenaarnetwerk beskikbaar by F:\Apps\Alginl\Etiekkom\Mense.doc. Die jongste weergawe van die vorm, soos op die netwerk beskikbaar, moet gebruik word.

Persoonlike besonderhede van projekleier/navorser

1. Titel, voorletters en van: Mev W H Oldewage-Theron.....
2. Volledige kwalifikasies: B Sc Dieetkunde Honneurs, Nagraadse Diploma in Hospitaal Dieetkunde, M Sc .....
3. Rang/pos beklee: Hoof van Departement: Voedsel, Vaaldriehoek Technikon .....
   ('n Volledige curriculum vitae moet aangeheg word deur aansoekers vir wie daar nie 'n CV sentraal aan die PU vir CHO beskikbaar is nie asook deur alle eerste aansoekers moet so 'n CV een keer per jaar aanheg).
4. Skool (vakgroep)/Instituut: Skool vir Fisiologie, Voeding en Gesinsekologie.....
5. Telefoon: (016) 950 9279..... (w) (016) 423 2660.....(h)
6. PU Bussie: N V T .....

Besonderhede van eksperiment

1. Titel van projek/proef:

   Evaluation of the fortification of tea and sugar with vitamin A and iron.....
2. Volle name, van, rang/pos en kwalifikasies van werklike toesighouer indien nie projekleier/navorser self nie:

   .....

   .....

   .....
3. Titels, voorletters, van en kwalifikasies van alle medewerkers:

   Me M Selepe, B Nutrition (US) .....

   Me E Dicks B Huishoudkunde Hons.....

Central University of Technology, Free State

4. Naam en adres van toesighoudende geneesheer:

.....

.....

(In alle gevalle waar noodsituasies moontlik kan ontstaan, word die fisiese teenwoordigheid van 'n geneesheer en 'n geregistreerde verpleegkundige vereis. Vir die onttrekking van bloedmonsters by diëetmanipulering en derglike studies kan met die teenwoordigheid van 'n geregistreerde verpleegkundige volstaan word.)

5. Beoogde aanvangsdatum: 10 Januarie 2000.....

6. Verwagte voltooiingsdatum: 1 Mei 2000.....

7. Plek waar eksperiment uitgevoer gaan word:
(Alle prosedures waarby noodsituasies kan ontstaan, moet binne 'n noodsorgruimte wat deur die toesighoudende geneesheer goedgekeur is, uitgevoer word.)

Vaaldriehoek Technikon.....

.....

8. Agtergrond:
(Beskryf kortliks die behoefte wat tot die betrokke eksperimentering aanleiding gegee het. Ondersteun u voorlegging met relevante literatuurverwysings.)

Kyk protokol aangeheg .....

.....

9. Doelstelling:
(Beskryf kortliks die doel wat met die proef nagestreef word)

Kyk protokol.....

.....

.....

10. Eksperimentele ontwerp en prosedures:
(Beskryf volledig hoe die eksperiment uitgevoer gaan word en watter prosedures gebruik gaan word. Dui alternatiewe prosedures aan, indien van toepassing, asook die statistiese beplanning. Indien daar van menslike weefsel of liggaamsvloeistowwe gebruik gemaak gaan word beskryf hoe u hierdie materiaal gaan wegdoen.)

Kyk protokol.....

.....

11. Eksperimentele medisyne:
(Gee die nodige besonderhede soos goedgekeurde naam, aanvaarde dosering, farmakologiese werking, ongewenste effekte, voorsorgmaatreëls, teenaanwysings en ander relevante inligting, om die Etiekkomitee in sy beoordeling van die aansoek te help. In die geval van bekende middels kan na handboeke verwys word.)

Geen.....

.....

.....

11.1 Is bogenoemde medisyne geregi.....

11.2 Indien nie, is goedkeuring vir die gebruik van ongeregistreerde medisyne vanaf die Medisynebeheerraad verkry? NVT.....

11.3 Indien "Ja" by 2.11.2 gee die datum van goedkeuring:

.....

(Finale goedkeuring van die aansoek deur die Etiekkomitee sal onderhewig wees aan goedkeuring van die proef deur die Medisynebeheerraad.)

12. Instansies wat eksperimentering borg.
(Gee, indien van toepassing, die naam en adres van alle instansies met 'n uiteensetting van die aard en omvang van die borgskap.)

Vaaldriehoek Technikon Sentrale Navorsingskomitee ± R 20 000

FRD (± R 40 000 aangevra).....

.....

12.1 Ontvang enige van die ondersoekers direk of indirek persoonlike vergoeding van die borg? Indien wel, spesifiseer.

Nee.....

.....

Annexure 1

Proefpersone

1. Ingeligte toestemming
   Die vorm vir ingeligte toestemming (hierby aangeheg) moet volledig ingevul en gehanteer word volgens die MNR-Riglyne ten opsigte van Etiese Beginsels in Mediese Navorsing, Hersiene Uitgawe (1987), Aanhangsel V.

   Vir nie-terapeutiese eksperimentering op proefpersone onder die ouderdom van 21 jaar is die skriftelike toestemming van sy/haar ouer of wettige voog nodig.

2. Ontvang die proefpersone vergoeding, en indien wel, hoeveel?

   Vervoer onkostes sal verhaal word, ± R 10 per persoon per dag. .....

   .....

   .....

   (U aandag word pertinent gevestig op bylae 5 van die Etiekkomitee se riglyne vir Eksperimentering met Mense en Diere, September 1988.)

3. Word studente as proefpersone gebruik? Ja, slegs 25 van die totale steekproef van 60 is studente by die Technikon.....
   (Studente mag nie individueel om deelname genader word nie en deelnemende studente moet verkieslik nie by die projekleier of sy medewerkers 'n kursus volg nie.)

Risikoversekering

1. Deur watter versekering word die risiko verbonde aan hierdie projek gedek? Gee volledige besonderhede.

   Lae risiko projek. 'n Geregistreerde, ervare verpleegsuster (W. Rademan) trek 5 ml volbloed (EDTA) en 20 ml serum per keer .....

   .....

2. Is die versekering voldoende?

   Ja.....

   .....

Aansoek en verklarings

1. Projekleier:
   Hiermee doen ek, die ondergetekende, aansoek om die uitvoering van die eksperiment soos beskryf in die voorafgaande protokol en verklaar dat:

Annexure 1

1.1.1 ek my deeglik vergewis het van die inhoud van 1) die Etiekkomitee se Riglyne vir die Eksperimentering met Mense en Diere (September 1988) en 2) die Mediese Navorsingsraad se Etiese Beginsels in Mediese Navorsing, Hersiene Uitgawe (1987) [dit is elektronies beskikbaar op die Pukweb by F:\Apps\Alginl\Etiekkom] en dat ek my by die riglyne soos vervat in hierdie twee dokumente sal hou;

1.1.2 ek elke proefpersoon wat aan die eksperiment deelneem, die meegaande vorm vir ingeligte toestemming sal laat onderteken en die skriftelike toestemming van die ouers of wettige voogde van alle minderjarige proefpersone sal verkry voordat die eksperiment 'n aanvang neem;

1.1.3 die inligting in hierdie aansoek na my beste wete juis is en dat geen etiese kodes met die proef geskend sal word nie;

1.1.4 ek nie van die goedgekeurde protokol sal afwyk nie;

1.1.5 alle vooraf-navorsing ter uitvoering van die proef volledig afgehandel is, en dat ek myself geskik en bekwaam ag om die navorsingswerk te doen en

1.1.6 ek jaarliks op die voorgeskrewe vorm aan die Etiekkomitee sal verslag doen aangaande etiese aspekte van die projek.

Volle name:
WilhelminaHendrikaOldewageTheron.....

Handtekening: ..... Datum:8 November 1999.....

2. Toesighoudende geneesheer
Die Etiekkomitee steun volkome op die professionele oordeel van die toesighoudende geneesheer met betrekking tot die aard en omvang van toesighouding asook die graad van risiko van elke projek.

2.1 Wat behoort na u mening die aard en omvang van toesighouding tydens die projek te wees?

.....

.....

2.2 Wat is na u mening die graad van risiko vir die proefpersone betrokke by die projek?

.....

.....
Let wel: Reëlings ter voldoening aan die toesigvereistes moet onderling tussen die geneesheer en die projekleier getref word.

2.3 Ondertekening van die protokol impliseer:

(a) dat u u voor die aanvang van die projek sal vergewis van die mediese geskiktheid van elke proefpersoon, en

(b) dat 'n mediese lêer (waar u dit nodig ag) vir elke proefpersoon aangehou sal word en

(c) dat u volledig op hoogte van die risiko verbonde aan die projek sal wees.

Volle name:.....

Handtekening: ..... Datum: .....

3. Skooldirekteur/Instituutdirekteur en Fokusareadirekteur:
Hiermee verklaar ek dat bogenoemde projek wetenskaplik verantwoord is, dat eksperimentering mag voortgaan indien dit deur die Etiekkomitee goedgekeur word en dat die projekleier/navorser oor genoegsame fisiese geriewe, toerusting en geld beskik om die proef uit te voer en te voltooi.

Skooldirekteur/Instituutdirekteur:

Volle name: .....

Handtekening: ..... Datum:.....

Fokusareadirekteur:

Volle name: .....

Handtekening: ..... Datum:.....

Central University of Technology, Free State

VERTROULIK

Vorm vir ingeligte toestemming
DEEL 1

1. Skool (vakgroep)/Instituut:

   .....

2. Titel van projek/proef: .....

   .....

3. Volle name, van en kwalifikasies van projekleier/navorser:

   .....

4. Rang/pos van projekleier/navorser:
   (bv. Professor, Lektor, Navorsingswetenskaplike ens..)

   .....

5. Volle name, van en kwalifikasies van persoon wat met die daadwerklike toesig oor die projek/proef belas sal wees:
   (Voltooi slegs as dit iemand anders is as die persoon in 4 hierbo genoem)

   .....

   .....

6. Naam en adres van toesighoudende geneesheer *(waar van toepassing)*:

   .....

7. Die doel van die projek/proef:

   .....

   .....

   .....

8. Verduideliking van die aard van alle prosedures wat gevolg sal word, insluitende identifisering van nuwe prosedures:

   .....

   .....

   .....

9. Beskrywing van die aard van die ongerief of gevare of waarskynlike permanente nagevolge vir proefpersone wat met die projek/proef gepaard mag gaan:
   (Insluitende moontlike newe-effekte van en interaksies tussen geneesmiddels asook radio-aktiewe isotope wat gebruik sal word.)

   .....

   .....

   .....

   .....

10. Voorsorg wat getref word om proefpersone te beskerm:

.....

.....

.....

.....

.....

11. Beskrywing van die voordele wat uit die resultate van die proef verwag kan word:

.....

.....

.....

.....

.....

12. Alternatiewe prosedures wat voordele vir die proefpersoon sal inhou:
(Voltooi slegs indien op die bepaalde projek van toepassing.)

.....

.....

.....

.....

Handtekening: ..... Datum: .....
Projekleier

Annexure 1
DEEL 2

Aan die ondertekenaar van die toestemming vervat in deel 3 van hierdie dokument:

U word uitgenooi om deel te neem aan die navorsingsprojek/proef soos genoem in paragraaf 2 van Deel 1 hiervan. Dit is belangrik dat u die volgende algemene beginsels, wat op alle deelnemers aan ons navorsingsprojekte van toepassing is, sal lees en verstaan:

1. Deelname aan die projek/proef is heeltemal vrywillig.
2. Dit is moontlik dat u persoonlik nie enige voordeel uit u deelname aan die projek/proef sal trek nie, alhoewel die kennis wat deur middel van die projek/proef opgedoen mag word andere tot voordeel kan strek.
3. Dit staan u vry om uself te enige tyd sonder opgawe van redes aan die projek/proef te onttrek. U word egter vriendelik versoek om nie sonder deeglike besinning aan die projek/proef te onttrek nie, aangesien dit o.a. die statistiese betroubaarheid van die projek/proef nadelig mag beïnvloed.
4. 'n Samevatting van die aard van die projek/proef, die vermeende risikofaktore, faktore wat moontlik ongerief of ongemak vir u kan veroorsaak, die voordele wat verwag kan word en die bekende en/of waarskynlike permanente nagevolge wat u deelname aan die projek/proef op u proefpersoon mag hê, word in Deel 1 hiervan vir u uiteengesit.
5. U word aangemoedig om op enige stadium enige vrae wat u in verband met die projek/proef en die prosedures in verband daarmee mag hê aan die projekleier of sy personeel te stel, wat u navrae graag sal beantwoord. Hulle sal ook die projek/proef volledig met u bespreek.
6. Indien u minderjarig is, is die skriftelike toestemming van u ouer of wettige voog nodig alvorens u aan hierdie projek mag deelneem.
7. U word daarop gewys dat van u vereis word om die Universiteit te vrywaar teen aanspreeklikheid weens benadeling wat as gevolg van die handelinge van die Universiteit of enige van sy werknemers of studente of ander proefpersone vir u of iemand anders mag ontstaan. Voorts dat u die Universiteit skadeloos moet stel in geval van enige aanspreeklikheid wat die Universiteit teenoor enigiemand mag oploop weens benadeling van uself of 'n ander deur of as gevolg van u deelname aan die projek/proef in Deel 1 hiervan uiteengesit. Laastens word van u vereis om afstand te doen van enige aanspraak wat u teen die Universiteit mag verkry as gevolg van benadeling van u of iemand anders, weens u deelname aan die projek/proef in Deel 1 uiteengesit.
8. Indien u getroud is, word van u eggenoot/e vereis om afstand te doen van enige eise wat hy/sy andersins teen die Universiteit sou kon hê as gevolg van enige benadeling of die dood van u weens die projek/proef in Deel 1 uiteengesit.

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Annexure 1

DEEL 3
Toestemming

Titel van projek: .....

.....

Ek, die ondergetekende ..... (volle name) het die voorafgaande gegewens in verband met die projek/proef genoem in DEEL 1 en DEEL 2 hiervan gelees en ook die mondelinge weergawe daarvan aangehoor en ek verklaar dat ek dit verstaan. Ek was die geleentheid gegun om tersaaklike aspekte van die projek/proef met die projekleier te bespreek en ek verklaar hiermee dat ek vrywillig aan die projek/proef deelneem. Ek gee hiermee my toestemming om as proefpersoon in bogenoemde projek op te tree.
Ek vrywaar hiermee die Universiteit asook enige werknemer of student van die Universiteit, teen enige aanspreeklikheid wat teenoor my, in die loop van die projek/proef mag ontstaan.
Ek onderneem verder om geen eise teen die Universiteit in te stel weens skade of persoonlikheidsnadeel wat ek weens die projek/proef mag ly nie, hetsy dit aan die nalatigheid van die Universiteit, sy werknemers of studente, of ander proefpersone mag onstaan nie.

(Handtekening van proefpersoon)

Onderteken te ..... op .....

GETUIES

1. .....

2. .....

Onderteken te ..... op .....

Vir nie-terapeutiese eksperimentering op proefpersone onder die ouderdom van 21 jaar is die skriftelike toestemming van die ouer of wettige voog nodig.

Hiermee gee ek ..... (volle name) ouer of wettige voog van die proefpersoon hierbo genoem toestemming dat hy/sy aan hierdie projek/proef mag deelneem en ek vrywaar hiermee die Universiteit asook enige werknemer of student van die Universiteit, teen enige aanspreeklikheid wat teenoor my in die loop van die projek/proef mag ontstaan.

Handtekening: ..... Datum: .....

Verwantskap: .....

Vir eksperimentering op getroude proefpersone is die onderstaande vrywaring deur die eggenoot/-e nodig:
Hiermee onderneem ek, ... (volle name), die eggenoot/-e van die proefpersoon in hierdie aansoek, om geen eis teen die Universiteit

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Annexure 1

in te stel vir behandeling weens beser[illegible] dood van die gemelde persoon as gevolg van die projek/proef in hierdie aansoek uiteengesit nie, hetsy sodanige besering, skade of dood veroorsaak is deur die nalatigheid van die Universiteit, sy personeel of sy studente of 'n ander proefpersoon, of op enige ander wyse.

Handtekening: ..... Datum: .....

Verwantskap: .....

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Annexure 2

INFORMED CONSENT : EVALUATION OF THE FORTIFICATION OF SUGAR WITH VITAMIN A

I, the undersigned.....(full names in print) have read the details of the project, or have listened to the oral explanation thereof, and declare that I understand it. I have had the opportunity to discuss relevant aspects with the researcher and declare that I voluntarily participate in the project. I hereby give consent to participate in the project and that blood samples may be taken from me.

I hereby indemnify the Technikon, or any employee of the Technikon, against any liability that may originate during my participation in this research project. I further undertake that I will not lay any claim against the Technikon or any Technikon employee for damage or personal disadvantages that I may suffer as a result of this research.

.....
Signature of volunteer

Signed at ..... on .....

Witnesses

Name ..... Name .....

Signature ..... Signature .....

Signed at ..... on .....

For subjects under the age of 21 years, signed consent of a parent or legal guardian is essential.

I, ...(full names), the parent/legal guardian of the person named above, hereby consent that she may participate in this research project and that blood samples may be taken from my child .

I hereby indemnify the Technikon, or any employee of the Technikon, against any liability that may originate during her participation in this research project. I further undertake that I will not lay any claim against the Technikon or any Technikon employee for damage or personal disadvantages that my child may suffer as a result of this research.

Signature ..... Relationship.....

Signed at ..... on .....

Address of volunteer: .....
.....
.....

Telephone number : .....

Central University of Technology, Free State

Request For Analysis

Date Received: March 1, 2000 Reference no: F 00-031

Requested For: Sugar Fortification Requested By: H Robertson

Product: Sugar

Batch / Ref Number	Analyze For	Declared Level	Results Found
Vitamin A Sugar 2 March	Vitamin A .	80 IU / g	143 IU / g
Sugar CSIR	Vitamin A	7.17 IU / g	9.4 IU / g

Date Completed: March 3, 2000

Analyst: M Kent

These results are reported in strict confidence and without further engagement on the part of Roche Products (Pty) Ltd.

Request For Analysis

Date Received: April 2, 2000 Reference no: F00-041

Requested For: Sugar Fortification Project Requested By: H Robertson

Product: Sugar

Batch / Ref Number	Analyze For	Declared Level	Results Found
17.Feb	Vitamin A	80 IU / g	89.8 IU / g
02.Mar	Vitamin A	80 IU / g	165 IU / g
28 March top	Vitamin A	80 IU / g	161 IU / g
28 March bottom	Vitamin A	80 IU / g	150 IU / g

Date Completed: April 12, 2000

Analyst: M Kent

These results are reported in strict confidence and without further engagement on the part of Roche Products (Pty) Ltd.

Declared levels of Vitamin A in the fortified sugar

Annexure 4

SUGAR INFORMATION

Please use our sugar

Do not give the sugar away

Do not sell the sugar

We will know if you use our sugar through blood analysis

All the persons living with you in the house may use our sugar

Please do not use more sugar than you would normally use

Use the sugar in all the food you normally use sugar in

If you cook food usually with sugar, use our sugar to cook with

It is important that you keep the sugar in the purple bag at all times

Close the bag each time after opening

We prefer that the sugar is stored in the purple bag in another container with a lid.

Do not store the sugar on the floor

Keep the sugar away from wet places

Store the sugar away from sunlight

We give enough sugar to be use in four week

Try not to drink tee with your meals, but rather in between the meals

Do not use non-calorie sweeteners e.g. Nutrasweet

Use the sugar when you bake cakes

Brush your teeth regularly to prevent dental caries

Do not use more sugar than normally to avoid obesity

Use sugar in moderation

If you are not sure of the correct use of the sugar please contact Mrs. Selepe at (016-950 9460)

Annexure 5

EVALUATION OF THE FORTIFICATION OF SUGAR WITH VITAMIN A

INSTRUCTION MANUAL

IMPORTANT

DO NOT START SAMPLING BEFORE YOU HAVE READ AND UNDERSTOOD THE FOLLOWING INSTRUCTIONS

INDEX

1 Importance of the study Page 2

2 Equipment per team
2.1 Team 1- Station 1
2.2 Team 2 – Station 2
2.3 Team 3 – Station 3
2.4 Team 4 – Station 4
2.5 Team 5 – Station 5
2.6 Team 6 – Station 6
2.7 Team 7 – Station 7

3 Instructions to fieldworkers
3.1 Team 1 – Station 1
3.2 Team 2 – Station 2
3.3 Team 3 – Station 3
3.4 Team 4 – Station 4
3.5 Team 5 – Station 5
3.6 Team 6 – Station 6
3.7 Team 7 – Station 7

References

Annexure 5

1 IMPORTANCE OF THE STUDY

In children three micronutrient deficiencies, namely vitamin A, iron and iodine, are considered to be a major health problem in developing countries. These are presently receiving high priority globally. Communities that are affected the most are those in situations where poverty, unemployment, civil unrest, war and exploitation remain endemic (SAVACG, 1995: 39; USAID, 1993: 2). World wide more than 250 million young children and many of their mothers are vitamin A deficient, increasing the severity of common illnesses and their risk of death. Vitamin A is a powerful "child survival tool", reducing child mortality by 23-34 % (Malanick, 1999: 1).

Growth retardation, brain damage, diminished cognitive function and diminished working capacity in children and adults, as well as increased susceptibility to and severity of infections, and mortality are the collective result of these micronutrient deficiencies (SAVACG, 1995: 39; USAID, 1993: 2).

A new concept is emerging in terms of micronutrient deficiencies. At a national level the constraints to make more vitamins and minerals available to the population can be largely addressed by implementing programmes designed to educate people. These could be diversifying their diets or by fortifying commonly eaten foods with the missing micronutrients or providing nutrient supplements through targeted distribution programmes (USAID, 1993: 7).

Many studies have been done to determine the effect of vitamin A supplementation on nutritional status of people. The purpose of this study is to determine the effect of fortified vitamin A on the nutritional status. Although a number of similar studies have been done in other countries, this is the first study in South Africa where vitamin A fortified sugar will be used. The results of this study will be published in national and international academic journals and also be presented at nutrition conferences as this will assist the policy makers on the effectiveness of fortification and the decision for a suitable vehicle for vitamin A fortification in South Africa.

The team leaders of this project are Ms W Oldewage-Theron, Ms E Dicks and Ms M Selepe. In order to ensure accurate and reliable data, the team leaders will supervise the data collection process and cross-check some of the information that you as field worker has obtained. If differences are found, you will be asked to repeat the observations in the presence of one of the team leaders in order to find the reasons for the differences.

All questions must be asked in the same way by all the field workers. Should questions be translated, please ensure that the meaning of the question is not changed by the way the question is rephrased. The team leaders will be available for assistance at all times during the trial.

Annexure 5

2 EQUIPMENT PER TEAM

The following equipment must be prepared and be available for the week that measurements will be done.

2.1 Team 1 - Station 1

- All the subject files
- 100 X Demography questionnaires
- 100 X Consent form
- Check lists
- 10 X Pens

2.2 Team 2 - Station 2

- 2 X Measuring tape
- 2 X Scales
- 2 X Pens
- Calculator

2.3 Team 3 - Station 3

- 100 X QFFQ's
- 100 X Food diary questionnaire
- 100 X Sugar consumption questionnaire
- 100 X KAB questionnaire
- 100 X Compliance questionnaire
- 10 X Pens

2.4 Team 4 - Station 4

- 1 X Blood pressure equipment
- 5 X Thermometers
- 20 X Pairs of surgical gloves (medium size)
- 100 X Green butterflies (21G)
- 100 X Webcol sterile preps
- 100 X syringe needles
- 100 X 5 ml Disposable syringes
- 100 X Elastoplast plasters
- Disinfectant
- 100 X Vacutainers
- 100 X 5 ml EDTA tubes (purple rubber top) contained in polystyrene trays as supplied by the manufacturers
- 100 X 10 ml plain tubes (red rubber top) contained in polystyrene trays as supplied by the manufacturers

Annexure 5

- 1 X pack of cotton wool (1 kg)
- 5 X Black plastic sheets (50 X 50 cm)
- 5 X Roller paper towels
- 4 X Cooler boxes with 3 ice packs each
- 1 X Waste disposal unit
- Pens

2.5 Team 5 - Station 5

- Fruit juice
- Bread
- Spreads
- Knives
- Paper plates
- Paper serviettes
- Pen

2.6 Team 6 - Station 6

- Training manual
- Flip chart
- Pens

2.7 Team 7 - Station 7

- Fortified sugar
- Unfortified sugar
- Pens

3 INSTRUCTIONS TO FIELDWORKERS

Each team consists of two people, except team 3 consisting of 4 people.

3.1 Team 1 - Station 1

At the beginning:

- Explain the routine for the day
- Register the subject on the attendance register as being present
- Complete the demography form (week 1 only)
- Ensure that the consent form is in the file (week 1 only)

At the end:

- Check that the station form is complete
- Collect the completed file from the subject
- Sign the subject out on the register

- Pay each subject R 10 for her expenses. Each subject must complete the claim form and sign for receipt of the money (weeks 0,1,4,8)
- Pay each subject R 60 for her expenses. Each subject must complete the claim form and sign for receipt of the money (week 12)

3.2 Team 2 - Station 2 (weeks 1,4,8,12)

Weight measurement:

- Place the scale on an uneven uncarpeted area. Ensure that the spirit level indication is in the middle.
- Switch the scale on and wait for the zero indication (0.0) as well as the stable indicator (° in the top left-hand corner of the display panel) to appear.
- Weigh the subjects with clothes, without shoes, after emptying their bladders
- Place the subject on the scale. They must stand upright in the middle of the platform, facing the fieldworker and looking straight ahead. Their feet must be flat and slightly apart. They must stand still until the measurement was recorded in the space provided on the station card.
- Let the subject step down from the scale and wait for the zero reading to appear on the digital display.
- Repeat the procedure. The reading should be within 100g of each other.

Height measurement:

- The subject must remove her shoes.
- Position the subject as follows:
  - facing the fieldworker
  - shoulders relaxed, with shoulder blades, buttocks and heels touching the measuring board
  - arms relaxed at the sides
  - legs straight and knees together
  - feet flat, heels touching
- The subject must look right ahead before the headpiece is slid down on the head. It should just touch the crown of the head.
- Record the reading in mm on the space provided on the station card.
- Repeat the procedure. The two readings should not vary by more than 5 mm.

Waist measurement:

- Position the subject as follows:
  - facing the fieldworker
  - standing erect with the abdomen relaxed, arms at the sides and feet together
- Place an inelastic tape around the subject, in a horizontal plane, at the level of the natural waist, which is the narrowest part of the torso
- Record the reading in mm on the space provided on the station card.
- Repeat the procedure. The two readings should not vary by more than 15 mm.

Annexure 5

Hip circumference:

- Position the subject as follows:
  - facing the fieldworker
  - standing erect with the abdomen relaxed
  - arms at the sides
  - feet together
  - no tensing of the gluteal muscles
- Stand at the side of the subject to ensure the tape is held in a horizontal plane
- Place an inelastic tape around the subject, in a horizontal plane, at the level of the greatest posterior protuberance of the buttocks, which usually corresponds anteriorly to about the level of the symphysis pubis.
- Record the reading in mm on the space provided on the station card.
- Repeat the procedure. The two readings should not vary by more than 15 mm.

Waist hip ratio:

- Calculate the body mass index by using the following formula:

$$\frac{\text{Body weight}}{\text{Square Body height}} = \frac{\text{kg}}{\text{m}^2}$$

- Record the BMI on the station card.

3.3 Team 3 - Station 3

- Complete the QFFQ (weeks 0,1,4,8,12 -fieldworker 1)
- Hand out the food diary and explain completion (weeks 0,1,4,8- fieldworker 2)
- Complete the sugar consumption questionnaire (weeks 0, 8 – fieldworker 3)
- Complete the KAB questionnaire (weeks 0, 4, 12 – fieldworker 4)
- Complete the compliance questionnaire (week 12 – fieldworker 2)

3.4 Team 4 - Station 4

Instructions for blood sampling:

- Ensure that the informed consent form has the authoritative signature
- Put on two pairs of surgical gloves.
- Take 20 ml blood from each subject.
- Write clearly on three labels the subject number and date of sampling.
- Retrieve sample tubes from the cooler box, replace lid and keep cooler box closed at all times.
- For each subject, select on tube with a purple rubber top, one with a blue rubber top and one with a yellow rubber top. Stick labels (one on each tube) directly over label already on tube.
- Slowly and carefully draw 20 ml sample of venous blood into the syringe using a butterfly.
- Write the time of sampling on the tube labels.

- Slowly and carefully transfer from the syringe 1 ml of the sampled blood into the tube with the purple rubber tube and the remaining blood into the tube with the red rubber top by piercing the rubber seals with the butterfly needle.
- Gently and repeatedly (5 X) invert both tubes. Do not shake.
- Place tubes in polystyrene trays. Keep tubes shielded from direct light or sunlight by covering the trays with the black plastic sheets.
- All blood samples drawn must be returned to the cooler box within two hours from the time the blood sample was drawn.
- Dispose of the syringe and butterfly in waste disposal unit.
- Proceed with the next subject by repeating the previous steps until the complete blood sample has been obtained from all the subjects.
- Dispose of the surgical gloves in the waste disposal unit and seal.
- Check that all the samples are complete and the details on the labels are complete.
- Close the cooler box and remove to the laboratory with the waste disposal unit.

3.5 Team 5 - Station 5

- Check that the subject has completed all the steps in stations one to four by checking the station card.
- Hand out a sandwich with a 250 ml fruit juice to each subject and sigh the station card.

3.6 Team 6 - Station 6

- Group 5 subjects together.
- Hand out a training manual to each of the subjects.
- Read and explain the training manual. Answer all questions and call a team leader should you not be able to answer the questions.
- Sign the station card for each subject.

3.7 Team 7 - Station 7

- Check that the subject has completed all the steps in stations one to six by checking the station card
- Check the code on the subject file and issue the sugar accordingly
- N001, N 095, etc = sugar bags marked with an "N"
- F020, F085 = sugar bags marked with an "F"
- Sign the station card

Annexure 5

REFERENCES

MALANICK, C. 1999. USAID's enhanced vitamin A effort: saving lives around the world. Washington D.C: USAID. 1 p.

THE SOUTH AFRICAN VITAMIN A CONSULTATIVE GROUP (SAVACG). 1995. Children aged 6 to 71 months in South Africa, 1994: Their anthropometric, vitamin A, iron and immunisation coverage status. Isando: SAVACG. 335 p.

USAID. 1993. Micronutrients. Increasing survival, learning and economic productivity. Washington D.C: USAID. 28 p.

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Annexure 6

VITAMIN A FORTIFICATION PROJECT

Subject name: _____ Subject number: _____

STATIONS	ACTIVITY	CHECK WEEK 0	CHECK WEEK 1	CHECK WEEK 4	CHECK WEEK 8	CHECK WEEK 12
Station 1 **Check/control**	Recruitment (Date)					
	Demography questionnaire					
	Consent form					
Station 2 **Antropometry**	Weight		kg	kg	kg	kg
	Height		m	m	m	m
	Waist		cm	cm	cm	cm
	Hip circumference		cm	cm	cm	cm
	Waist Hip Ratio		cm	cm	cm	cm
Station 3 **Questionnaires**	QFFQ					
	Food Diary	Out	In Out	In Out	In Out	In
	Sugar consumption					
	KAB	2X				
	Compliance					
Station 4 **Clinical signs and blood samples**	Blood pressure	mm Hg	mm Hg	mm Hg	mm Hg	mm Hg
	Oral temperature	°C	°C	°C	°C	°C
	Vitamin A					
	Citrate (5ml EDTA)					
	Full blood (20ml)					
Station 5 **Café**	Snacks					
Station 6 **Education program**	Training session					
Station 7 **Store**	Issuing of sugar					
Station 1 **Check/control**	Back to check/control (Sign completed form)					

Annexure 8

HEALTH QUESTIONNAIRE: EVALUATION OF THE FORTIFICATION OF SUGAR WITH VITAMIN A

A

Surname		ID number	
First Names		Age	

B

ARE YOU SUFFERING OR HAVE YOU SUFFERED FROM	YES	NO	IF ANY ANSWER IS YES, GIVE DETAILS OF THE NATURE, SEVERITY AND DURATION OF ILLNESS
1. Any skin disease?			
2. Any affection of the skeleton and/or joints?			
3. Any affection of the eyes, ears, nose or teeth?			
4. Any affection of the heart or circulatory system?			
5. Any affection of the chest or respiratory system?			
6. Any affection of the digestive system?			
7. Any affection of the urinary system and/or genital organs?			
8. Any nervous affection or mental abnormality?			
9. Any other illness?			

C

	YES	NO
1. Do you suffer from any defect of hearing, speech or sight?		
2. Are you physically disabled and do you use artificial limbs?		
GIVE DETAILS OF THE NATURE AND SEVERITY OF THE DISABILITY		

Annexure 8

D

	YES	NO
Have you undergone any operations?		
GIVE DETAILS OF THE NATURE AND DATE OF THE OPERATION/S		

E

I declare that the above-mentioned information is true and correct and that I have not withheld any information regarding my health.
Signature.....Date.....

Annexure 9

MEDICATION QUESTIONNAIRE: EVALUATION OF THE FORTIFICATION OF SUGAR WITH VITAMIN A

A

Surname		ID number	
First Names		Age	

B

1. Do you use any medication?	Yes	No
2. If no, go to section C.		
3. If yes, what for/why?		
4. What is the name of the medication you are taking?		
5. What is the dosage and how often do you take this medication?.	Dosage	How often?

C

I declare that the above-mentioned information is true and correct and that I have not withheld any information regarding my medication usage.
Signature.....Date.....

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Subject number_____ **Interviewer**_____

QUANTITATIVE FOOD FREQUENCY QUESTIONNAIRE

INTRODUCTION:

Greeting

Thank you for giving up your time to participate in this study. I hope you are enjoying it so far. Here we want to find out what people living in this area eat and drink. This information is important to know as it will tell us if people are eating enough and if they are healthy.

Please think carefully about the food and drink you have consumed during the past four weeks. I will now go through a list of foods and drinks with you and I would like you to tell me:

- if you eat the food
- how the food is prepared
- how much of the food you eat at a time
- how many times a day you eat it and if you do not eat it every day, how many times a week or a month you eat it.

To help you to describe the amount of a food you eat, I will show you pictures of different amounts of the food. Please say which picture is the closest to the amount you eat, or if it is smaller, between sizes or bigger than the pictures.

THERE ARE NO RIGHT OR WRONG ANSWERS.

EVERYTHING YOU TELL ME IS CONFIDENTIAL. ONLY YOUR SUBJECT NUMBER APPEARS ON THE FORM.

IS THERE ANYTHING YOU WANT TO ASK NOW?

ARE YOU WILLING TO GO ON WITH THE QUESTIONS?

FOOD	DESCRIPTION	Amount	TIMES EATEN				CODE	AMOUNT/DAY
			Per day	Per week	Per month	Seldom/ Never		
	Fresh/long life whole						0006	
	Sour / Maas						0006	
Milk drinks Brand	Nestle_____ Milo_____ Flavoured milk_____ Other_____						0023	
Yoghurt	Drinking yoghurt Thick yoghurt						0044 0020	
Squash	SweetO SixO Oros/Lecol with sugar - artificial sweetener Kool Aid Other						9013 9013 9002 9013 9002	
Fruit juice	Fresh/Liquifruit/Ceres						0535	
	Tropica Show examples						0089	
Fizzy drinks Coke, Fanta	Sweetened Diet						9001 9013	
Mageu/Motogo							9562	
Home brew							9516	
Tlokwe							9516	
Beer							9506	
Spirits							9510	
Wine red							9508	
Wine white							9518	
Other specify								

SNACKS AND SWEETS:

Potato crisps							8049	
Peanuts	Raw Roasted						6001 6007	
Cheese curls: Niknaks etc.							4076	
Raisins							7022	

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INSTRUCTION

Circle the subject's answer. Fill in the amount and times eaten in the appropriate columns.

I shall now ask you about the type and the amount of food you have been eating in the last few months. Please tell if you eat the food, how much you eat and how often you eat it. We shall start with maize meal porridge.

Do you eat maize meal porridge? YES 1 NO 2

If YES, what type do you have at home now?

Brand name_____

Don't know_____2

Grind self_____3

If brand name given, do you usually use this brand YES 1 NO 2 DON'T KNOW 3

Where do you get your maize-meal from? (May answer more than one)

Shop ☐ 1

Employer ☐ 2

Harvest and grind self ☐ 3

Other - specify _____ ☐ 4

Don't know ☐ 5

FOOD	DESCRIPTION	Amount	TIMES EATEN				CODE	AMOUNT/DAY
			Per day	Per week	Per month	Seldom/ Never		
Maize-meal porridge	Stiff (pap)						e4225 4250	
Maize-meal porridge	Soft (slappap)						e4225 4250	
Maize-meal porridge	Crumbly (phutu)						e4225 4250	
Ting								
Mabella Coarse Fine Rice	Stiff						4082	
Mabella	Soft						4082	
Oats							4032	

FOOD	DESCRIPTION		TIMES EATEN				CODE	AMOUNT/DAY
			Per day	Per week	Per month	Seldom/ Never		
Breakfast cereals	Brand names of cereals at home now: (5) Don't know							

Do you pour milk on your porridge or cereal? YES 1 NO 2

If YES, what type of milk (whole fresh, sour, 1%, fat free, milk blend.)_____

INSTRUCTION: Show subject examples.

If YES, how much milk?							

Do you pour sugar on your cereal/porridge/mabella YES 1 NO 2

If YES, how much sugar?							9012	
Samp	Bought Self ground						4077 4073	
Samp and beans							A014	

Are the amounts of samp and beans the same as in the picture? YES NO

If no, do you use more beans than in the picture or less? MORE LESS

Samp and peanuts							A013	

Are the amounts of samp and peanuts the same as in the picture? YES NO

If no, do you use more peanuts than in the picture or less? MORE LESS

Rice	White Brown Maize rice						4040 4134 4043	
Pastas	Macaroni Spaghetti Other:						4062	

You are being very helpful. Can I now ask you about meat?

CHICKEN, MEAT, FISH

FOOD	DESCRIPTION	Amount	TIMES EATEN				CODE	AMOUNT/DAY
			Per day	Per week	Per month	Seldom/ Never		
Chicken	Boiled						1521	
	Fried: in batter/crumbs Not coated						1634 1520	
	Roasted/grilled						1520	

Do you eat chicken skin ALWAYS 1 SOMETIMES 2 NEVER 3

Chicken bones stew							A083	
Chicken feet							A084 1582	
Chicken offal							1610	
Red meat:	How do you like meat? With fat Fat trimmed							
Red meat	Fried							
	Stewed						A001	
	Mince with tomato and onion						1585	
Beef Offal	Intestines: boiled, nothing added						1616	
	Stewed with vegetables							
	Liver						1515	
	Kidney						1518	
	Other specify:							
What vegetables are usually put into meat stews?								
Wors / sausage	Fried						1526	
Bacon							1581	
Cold meats	Polony						1514	
	Ham						1564	
	Viennas						1531	
	Other - specify							
Canned meat	Bully beef						1535	
	Other specify:							
Meat pie	Bought						1548	
Hamburger	Bought						A015	

FOOD	DESCRIPTION	Amount	TIMES EATEN				CODE	AMOUNT/DAY
			Per day	Per week	Per month	Seldom/ Never		
Dried beans/peas/ lentils (10)	Soup Salad						3033 3508	
Soya products eg. Toppers	Brands at home now (5) Don't know_____ Show examples						3527	
Pilchards in tomato/chilli/ brine	Whole						2557	
	Mashed with fried onion						A005	
Fried fish	With batter/crumbs						2509	
	Without batter/crumbs						2523	
Other canned fish	Tuna						2547	
	Pickled fish Other:						2562	
Fish cakes	Fried						2531	
Eggs	Boiled/poached Scrambled Fried						1001 1025 1003	

WE NOW COME TO VEGETABLES AND FRUIT

FOOD	DESCRIPTION	Amount	TIMES EATEN				CODE	AMOUNT/DAY
			Per day	Per week	Per month	Seldom/ Never		
Cabbage	How do you cook cabbage?							
	Boiled, nothing added						8066	
	Boiled with potato and onion and fat						A006	
	Fried, nothing added						A007	
	Boiled, then fried with potato, onion						A006	
	Other: Don't know							

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FOOD	DESCRIPTION	Amount	TIMES EATEN				CODE	AMOUNT/DAY
			Per day	Per week	Per month	Seldom/ Never		
Spinach/morogo/ other green leafy	How do you cook spinach?							
	Boiled, nothing added						8071	
	Boiled fat added						8209	
	Boiled with onion/tomato and fat						A011	
	- onion, tomato & potato							
	- with peanuts							
	Other: Don't know							
Tomato and onion 'gravy'	Home made - with fat - without fat						A012 A016	
	Canned						8221	
Pumpkin	How do you cook pumpkin?							
	Cooked in fat & sugar						A010	
	Boiled, little sugar and fat						A009	
	Other: Don't know							
Carrots	How do you cook carrots?							
	Boiled, sugar & fat						8129	
	With potato/onion						A008	
	Raw, salad						8015	
	Chakalaka							
	Other: Don't know							
Mealies/Sweet corn	How do you eat mealies?							
	On cob						8033	
	Off cobb - creamed sweet corn - whole kernel						8034 8261	
Beetroot salad	Home made Bought						8005	

FOOD	DESCRIPTION	Amount	TIMES EATEN				CODE	AMOUNT/DAY
			Per day	Per week	Per month	Seldom/ Never		
Potatoes	How do you cook potatoes?							
	Boiled/baked with skin						8046	
	- without skin						8045	
	Mashed						8187	
	Roasted						8189	
	French fries						8048	
	Salad Other:						8236	
Sweet potatoes	How do you cook sweet potatoes?							
	Boiled/baked with skin						8037	
	- without skin						8214	
	Mashed							
	Other: Don't know							
Salad vegetables	Raw tomato						8059	
	Lettuce						8031	
	Cucumber						8025	
Other vegetables, specify:								

FRUIT:

Do you like fruit? YES NO

Apples/Pears	Fresh Canned pears						7001 7054	
Bananas							7009	
Oranges/naartjie							7031	
Grapes							7020	
Peaches	Fresh Canned						7036 7038	
Apricots	Fresh Canned						7003 7004	
Mangoes	Fresh						7026	

FOOD	DESCRIPTION	Amount	TIMES EATEN				CODE	AMOUNT/DAY
			Per day	Per week	Per month	Seldom/ Never		
Guavas	Fresh Canned						7021 7023	
If subject eats canned fruit: Do you have custard with canned fruit:			YES 1		NO 2			
Custard	Home made Ultramel						0004	
Wild fuit/berries	Specify type						7030	
Dried fruit	Types:							
Other fruit								

BREAD AND BREAD SPREADS

Bread/Bread rolls	White						4001	
	Brown						4002	
	Whole wheat						4003	

Do you spread anything on the bread? ALWAYS 1 SOMETIMES 2 NEVER 3

Margarine	What brand do you have at home now? _____ Don't know_____ Show examples							
Peanut butter							6509	
Jam/syrup/honey							9008	
Marmite/Fray Bentos							9501	
Fish/meat paste							1512	

FOOD	DESCRIPTION	Amount	TIMES EATEN				CODE	AMOUNT/DAY
			Per day	Per week	Per month	Seldom/ Never		
Cheese	Type:						0010	
Achaar							A017	
Other spreads:	Specify							
Dumpling							4001	
Vetkoek							4057	
Provita, crackers, etc.								
Mayonnaise/salad dressing	Number of spoons_____/ number in family						6573	

DRINKS:

Tea							9514	
Coffee							9513	
Sugar/cup tea or coffee							9012	
Milk/cup tea or coffee	What type of milk do you use in tea and coffee?							
	Fresh/long life whole						0006	
	Fresh/long life 2%						0069	
	Fresh/long life fat free						0072	
	Whole milk powder Brand						0009	
	Skimmed milk powder Brand						0008	
	Milk blend Brand						0068	
	Whitener Brand						0039	
	Condensed milk						0002	
	Evaporated milk						0003	
	None							
Milk as such	What type of milk do you drink as such?							

FOOD	DESCRIPTION	Amount	TIMES EATEN				CODE	AMOUNT/DAY
			Per day	Per week	Per month	Seldom/ Never		
Peanuts and raisins							6007 7022	
Chocolates	Name						9024	
Candies	Sugus, gums, hard sweets						9009	
Sweets	Toffees, fudge, caramels						9014	
Biscuits	Type							
Cakes & tarts	Type							
Scones							4029	
Rusks							4160	
Savouries	Sausage rolls Samoosas Biscuits eg bacon kips Other:						1534 4196 4162	
Jelly							9004	
Baked pudding							4181	
Instant pudding							4066	
Ice cream Sorbet							6507 6516	
Other Specify:								

SAUCES / GRAVIES / CONDIMENTS

Tomato Sauce Worcester sauce							9505	
Chutney							9524	
Pickles							8176	
Packet soups							4069	
Others:								

WILD BIRDS, ANIMALS OR INSECTS (hunted in rural areas or on farms)

Wild fruit								

Central University of Technology, Free State

MISCELLANEOUS: Please mention any other foods used more than once/two weeks which we have not talked about:

FOOD	DESCRIPTION	Amount	TIMES EATEN				CODE	AMOUNT/DAY
			Per day	Per week	Per month	Seldom/ Never		

SALT USE:

What type of salt do you use?_____

The next few questions are to find out if you use salt, where you use it and how much you use?

Do you add salt to food while it is being cooked?

Always 1	Sometimes 2	Never 3	Don't know 4

Do you add salt to your food after it has been cooked?

Always 1	Sometimes 2	Never 3

Do you like salty foods eg. salted peanuts, crisps?

Very much 1	Like 2	Not al all 3

Do you use any of the following:

	Name of product	Amount/day
Vitamins/vitamins & minerals		
Tonics		
Health foods		
Body building preparations		
Dietary fibre supplement		
Other: specify		

THANK YOU FOR YOUR COOPERATION AND PATIENCE

GOOD-BYE!

Annexure 11

Central University of Technology, Free State

FOOD DIARY OF VITAMIN A - RICH AND SUGAR CONTAINING FOODS

Dear Friend,

The purpose of this survey is to obtain information on the food that you eat, especially those that are rich in Vitamin-A and those that have sugar added.
Please tick on appropriate column and also show the amounts that you eat for breakfast (B/F), lunch (L) and supper (S).
Please be as honest as possible.
We thank you for your co-operation!

		Friday			Saturday			Sunday			Monday		
Type of food	**Amount**	**B/F**	**L**	**S**	**B/F**	**L**	**S**	**B/F**	**L**	**S**	**B/F**	**L**	**S**
whole milk													
butter													
fortified margarine													
whole milk cheese													
liver													
egg yolk													
spinach													
beet greens													
broccoli													
carrots													
sweet potatoes													
squash													
pumpkin													
apricots													
peaches													
cream													
How much sugar do you pour in/over the following food?													
soft porridge /oats													
tomato & onion													
tea / coffee													
baked products													
cereals													
custard													
Specify any other food:													

Annexure 12

CONFERENCE PAR[illegible] & PUBLICATIONS

1 **From Lab To Land National Nutrition Conference From 15-18 August 2000, Durban SA**

- Oldewage-Theron, W.H; Dicks, E; Selepe, M; Grobler, C.J; van Rensburg, J; Hanekom, S.M. & Vorster, H.H. Demographic profile and health status of females aged 13-25 years old in the Vaal Triangle (poster).
- Grobler, C.J; van Rensburg, J; Oldewage-Theron, W.H; & Selepe, M. Haematological iron related parameters of females aged 13-25 years old in the Vaal Triangle (poster).

2 **XVIth International Fibrinogen Workshop From 23-26 August 2000, Leiden The Netherlands**

- Grobler, C.J; Oldewage-Theron, W.H;Vorster, H.H; Veldman, D. The effect of fortified vitamin A on fibrinogen levels of females aged 13-25 years in the Vaal Triangle South Africa (poster).

3 **16th National Congress of the society of Medical Laboratory Technologists of South Africa from 1-4 May 2001, Eskom Conference Centre, Midrand Gauteng**

- Grobler, C.J; Oldewage-Theron, W.H;Vorster, H.H; Veldman, D. The effect of fortified vitamin A on fibrinogen levels of females aged 13-25 years in the Vaal Triangle South Africa (oral).

4 **7th Congress of the European haematology association from 6-9 June 2002, Florence Italy**

- Grobler, C.J; Oldewage-Theron, W.H;Vorster, H.H; Veldman, D. The effect of fortified vitamin A on fibrinogen levels of females aged 13-25 years in the Vaal Triangle South Africa (poster).

5 **1st Africa nutritional epi[illegible]rence from 16-21 August 2002, Vanderbijlpark South Africa**

- Grobler, C.J; Oldewage-Theron, W.H;Vorster, H.H; Veldman, D. The effect of fortified vitamin A on fibrinogen levels of females aged 13-25 years in the Vaal Triangle South Africa (oral).

6 **Abstracts published in the SA Journal of Clinical Nutrition, August 2000**

- Oldewage-Theron, W.H; Dicks, E; Selepe, M; Grobler, C.J; van Rensburg, J; Hanekom, S.M. & Vorster, H.H. Demographic profile and health status of females aged 13-25 years old in the Vaal Triangle.
- Grobler, C.J; van Rensburg, J; Oldewage-Theron, W.H; & Selepe, M. Haematological iron related parameters of females aged 13-25 years old in the Vaal Triangle.

7 **Abstract published in The Haematology Journal – Official Journal of the European Haematology Association, June 2002 (volume 3 – Supplement 1: p454)**

- Grobler, C.J; Oldewage-Theron, W.H;Vorster, H.H; Veldman, D. The effect of fortified vitamin A on fibrinogen levels of females aged 13-25 years in the Vaal Triangle South Africa (oral).

Annexure 13

RESEARCH PROJECT: EVALUATION OF THE FORTIFICATION OF SUGAR WITH VITAMIN A

WHAT IS THIS PROJECT?

The major objective of this project is to perform a clinical intervention trial under controlled conditions to examine the effect of fortified vitamin A in young, African females in order to answer the following questions:

- Is sugar a suitable vehicle for vitamin A fortification in terms of dietary intake, bio-availability and consumer acceptance?
- What effect does vitamin A fortification have on the vitamin A- and iron status of young, African females?
- What effect does fortified vitamin A have on the fibrinogen levels?

WHY IS THIS PROJECT IMPORTANT?

In children three micronutrient deficiencies, namely vitamin A, iron and iodine, are considered to be a major health problem in developing countries. These are presently receiving high priority globally. Communities that are affected the most are those in situations where poverty, unemployment, civil unrest, war and exploitation remain endemic (SAVACG, 1995: 39; USAID, 1993: 2). Growth retardation, brain damage, diminished cognitive function and diminished working capacity in children and adults, as well as increased susceptibility to and severity of infections, and mortality are the collective result of these micronutrient deficiencies (SAVACG, 1995: 39; USAID, 1993: 2).

PROCEDURE

The project will take place over a period of 14 weeks. You will be requested to report to us five times during the 14-week period. You will be supplied with the dates to report to the Vaal Triangle Technikon to participate in the project.

WHAT WILL BE MEASURED IN THE PROJECT?

- Eating and drinking habits
- Medical history
- Weight, height, waist and hip circumference
- Clinical signs of vitamin A deficiency
- Blood sample: markers of nutritional status. PLEASE NOTE, **NO HIV OR AIDS** testing
- Blood pressure

Annexure 13

WHO MAY PARTICIPATE?

Healthy African females living in the Vaal Triangle who are between 13 and 25 years of age. People will be asked to participate and may refuse. Therefore, only **volunteers** will be asked to sign the informed consent form to participate.

WHAT ARE THE BENEFITS FOR YOU?

Many healthy and nutritional status indicators of yourself will be measured. You will receive feedbacjk during which a member of the investigation team will explain your health risk to you. You will receive dietary advice and will be referred to your clinic or doctor if necessary. A doctor will be supervising the research project and it involves a low risk.

WHAT DO WE EXPECT OF YOU?

- Please bring your ID, we need to know your birth date.
- We will appreciate it if you will report fasting on the day of your participation. It means that for 10-12 hours before your blood sample is taken, you must not eat or drink anything but pure water.
- You will be asked to sign a form giving consent to participate in the project.
- We will ask you a number of questions regarding your health, age, income, family, smoking and drinking habits.
- Then you will receive a **reference number** for the project.
- You will be weighed and measured.
- We will take your blood pressure to determine stroke risk.
- Your temperature will be taken orally.
- You will be questioned in detail about your eating habits.
- You will be given sugar samples to consume for a period of 6 weeks at a time. We would like you to use this sugar and to answer questions about the acceptability of the sugar.
- Blood will be taken from you 5 times in a period of 14 weeks by a registered nursing sister.
- You will receive journals to read while you wait.
- You will receive a snack after blood has been taken.

If you have any questions about the project, please do not hesitate to ask any one of the field workers at any time.

Thank you for your participation.

Wilna Oldewage-Theron
Head of Department: Food