EVALUATION OF THE FORTIFICATION OF SUGAR WITH VITAMIN A

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DEDICATED TO

My loving and supportive family –my parents, Herby and Babs Paul, and my husband, Chris Theron.

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ABSTRACT

Background, motivation and hypothesis: A high prevalence of micronutrient deficiencies has been described in the South African black population, especially vitamin A deficiency and iron deficiency anaemia (IDA). However, iron overload is also a problem in South African blacks. The literature indicated that while there is a high prevalence of IDA in especially young African girls, there is also an increased susceptibility to iron overload – probably not only of increased intakes by men of beer brewed in iron containers, but also because of a genetic factor. It seems that in South African blacks, the prevalence of the homozygous state for haemochromatosis may be more than ten times higher than in European populations. The role of vitamin A in iron metabolism has received increased attention during recent years. Studies have shown that vitamin A has protective effects during iron supplementation such as an increased mobilisation of iron. It would thus be of benefit for prevention and intervention programmes to know if vitamin A fortification/supplementation would improve iron status in vulnerable groups at lower, safer levels of iron supplementation/fortification.

The emergence of chronic diseases of lifestyle in Africans in transition (urbanisation), especially obesity and stroke, is causing a double burden of disease. One of the consequences of obesity is raised plasma fibrinogen levels, which is a risk factor for stroke and coronary heart disease. Stroke is known to be the first chronic disease of lifestyle to emerge in the South African black population during urbanisation and is thought to be related to high incidences of hypertension and hyperfibrinogenaemia in this population. Epidemiological studies indicated that vitamin A intake and status are associated with plasma fibrinogen levels.

The hypothesis developed for this study, is that increased vitamin A intake through consumption of vitamin A fortified sugar will improve iron status and decrease plasma fibrinogen levels of a group of black South African women. This hypothesis is based on the evidence that there is an interaction between vitamin A and iron absorption, transport and function, a possible interaction between plasma fibrinogen and vitamin A status, and therefore also a possible relationship between iron status and fibrinogen.

The major objective of this project was therefore to prove that iron deficiency can be addressed by increasing vitamin A status of young black South African women. If this is true it could mean that lower levels of iron fortification or supplementation may be necessary to address iron deficiency problems, without increasing the risk of an iron overload when genetically susceptible individuals consume iron fortified products. Another objective was to determine the effect of vitamin A supplementation on the fibrinogen levels in the same sample.

Methods: To test the hypothesis, the study was designed in different phases:

Phase 1 : In this phase, the suitability of the habit of tea drinking as a vehicle for fortification by determining the amount of tea consumed, the type of tea mostly consumed, milk and sugar additions, when tea is consumed, the reasons for tea consumption and whether people like tea, were examined.

Phase 2 : During phase 2, the fortification levels and fortifying the sugar, as well as compliance and consumer acceptability of the vitamin A fortified sugar, were determined.

Phase 3 : In phase 3 the effects of vitamin A fortified sugar on iron status and fibrinogen levels by means of a double blind, placebo controlled parallel study in 13-25 year old black South African, non-pregnant, non-lactating, apparently healthy female volunteers (n=100) were tested. Measurements were taken twice at baseline and thereafter during weeks 4, 8 and 12. Variables measured included:

- Anthropometry (Body mass index (BMI), waist-to-hip ratio)
- Serum retinol (High performance liquid chromatography – HPLC)
- Haemoglobin (Cyanmethaemoglobin-colorimetry)

- Haematocrit (Numeric integration)
- Mean cell volume (Impulse generating)
- Red blood cell and white blood cell count (Cell counting - autoanalyser)
- Serum iron (Colorimetry)
- Serum ferritin and transferrin (Immunoturbidity)
- Full blood count (Coulter counter)
- Fibrinogen (Modified Clauss method)

Results: In phase 1 it was found in a sample of 500 subjects that at least one cup of tea was consumed by the 92,9 % of the participants in this study, with rooibos tea selected as the most popular (50 % of tea consumed). Sugar was chosen by 40,4 % and milk by 37,0 % of the sample to be the preferential ingredient added to tea. According to the preference scales of these respondents, tea was the third most consumed beverage in summer and the first most consumed beverage in winter.

Phase 2 indicated that sugar fortified with vitamin A seemed to be acceptable, as no differences in the colour and taste of the fortified sugar were noted. A statistically significant difference was, however, noted in the smell of the sugar that was fortified to a level of 100 % of the recommended daily allowance of the sample population. Compliance to the intake of the fortified sugar was good as 93 % of the participants in this study used white sugar on a daily basis. This may indicate that sugar is a suitable vehicle for vitamin A fortification.

The main results of phase 3 indicated that vitamin A intakes influenced serum iron, but not to the extent that it could rectify iron deficiency at the levels of iron consumption of these subjects. It further showed significant correlations between serum retinol and iron status variables in this homogeneous group of subjects, confirming a relationship between vitamin A and iron status. 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 and weight.

Conclusions: From the results of this study, the following conclusions can be drawn:

- In a random sample (n = 100) of 13-25 year old black South African girls and women, 12 % had an unacceptable low vitamin A status (serum vitamin A $\leq$ 30 μg/dL) and 58 % had low iron status (based on a variety of iron status variables). Clearly, micronutrient deficiencies are prevalent in these urban women who follow a western-type of diet.
- Compliance to the intake of the fortified sugar was good and this indicates that sugar is a suitable vehicle for vitamin A fortification.
- Although dietary intake of vitamin A fortified sugar did not result in clinical improvements of iron status variables, statistically significant changes were observed in serum iron and the results thus suggest that the additional vitamin A consumed influenced iron metabolism. The significant correlations observed between serum retinol and iron status variables in this homogeneous group of subjects, confirm a relationship between vitamin A and iron status.
- 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.

Recommendations: For practical implications, the results of this project may impact on public health policy regarding the treatment of micronutrient (specifically vitamin A and iron) deficiencies. Programmes aimed at the prevention of micronutrient deficiencies, rather than treatment, may help to prevent the rising health costs in this country.

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

- The mechanism by which vitamin A and iron interact need to be studied in order to plan future intervention programmes.
- Optimal fortification levels need to be determined through more research in order to identify safe iron and vitamin A levels, which will rectify deficiency problems without increasing risk of haemochromatosis in Africans.
- Cost-effective analysis should be done to determine the most cost-effective interventions for the situation in South African rural, urban and poorest rural areas. A mix of strategies would probably be needed to ensure adequate vitamin A and iron intakes for all people.
- The results of this study regarding the high prevalence of especially iron deficiency in young, black women, as well as the possibility that sugar could be a fortification vehicle should be communicated to policy makers in the South African Department of Health (Directorate Nutrition) and should be used in nutrition education of the public.

OPSOMMING

Agtergrond, motivering en hipotese: 'n Hoë voorkoms van mikronutriënttekorte is beskryf onder die Suid-Afrikaanse swart bevolking, veral vitamien A-tekort en ystergebrekanemie. Ysteroorlading is egter ook 'n probleem in die Suid-Afrikaanse swart bevolking. Die literatuur dui aan dat alhoewel 'n hoë voorkoms van ystergebrekanemie veral onder jong swart meisies voorkom, 'n verhoogde geneigdheid tot ysteroorlading ook sigbaar is – nie slegs as as gevolg van 'n verhoogde inname van bier gebrou in ysterhouers nie, maar ook as gevolg van 'n genetiese faktor. Dit lyk asof die voorkoms van die homosigotiese vorm vir hemochromatose hoër in die Suid-Afrikaanse swart bevolking as in Europese nasies is. Die rol van vitamien A in ystermetabolisme het al meer aandag geniet gedurende die afgelope paar jaar. Studies het getoon dat vitamien A 'n beskermende effek soos 'n verhoogde mobilisasie van yster toon gedurende ystersupplementasie. Dit sou dus voordelig wees vir voorkoming- en intervensie programme om te weet of vitamien A fortifikasie/supplementasie ysterstatus in kewsbare groepe sou verbeter met laer, veiliger vlakke van ystersupplementasie/fortifikasie.

Die voorkoms van chroniese lewenstylsiektes, veral vetsug en beroerte, in die swart bevolking tydens oorgang (verstedeliking) veroorsaak 'n dubbele siektelas. Een van die gevolge van vetsug is verhoogde plasmafibrinogeenvlakke, 'n risikofaktor vir beroerte en koronêre hartvatsiektes. Beroerte is bekend as die eerste chroniese lewenstylsiekte wat onder die Suid-Afrikaanse swart bevolking tydens verstedeliking voorkom en word geassosieer met 'n hoë voorkoms van hipertensie en hiperfibrinogenemie in hierdie populasie. Epidemiologiese studies het aangetoon dat vitamien A-inname en -status met plasmafibrinogeenvlakke geassosieer word. Die hipotese ontwikkel vir hierdie studie, is dat verhoogde vitamien A-inname deur vitamien A gefortifiseerde suiker ysterstatus sal verbeter en plasmafibrinogeenvlakke verlaag in 'n groep Suid-Afrikaanse swart vroue. Hierdie hipotese is gebaseer op die bewyse van 'n interaksie tussen vitamien A en ysterabsorpsie, vervoer en funksie, 'n moontlike interaksie tussen plasma- fibrinogeen en vitamien-A status, en dus ook 'n moontlike verband tussen ysterstatus en fibrinogeen.

Die hoofdoelwit van hierdie projek was dus om te bewys dat ystergebrek aangespreek kan word deur verbeterde vitamien A status van jong, swart Suid-Afrikaanse vroue. Indien dit waar is, kan dit beteken dat laer vlakke van ysterfortifikasie of supplementasie nodig mag wees om yster- tekorte aan te spreek sonder 'n verhoogde risiko van ysteroorlading wanneer geneties vatbare individue ystergefortifiseerde produkte verbruik. 'n Ander doelwit was die bepaling van die effek van vitamien A supplementasie op die fibrinogeenvlakke in dieselfde steekproef.

Metodes: Vir die toetsing van die hipotese is die studie in verskillende fases verdeel:

Fase 1 : In hierdie fase is die aanvaarbaarheid van tee as 'n geskikte draer vir fortifisering vasgestel deur die daaglikse hoeveelheid en tipe tee meestal verbruik, wanneer tee gedrink word en hoe dit gedrink word, met betrekking tot melk en suiker byvoegings, te bepaal. Die redes waarom tee gedrink word is ook bepaal.

Fase 2 : Gedurende fase 2 is die fortifiseringsvlakke bepaal en die suiker gefortifiseer. Voorskrifnakoming en aanvaarbaarheid van die gefortifiseerde suiker vir die verbruiker is vasgestel.

Fase 3 : In fase 3 is die effek van vitamien A-gefortifiseerde suiker op ysterstatus en fibrinogeenvlakke deur middel van 'n dubbelblinde, plasebo-gekontrolleerde parallel-studie in 13-25 jaar oue Suid-Afrikaanse swart, nie-swanger, nie-lakterende, oënskynlik gesonde vroulike vrywilligers (n=100) getoets. Metings is twee keer gedurende basislyn gedoen en daarna gedurende week 4, 8 en 12. Veranderlikes gemeet sluit in:

- Antropometrie (liggaamsmassa indeks (LMI), middel-tot-heup verhouding)
- Serumretinol (Hoë doeltreffendheid vloeistofchromatografie)
- Hemoglobien (Siaanmethemoglobien-kolorimetrie)
- Hematokrit (Numeriese integrasie)
- Gemiddelde selvolume (Impulsgenerasie)
- Rooibloedsel- en witbloedseltelling (Seltelling – outo-analiseerder)
- Serumyster (Kolorimetrie)
- Serumferritien en transferrien (Immunoturbiditeit)
- Volbloedtelling (Coulter-teller)

- Fibrinogeen (Gemodifieerde Clauss-metode)

Resultate: In fase 1 is gevind dat in die steekproef bestaande uit 500 proefpersone, 92,9 % ten minste een koppie tee per dag gedrink het met rooibostee geselekteer as die populêrste (50 % van alle tee verbruik). Suiker is deur 40,4 % en melk deur 37,0 % van die proefpersone aangedui as die mees geskikte byvoeging by tee. Na aanleiding van die voorkeurskale van die proefpersone, word tee die derde meeste in die somer en die meeste in die winter gedrink.

Fase 2 het aangedui dat vitamien A gefortifiseerde suiker aanvaarbaar was aangesien geen verskille in kleur en smaak in die gefortifiseerde suiker opgemerk is nie. 'n Statisties betekenisvolle verskil was egter in die reuk van die suiker, gefortifiseer tot 100 % van die daaglikse aanbeveling vir die steekproef, merkbaar. Voorskrifnakoming van die gefortifiseerde suiker was goed aangesien 93 % van die proefpersone in hierdie studie wit suiker op 'n daaglikse basis gebruik. Hierdie resultate dui daarop dat suiker 'n geskikte draer vir vitamien A- fortifisering mag wees.

Die belangrikste resultate van fase 3 het aangetoon dat die vitamien A-innames in hierdie hoeveelhede serumyster beïnvloed het, maar nie tot so 'n mate dat dit ystergebrekanemie kon verbeter met vlakke ingeneem deur hierdie proefpersone nie. Dit het verder gedui op statisties betekenisvolle korrelasies tussen serumretinol en ysterstatusveranderlikes in hierdie homogene groep proefpersone. Die verband tussen vitamien A en ysterstatus is dus bevestig. Hierdie intervensie-studie bewys tot 'n mate dat verhoogde innames van vitamien A deur proefpersone met aanvaarbare vitamien A-status, klein, maar statisties betekenisvolle verlagings in plasma- fibrinogeen tot gevolg het. Hierdie verlagings was egter nie volgehou nie, moontlik as gevolg van die waargenome verhogings in LMI en massa.

Gevolgtrekkings: Die volgende gevolgtrekkings kan van die resultate van hierdie studie afgelei word:

- In 'n ewekansige steekproef (n = 100) van 13-25 jaar oue swart Suid-Afrikaanse meisies en vroue het 12 % 'n onaanvaarbare lae vitamien A-status (serumvitamien A ≤ 30 μg/dL) en 58 % 'n lae ysterstatus (gebaseer op 'n verskeidenheid ysterstatusveranderlikes) gehad. Dit is duidelik dat mikronutriënttekorte voorkom in hierdie stedelike vroue met 'n westerse-tipe dieet.
- Voorskrifnakoming tot die inname van die gefortifiseerde suiker was goed en dit dui daarop dat suiker 'n geskikte draer vir vitamien A-fortifisering is.
- Alhoewel dieetinname van die vitamien A gefortifiseerde suiker nie kliniese verbeterings in ysterstatusveranderlikes tot gevolg gehad het nie, is statisties betekenisvolle veranderings in serumyster gevind en die resultate dui dus daarop dat die addisionele vitamien A ingeneem, ystermetabolisme beïnvloed het. Die betekenisvolle korrelasies tussen serumretinol en ysterstatusveranderlikes waargeneem in hiedie homogene groep proefpersone, bevestig die verhouding tussen vitamien A en ysterstatus.
- Bewyse bestaan tot 'n sekere mate dat verhoogde inname van vitamien A in persone met aanvaarbare vitamien A-status, klein, maar statisties betekenisvolle verlagings in plasma- fibrinogeen tot gevolg het. Hierdie veranderinge was egter nie volgehou nie, moontlik as gevolg van die verhogings in LMI en massa.

Aanbevelings: Uit 'n praktiese oogpunt mag die resultate van hierdie projek 'n impak maak op die publieke gesondheidsbeleid met betrekking tot die behandeling van mikronutriënt (spesifiek vitamien A en yster) tekorte. Programme met die doel om mikronutriënttekore te voorkom eerder as te behandel, mag bydra om die verhoogde gesondheidskostes in hierdie land te voorkom.

Die resultate van hierdie studie dui daarop dat verdere navorsing nodig is om die volgende punte aan te spreek:

- Die meganisme van interaksie tussen vitamien A en yster moet bestudeer word vir die beplanning van toekomstige intervensieprogramme.
- Optimale fortifikasievlakke moet deur middel van navorsing vasgestel word om veilige yster- en vitamien A-vlakke te identifiseer. Moontlike probleme geassosieer met gebreke sonder die verhoogde risiko vir hemochromatose in swartes kan dus uitgeskakel word.
- Koste-effektiewe analises behoort gedoen te word om die mees koste-effektiewe intervensies vir die situasie in Suid-Afrikaanse plattelandse, stedelike en armste plattelandse areas te bepaal. 'n Verskeidenheid strategiee sou moontlik nodig wees om voldoende vitamien A- en ysterinnames vir alle persone te verseker.
- Die resultate van hierdie studie aangaande die hoë voorkoms van veral ystertekort in jong, swart vroue, sowel as die moontlikheid dat suiker 'n draer van fortifisering kan wees, behoort

aan die beleidmakers in die Suid-Afrikaanse Departement van Gesondheid (Direktoraat: Voeding) gekommunikeer te word en moet in voeding- voorligting van die publiek gebruik word.

CHAPTER 1

INTRODUCTION

1 BACKGROUND TO THE PROBLEM

1.1 Introduction

The main focus of the thesis is to examine the possibility that the consumption of vitamin A fortified sugar will also benefit the iron status of young South African women. Therefore, in this introductory chapter the problem of vitamin A and iron deficiencies will be briefly highlighted. However, it will be discussed in more detail in the literature survey, Chapter 2 of this thesis. The possible interaction between vitamin A and iron metabolism and status will receive more attention in this chapter as the background that motivated this study.

The growing perception that under- and overnutrition often co-exist in developing populations also motivated the measurement of the effect of consumption of vitamin A fortified sugar on plasma fibrinogen. The latter is accepted, together with hypertension, as a major risk factor for stroke. This nutrition-related chronic disease of lifestyle emerges early in black South Africans when they adopt modern or western lifestyles (Vorster *et al.*, 1998:174). Plasma fibrinogen, as a risk factor for cardiovascular disease (CVD), will be reviewed in the literature survey.

1.2 Micronutrient deficiencies

Good nutrition is universally accepted as a basic human right, but it is estimated that globally more than 800 million people suffer from malnutrition and that in developing countries, more than 20 % of the populations are hungry (reviewed by Vorster *et al.*, 1997c:1). The United Nation's Children Fund (UNICEF) estimated that 190 million children younger than five years of age are chronically malnourished and are trapped early in life in a pattern of poor health and development (UNICEF & WHO, 1994:3).

At the World Summit for Children held in New York during December 1990, political leaders from 123 countries around the world endorsed the "World Declaration on the Survival, Protection and Development of Children". This was done after the significant and adverse effects of malnutrition, as well as micronutrient malnutrition on health and development of children was highlighted. This Declaration was subsequently signed by President Mandela and Deputy President de Klerk. The Declaration specifically targets the year 2000 for the virtual elimination of vitamin A deficiency (VAD) and iodine deficiency, and a one-third reduction of iron deficiency anaemia in affected women (SAVACG, 1995: 41; USAID, 1993:3). Furthermore, the rights, needs and the growth and development of children are clearly identified priorities in the South African (SA) Government's Reconstruction and Development Programme (RDP). The RDP emphasises that the "needs of children

must be paramount throughout all programmes aimed at meeting basic human needs and socio-economic upliftment" (SAVACG, 1995:41).

In Africa it was reported that 1,3 million children younger than five years were affected by VAD. Iron deficiency affected 59,4 million women between 15 and 49 years old. The causes of these deficiencies are multiple. Low absorption of iron from foods, parasitic infections, or incorrect breastfeeding practices in infants are the main causes of iron deficiency. VAD is primarily due to inadequate dietary intake and/or impaired absorption of vitamin A. Iodine deficiency is largely an environmental problem in regions with iodine deficient soil and/or water supplies, resulting in low iodine intakes (SAVACG, 1995:39).

1.3 Global prevalence of iron and vitamin A deficiencies

Iron deficiency is a major health problem world-wide, with a total prevalence estimated at about 40 % of the world's population (Gillespie, 1998:9). Underwood (2000:356) mentioned that 3,5 - 5 billion people are iron deficient. Anaemia is affecting up to 10 % of the world population (Barasi, 1997:186). Infants, children, teenagers and women of childbearing age, constitute the main vulnerable groups (Barasi, 1997:187; SAVACG, 1995: 160).

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).

1.4 Prevalence of iron and vitamin A deficiencies in South Africa

The findings of the SAVACG study indicated that 33,3 % of SA children have a marginal vitamin A status and thus identified SA as having a serious public health problem of VAD. The most disadvantaged are the children in the 12-71 months age group, those living in informal type housing and whose mothers are poorly educated. Only 1 % of children had serum vitamin A concentrations higher than 50 µg/dL (SAVACG, 1995:137). The study further showed that 3 to 3,9 year old children and those from Kwazulu Natal and Northern Province had the highest incidence of VAD (Vorster *et al.*, 1997c:11).

With regard to iron deficiency, an overall anaemia prevalence of 21 % is present, with the lowest prevalence in Kwazulu Natal (10 %) and the highest in the Northern Province (34 %). The SAVACG study indicated differences between rural versus urban and between boys and girls. On a national level 9,8 % of the children had serum ferritin levels below 12 µmol/L (Vorster *et al.*, 1997c:11).

Little information and no recent studies on the serum micronutrient concentrations of white, coloured and Indian adolescents are available. The low mean concentrations of iron status variables of Indian children could be an indication of a possible iron deficiency in this group. The iron and folate deficiencies seen in younger African children are still present in adolescents (Vorster *et al.*, 1997c:12).

A meta-analysis of the South African literature indicated that mean nutritional status variables of whites were within normal ranges. However, a 23 % prevalence of low iron status was present in a small

group of 20-year old white women. Iron deficiency anaemia is present in many adult black South Africans (Vorster *et al.*, 1997c:12).

1.5 Health consequences associated with micronutrient deficiencies

In children three micronutrient deficiencies of vitamin A, iron and iodine, are considered to be major health problems in developing countries. These are presently receiving high priority globally. Communities that are affected most are those in situations where poverty, unemployment, civil unrest, war and exploitation remain endemic (SAVACG, 1995:39; USAID, 1993:2).

As mentioned in 1.3, 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 (Fishman, 2000:125; SAVACG, 1995:39; USAID, 1993:2).

1.6 Economic costs of micronutrient deficiency

The economic losses attributed to malnutrition, were calculated by the World Bank. They are ranging between 6 and 12 % of the Gross National Product (GNP) of developing countries. If a figure of 6 % loss is applicable to SA, the costs of undernutrition alone could amount to at least four billion Rand, and may be higher if the cost of undernourished chronically and acutely ill patients is taken into account (SAVACG, 1995:37).

Lynch (1999:102) suggested that for a 1 % decrease in haemoglobin (Hb), the resultant decrease in work capacity is 1,5 %, and for work output it is 1-2 %. He further suggested that if the average productivity is decreased by 20 % for anaemic individuals and if 50 % of women and 20 % of men are affected, the national economic output will be decreased by 5-7 %.

Therefore, the prevention of malnutrition and its negative effects on quality of individual and community life, together with the implications for national productivity and socio-economic development , amount to the strongest argument for any government to afford this the highest priority (SAVACG, 1995:37). Social and political development cannot occur without an adequately nourished, healthy and productive population (USAID, 1993:2). In the short term, nutrition intervention programmes should be seen as a national priority until such time that the infrastructure is realised to prevent protein-energy malnutrition (PEM) (SAVACG, 1995:37).

1.7 Treatment of micronutrient deficiency

New concepts and perceptions are emerging in terms of micronutrient deficiencies. At a national level more vitamins and minerals can be made available to the population by implementing suitable and appropriate intervention programmes. These could be education in diversifying diets, or by fortifying commonly eaten foods with the missing micronutrients or providing nutrient supplements through targeted distribution programmes (USAID, 1993:7). For the purpose of this study, only fortification will be examined, as this will be the main focus of the empirical work of this study.

Political and public investment in national programmes, which call for dietary diversification, fortification or supplementation is essential. Micronutrient interventions can be integrated into primary health care, family planning, agriculture, or basic education programmes and can be sustained with minimal long-term programme costs after initial investments have been made (USAID, 1993:7).

1.8 Vitamin A and iron interaction

Since the first study by Findley and MacKenzie in 1922, several reports have suggested interdependence between vitamin A and iron (Bloem, 1995:501). Vitamin A and its derivatives are not only important for normal eye functioning, but also for normal differentiation of several tissues (Bloem *et al.*, 1989:332).

Findley and MacKenzie reported in 1922 that vitamin A deficient rats developed patches of gelatinous degeneration in their bone marrow, and that in animals surviving the longest, the haematopoietic tissue had been replaced almost completely with fibrous stroma. A subsequent study by Wolbach and Howe in 1925 did not report characteristic changes in the bone marrow, but in a few cases reductions in haematopoietic cells were apparent (Bloem, 1995:501; Hodges *et al.*, 1978:882). In an experiment involving a vitamin A deficient group of otherwise healthy volunteers an association between iron metabolism and hypovitaminosis A was established. The experimental group developed anaemia that was responsive to vitamin A (Bloem *et al.*, 1989:335).

In 1926, Koessler *et al.* (1926:481) examined the consequences of chronic vitamin A underfeeding. Through clinical observations, experimental work and theoretical considerations they found that vitamin A deficiency and underfeeding may be a factor of primordial importance in the production of severe anaemia in children and adults. The pernicious megaloblastic and aplastic type of anaemia may be intimately related to a vitamin A deficiency. The conclusions that were reached in this study are as follows:

- Blood regeneration cannot take place in the absence of vitamin A.
- The addition of vitamin A to the diet of vitamin A deficient animals, resulted in rapid formation of new blood cells.
- The rate and intensity of blood regeneration is related to the quantity of vitamin A present in the body.
- A definite relationship exists between a state of chronic vitamin A deficiency and certain anaemias (Koessler *et al.*, 1926:481).

Anaemia, as well as haemosiderosis of the spleen and liver, in vitamin A-deficient patients was demonstrated by Blackfan and Holbach in 1933. Repletion with vitamin A led to the regeneration of the bone marrow, disappearance of the haemosiderin from the spleen and liver, and erythroblastic activity. In 1940 Wagner showed that patients maintained on a vitamin A deficient diet for a period of six months developed low haemoglobin (Hb) and packed cell volume levels, and concluded that haemopoiesis was impaired (quoted by Bloem, 1995: 502; Hodges *et al.*, 1978:882).

When comparing these studies, it seems that vitamin A is essential for normal haematopoiesis (Hodges *et al.*, 1978:882; Semba *et al.*, 1992:474). Suharno and colleagues proved that small daily vitamin A supplements have haemopoietic effects additive to those of iron when given to pregnant women

(Thurnham, 1993:1312). A number of studies in animals and humans suggest that there may be a direct interaction between vitamin A nutritional status and the ability to effectively utilise both dietary and stored iron for haemoglobin formation (Ahmed et al., 1996:346; Meija & Chew, 1988:599). In 1970 a study by Amine (1970:1038) compared the haematologic effects of iron deficiency and vitamin A deficiency in rats. This study demonstrated that iron deficiency results in the familiar picture of microcytic, hypochromic anaemia, whilst vitamin A deficiency results in microcytic, hypochromic polycythemia. When both vitamin A and iron deficiencies are present, a normocytic, hypochromic anaemia is the result (reviewed by Bloem et al., 1989:336; Hodges et al., 1978:883).

In 1977 a positive correlation between serum retinol and Hb was found in children aged between five and 12 years old (Ahmed et al., 1996:346; Lynch, 1997:106; Meija et al., 1997:1175), and in pregnant women (Ahmed et al., 1996:347; Lynch, 1997:106). During the last decade several cross-sectional studies confirming this relationship have been reported from Thailand, Ethiopia and Indonesia. In 1989 Bloem showed that serum retinol was significantly associated with packed cell volume, serum iron, ferritin, transferrin and saturation of transferrin. No correlation, however, was found between serum retinol and haemoglobin (Bloem, 1995: 503).

The role of vitamin A in iron metabolism has received increased attention during recent years (Van Stuijvenberg et al., 1997:41). A common link in the metabolism of iron and retinol is the fact that both retinol and iron are transported by the "negative" acute phase proteins, retinol binding protein (RBP) and transferrin. Infections result in a depressed synthesis of both these proteins. Both infections and iron deficiency are widespread in the developing world and it seems plausible that chronic gastrointestinal and respiratory infections may play a role in the aetiology of nutritional anaemias. One very rapid response to infection is an increase in endothelial permeability. The reduced synthesis of small molecular weight proteins such as RBP, transferrin and albumin is thus a short-term measure to reduce urinary losses of these compounds. Low retinol concentrations do not seem to offer any biological advantage in the long term, but it may be a feature of the overwhelming presence of infections and retinol, like iron, may be trapped in the liver. Iron accumulation in the liver and spleen is a common response to chronic infection and the accompanying depression of RBP may further exacerbate the infection. Administering oral vitamin A may break this vicious cycle. The anti-infective properties of vitamin A are widely acknowledged and suppression of infection will immediately stimulate a resumption of transferrin and RBP synthesis, thereby releasing the trapped iron and retinol (Thurnham, 1993:1313).

Although there is strong evidence for an association between vitamin A and anaemia, the underlying biochemical mechanism is still unsure (Bloem, 1995:504; Tanimuhardjo et al., 1996:32). It is thought that the association is causal, as there is evidence for an interaction between iron and vitamin A metabolism. VAD has been associated with an impaired ability to utilise endogenous iron stores, and vitamin A deficient patients have been found to be unresponsive to dietary iron supplementation (Ahmed et al., 1996:347; Lynch, 1997:107; Mohanram et al., 1977: 389).

Marginal vitamin A status is common among children and women of childbearing age in developing countries. As a consequence, the World Health Organisation (WHO) has suggested vitamin A supplementation (200 000 IU after 4 weeks of delivery) to lactating women in high-risk areas. Lactating Indonesian women have shown a significant improvement in serum retinol concentrations after high-dose supplementation soon after delivery. Vitamin A supplementation also resulted in increased haemoglobin concentrations in both children and pregnant women. The mechanism

suggested for this increase was that vitamin A may act by down-regulating the acute-phase response, which ultimately should lead to increased transferrin synthesis, augmented iron transport from liver to bone, and thus enhanced haemoglobin formation (De Pee et al., 1995:75; Tanimuhardjo et al., 1996:32).

Pakistan is predominantly a rural third world country and UNICEF estimates the death rates of children, 1-4 years to be 16,2 % in Pakistan, with diarrhoea and respiratory diseases the leading causes of mortality (60 %). Basic health and education facilities are limited and very little emphasis is placed on nutrition, hygiene and sanitation. The health and nutritional status of pregnant and lactating women, and pre-school children is poor and the prevalence of malnutrition in children under five years of age is estimated to be 67 %. Iron deficiency is considered the most important nutritional deficiency among infants in Pakistan (Northrop-Clewes et al., 1996:694).

In Pakistan vitamin A deficiency is not such a serious problem as in parts of India and Indonesia. However, in a study performed in 1996 in which 300 breastfed infants under two years of age participated, the retinol values of Pakistani infants showed a median value of 0,61-0,65 µmol/L. Of the 300 children participating in the study, 59 % had marginal retinol values (< 0,7µmol/L) that may be insufficient for maintaining normal epithelial integrity of the eye, gut and respiratory tract tissues. In addition, ± 7 % of the infants were biochemically deficient with retinol concentrations < 0,35 µmol/L. In this study 15 milligram (mg) ferrous iron sulphate (FeSO4) was administered orally to the treatment group on a daily basis. The control group received a placebo. This study was done during the months of July to September when seasonal foods included mangoes, spinach and peaches. The median retinol concentrations of both groups of infants increased significantly from 0,64 to 0,75mol/L at the end of the 12-week study. Although plasma lutein concentrations increased significantly to 0,19 µmol/L in both groups, there were no significant changes in plasma beta-carotene. The significant increase in plasma retinol, lutein and alpha-tocopherol seen after supplementation could have been due to an increased availability of carotenoid-rich vegetables and fruit. The median plasma ferritin levels increased from 6,7-7,2 µg/L before supplementation to 12,9 µg/L after supplementation. This was the first study to describe the protective effect of vitamin A during iron supplementation (Northrop-Clewes et al., 1996:697).

This protective effect of vitamin A during iron supplementation and an increased mobilisation of iron after vitamin A supplementation are now widely recognised. Small daily supplements of vitamin A have haematopoietic effects that are additive to those of iron when given to pregnant women (Northrop-Clewes et al., 1996:698; Ribayo-Marcado, 1997:306). As mentioned above, vitamin A may act by down-regulating the acute-phase response, which should ultimately lead to increased transferrin synthesis, augmented iron transport from the liver to the bone marrow, and enhanced haemoglobin formation (Tanimuhardjo et al., 1996: 32). The response to iron supplementation is greater when vitamin A is given in conjunction with iron (Van Stuijvenberg et al., 1997:47).

In 1998 Venezuelan researchers found that when vitamin A or beta-carotene is added to iron-fortified rice, wheat or corn, the iron absorption is increased. Both beta-carotene and vitamin A can prevent the inhibitory effects of phytates on the absorption of non-haem iron from grain products by forming a complex with iron and thus keeping it soluble in the intestinal lumen (Garcia-Casal et al., 1998:650).

Nutritional anaemia and vitamin A deficiency have been identified as two of the major public health problems in Bangladesh. The anaemia, specifically common amongst women, appears to be caused by the iron deficiency. The per capita intake of iron, however, is high and therefore the possibility exists that other factors of importance may influence the metabolic availability of iron. The relevance of vitamin A has been considered. A number of studies in animals and humans suggested a direct interaction between vitamin A status and the ability to effectively utilise both dietary and stored iron for haemoglobin formation. When a higher serum retinol concentration is present, there appeared to be significantly greater iron availability in circulation, suggesting that vitamin A contribute to the aetiology of iron deficiency anaemia (Ahmed *et al.*, 1995: 350).

A study where pregnant women were supplemented with iron for a period of 12 weeks indicated that iron supplementation resulted in the improvement of iron status, and was also beneficial in improving the vitamin A status, even in the absence of vitamin A supplementation. It was possible that iron supplementation resulted in better absorption of dietary vitamin A by improving the intestinal mucosal function usually compromised by iron deficiency (Shatrugna *et al.*, 1997:147).

A logical approach to control both vitamin A and iron deficiencies is the fortification of food with both vitamin A and iron. The food to be fortified must, however, reach the population groups at risk and meet the criteria for an acceptable fortification vehicle. Sugar has been shown to be an adequate vehicle for vitamin A fortification in Guatemala and it does not interfere with iron absorption. Sodium iron ethylene diamine tetra-acetic acid (NaFeEDTA) was added to sugar at 13 mg iron/kg to provide an extra 4 mg iron per person per day (Hurrell, 1997:218; Viteri *et al.*, 1995:1553; Yip, 1995:1165).

A study was done in which sugar was fortified with vitamin A and FeNaEDTA amongst low-income, semi-rural communities in Guatemala, where iron deficiency and its consequent anaemia were prevalent. All aspects of this study were successful in proving the feasibility, organoleptic advantages, simplicity of execution and biological effectiveness of FeNaEDTA and vitamin A fortified sugar in controlling iron deficiency in Guatemala (Viteri *et al.*, 1995:1160). The results of this study demonstrated the effectiveness of fortifying sugar with FeNaEDTA as it proved to be stable and safe for consumption. This study also resulted in a decline in the prevalence of mild to moderate anaemia from 27,3 % at baseline measurement to 13,9 % after a 32-month period of consuming vitamin A and FeNaEDTA fortified sugar (Viteri *et al.*, 1995:1162).

1.9 Objectives of the study

The question asked in this study was whether vitamin A supplementation of the diet of young black women, a vulnerable group for iron deficiency, will improve vitamin A and iron status concomitantly. Because African women is known to have high plasma fibrinogen levels, and therefore a high risk for stroke (Vorster *et al.*, 1998:173), and because low vitamin A status has been associated with high plasma fibrinogen (reviewed by Vorster *et al.*, 1997b:129) the effect of vitamin A supplementation on plasma fibrinogen was also examined. To reach these objectives, a series of experimental steps were followed. The first step was a preliminary study (described in Chapter 3) to determine the suitability of tea as a fortification vehicle. Tea is apart from water, the most widely consumed drink globally (Vorster *et al.*, 1996:58). Furthermore, the Transition and Health during Urbanisation in Southern Africa (THUSA) study in the North West Province of South Africa

indicated that, except for maize products, tea was the dietary item that was consumed in the largest quantities by this population (MacIntyre, 1998:258).

The objective of this preliminary study was to identify whether tea with sugar was consumed in sufficient quantities to justify added sugar as vehicle for vitamin A fortification in these young African women.

The second step was to assess the total nutrient intake of these potential subjects to decide on the necessary levels of fortification. Therefore, dietary intake was measured and nutrient intake calculated to determine the total iron and vitamin A intake. Although there are a number of ways in which dietary data can be collected, no ideal instrument exists. Suitable visual aids to assist the respondents in describing foods and portion sizes have to be used, especially for the illiterate and younger respondents. Food models and actual food and containers were used to assist with the portion sizes to be stated in the quantitative food frequency questionnaire (QFFQ) used to measure habitual food intake.

After completion of the preliminary study in which sugar was identified as a suitable fortification vehicle for vitamin A, the third step was a matched placebo-controlled clinical intervention trial for a period of 12 weeks to determine the effect of the fortified vitamin A sugar on the vitamin A and iron status, as well as the fibrinogen levels of 100 subjects in a randomly selected sample population. The purpose of the total study was, therefore, to develop a suitable vitamin A fortified product and then to test the effect of the fortified product on vitamin A and iron status, as well as on the relationship between vitamin A status and fibrinogen levels.

In summary, the objectives of this study were to:

1. assess tea and sugar consumption in young African women, aged 13-25 years, living in the Vaal Triangle, Gauteng Province, in South Africa (SA);
2. describe habitual nutrient intakes of this sample population;
3. fortify sugar with appropriate levels of vitamin A;
4. measure acceptability of vitamin A fortified sugar in this sample population;
5. conduct a placebo-controlled, randomised trial to assess the physiological and biochemical effects of consumption of fortified sugar by young women. During this clinical intervention trial, the compliance of consumption of the fortified sugar was assessed. Blood analyses, including a large number of variables indicative of vitamin A and iron status, as well as plasma fibrinogen, were done during a double baseline and after 4, 8 and 12 weeks of the intervention.

1.10 Relevance of the study

This project provides information regarding the effect of fortified vitamin A on the iron and vitamin A status of young females with a high risk for iron deficiency. It is also the first intervention study to determine the effect of a vitamin A fortified product on fibrinogen levels, and thus the risk of CVD. The study will therefore indicate whether sugar, fortified with vitamin A, will influence nutritional status of young African women. The results should therefore have an impact on public health policy regarding the treatment of micronutrient, specifically vitamin A and iron deficiencies. Programmes aimed at the prevention of micronutrient deficiency, rather than treatment, may help to prevent the rising health costs in the country.

1.11 Organisation of the thesis

Each of the objectives of the present study constituted a study in its own right. The total study thus comprised several substudies. This thesis has been organised in such a way that each chapter deals with one aspect to form a complete entity. Each substudy is thus presented with its objectives, methods, results, discussion and conclusion in the format of a scientific publication. The final chapter demonstrates how the various parts of the project are linked together.

Following the introductory chapter, a review of the literature is presented in Chapter 2. This chapter provides background information on the global and South African prevalence of micronutrient deficiencies, specifically vitamin A and iron deficiencies, as well as the causes, and intervention strategies to address these, with emphasis on fortification. The importance of reducing plasma fibrinogen level and the possible effect of vitamin A in this regard is also discussed.

The different steps of this research project are presented as separate manuscripts that can be read independently, except for Chapter 4. Therefore, the introduction to each will necessarily overlap with the first part of the literature survey (Chapter 2.1 to 2.7). The development of the hypothesis that motivated and was tested in this study is given in Chapter 2, Section 2.8.

Chapter 3 describes the preliminary study that was undertaken to determine whether the habit of tea consumption and therefore sugar added to tea, was a suitable vehicle for vitamin A fortification. The study design of the intervention study will be described in Chapter 4 and Chapter 5 will deal with the compliance and acceptability of sugar as a vitamin A fortification vehicle. Chapter 6 describes the effect of fortified vitamin A on the iron and vitamin A status, and Chapter 7 describes the effect of vitamin A fortification on fibrinogen levels. In Chapter 8 the results are summarised, discussed, conclusions drawn and recommendations made.

CHAPTER 2

LITERATURE REVIEW

2.1 Introduction

Vitamins and minerals are micronutrients that are making headlines globally as evidence grows that they assist in reducing the risk of heart disease, cancer and other chronic illnesses. In the developing world where approximately 800 million people go hungry and childhood deaths far overshadow those due to chronic illnesses, the micronutrients play an undisputed life-saving role (USAID, 1993:1). Since the discovery that micronutrients were essential nutrients, the growth of this field has been the subject of temporary excitement, continuing controversy, ridicule and confusion for the public as well as scientists. Intensive research over the past years has provided an appropriate perspective regarding some aspects of the essentiality of micronutrients and their role in relation to deficiencies and also in the prevention of disease. As such, early, mostly anecdotal claims in the fields of therapeutics and prevention have largely been replaced by credible research data obtained from long-term studies with clinical outcomes based on evidence (Blaauw, 1999:29; Labadarios, 1999:4). In the following sections, data on combined or general micronutrient deficiencies will be discussed first, after which special attention will be given to vitamin A and iron deficiencies.

2.2 Micronutrient deficiency

2.2.1 Global prevalence of micronutrient deficiencies

Good nutrition is universally accepted as a basic human right, but it is estimated that more than 800 million people suffer from malnutrition globally and that in developing countries more than 20 % of the populations are hungry (reviewed by Vorster *et al.*, 1997c:1). UNICEF estimated that 190 million children younger than five years of age are chronically malnourished and are trapped early in life in a pattern of poor health and development (quoted by SAVACG, 1995:98).
The prevention of malnutrition and its negative effects on the quality of individual and community life, together with the implications for national productivity and socio-economic development, therefore, amount to the strongest argument for any government to afford this the highest priority (SAVACG, 1995:37). Social and political development cannot occur without an adequately nourished, healthy and productive population (USAID, 1993:2). In the short term, nutrition intervention programmes should be seen as a national priority until such time that the infrastructure is realised to prevent protein energy malnutrition (PEM) (SAVACG, 1995:37).

At the World Summit for Children held in New York during December 1990, political leaders from 123 countries around the world endorsed the "World Declaration on the Survival, Protection and Development of Children". This was the largest gathering of heads of state ever to take place. They helped to form a global political consensus that some specific reductions in micronutrient deficiencies had to be achieved (Alnwick, 1998:137; SAVACG, 1995:41; USAID, 1993:3). This was done after the

significant and adverse effects of malnutrition, as well as of micronutrient malnutrition on health and development of children was highlighted. President Mandela and Deputy President de Klerk subsequently signed this Declaration. The Declaration specifically targeted the year 2000 for the virtual elimination of VAD and iodine deficiency, and a one-third reduction of iron deficiency anaemia in affected women (SAVACG, 1995:41; USAID, 1993:3). Furthermore, the rights, needs and the growth and development of children are clearly identified priorities in the SA Government's Reconstruction and Development Programme (RDP). The RDP emphasises that the "needs of children must be paramount throughout all programmes aimed at meeting basic human needs and socio-economic upliftment". In addition the goal is to achieve a 90 % immunisation coverage amongst younger than one year old children, the eradication of polymyelitis, the elimination of neonatal tetanus, a 90 % reduction in measles cases and a 95 % reduction in deaths caused by measles (SAVACG, 1995:41).

In the ten-point plan for 1999 to 2000, the Department of Health (http://www.hst.org.za/doh/stratplan.htm) identified improved nutrition through the implementation of the Integrated Nutrition Programme (INP) as one of the strategic interventions to decrease morbidity and mortality in SA.

Encouraged by Congress, the United Stated (US) Agency for International Development (USAID) played an indispensable role in guiding and funding research that established micronutrient interventions as effective, affordable and sustainable, and thus built much of the scientific platform for the World Summit and International Congress of Nutrition (ICN) goals (USAID, 1993:3).

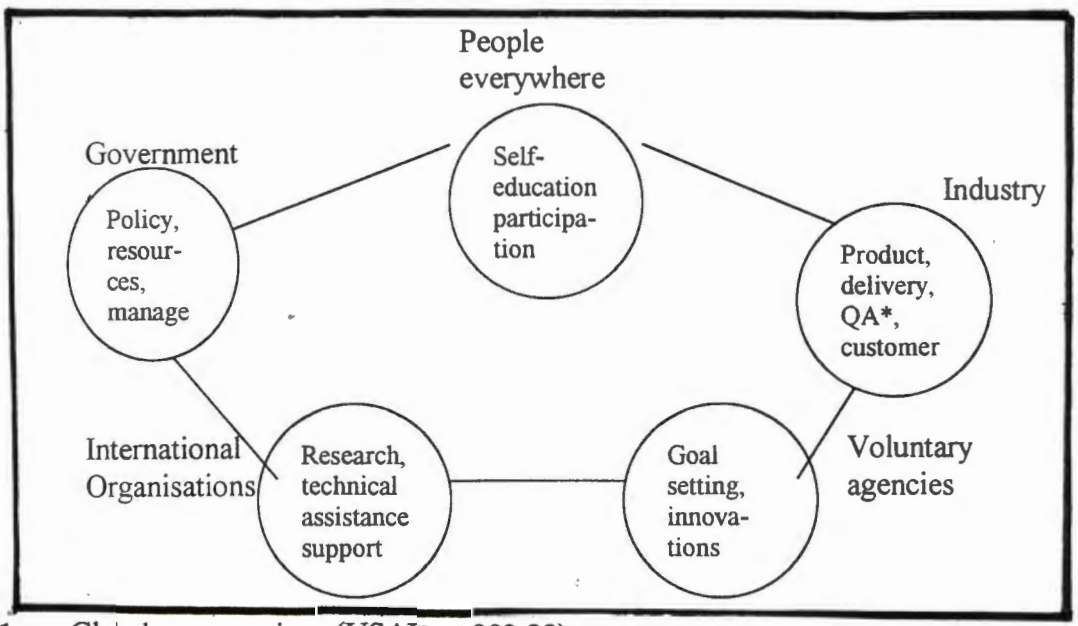

Figure 2.1 Global co-operation (USAID, 1993:28)
* QA = Quality Assurance

The USAID micronutrient programme, MOST, is focusing its attention on selected countries in Africa, Asia and the Near East, Latin America and the Caribbean. MOST is the USAID flagship programme for the promotion of activities designed to improve the micronutrient status of at-risk populations globally. The MOST strategy is built upon a framework of global and country-level results that will ultimately lead to the achievement of project goals and objectives, when attained. The global initiative focuses on collaborating with other organisations in promoting a revised global agenda, translating

scientific knowledge into policy and programme action, and maximising the use of lessons learned through field programme experience. The main emphasis of MOST activities is to reduce mortality through the reduction of VAD and morbidity (MOST, 1998:6).

The INP in SA aims to facilitate a co-ordinated inter-sectoral approach to solving problems in SA. The emphasis is on building long-term capacity of communities to be self-sufficient in terms of their food and nutrition needs while at the same time protecting and improving the health of the most vulnerable parts of the population, namely women and young children (DOH, 1997:2).

It was reported that in Africa 1,3 million children younger than five years were affected by VAD, and 59,4 million women between 15 and 49 years old, were affected by iron deficiency. The causes of these deficiencies are region and nutrient dependent. Low absorption of iron from foods, parasitic infections, or incorrect breastfeeding practices in infants are the main causes of iron deficiency. VAD is primarily due to inadequate dietary intake and/or impaired absorption. Iodine deficiency is largely an environmental problem in regions with iodine deficient soil and/or water supplies (SAVACG, 1995:39).

2.2.2 Prevalence of micronutrient deficiencies in South Africa

2.2.2.1 Introduction

The assessment of micronutrient status is based on the definition of the dietary intake of these nutrients by individuals or population groups, the determination of blood values and the occurrence of clinical deficiency manifestations. Very little evidence of the micronutrient status of population groups in SA is available. Clinical deficiency manifestations are occasionally seen in the severely malnourished individuals. In public health terms studies in this field have been limited, and the few available have been done on a small scale and have addressed primarily local or regional issues. Only recently have data been analysed collectively and national surveys been commissioned (Labadarios, 1999:4). All available literature on the nutritional status of South Africans (from 1975 to 1996) has been summarised by Vorster *et al.* (1997c).

Measurements of dietary intake and analyses of diets to obtain nutrient intakes can give valuable information on nutritional status. There has never been a national SA survey of food and nutrient intakes, except for a study on intakes of children aged 1-9 years, commissioned by the Department of Health in which nutritionists from nine universities co-operated (Labadarios *et al.*, 2000:98; Steyn *et al.*, 2000:99). The results of 55 studies which met the inclusion criteria for a meta-analysis, namely randomisation, stratification for ethnicity, age, and the type of database used for the analysis of the dietary data obtained, were combined in a meta-analysis by the South African Nutrition Survey Group (SANNS). This meta-analysis (Labadarios, 1999:4; Vorster *et al.*, 1997c:14-19) indicated the following:

Calcium	-	Low intakes due to low milk consumption, especially in Africans.
Iron	-	Urban black infants, a small group of black rural children, girls aged 11 to 15,9 years and adult women aged 16 to 64,9 years took in less than 67 % of recommended daily allowance (RDA).
Magnesium	-	Adult men and women had low intakes.
Potassium	-	Adequate for all groups.

Zinc (Zn)	-	Many groups had a low intake and as Zn plays an important role in metabolism, it may need immediate attention.
Phosphorus	-	Adequate in all groups, except black children.
Sodium	-	All groups had higher mean intakes than RDA's.
Thiamin	-	Adequate in all groups.
Riboflavin	-	Coloured, Indian and rural blacks had inadequate intake.
Niacin	-	Adequate intakes in all groups.
Vitamin B6	-	Black, coloured and Indian had intakes less than 67 % of RDA.
Folate	-	Low intakes in Indians and rural black women in reproductive years.
Vitamin B12	-	Adequate in all groups.
Vitamin C	-	Inadequate in most black and Indian groups.
Vitamin A	-	Adequate intakes in all groups, except black children.
Vitamin D	-	Adequate intakes.

2.2.2.2 Prevalence of micronutrient deficiencies in South African infants and children aged 0-6 years

The above-mentioned meta-analysis found that urban black children had normal mean levels of most of the nutritional status variables measured, but that mean serum retinol and iron were low, with borderline low Hb levels. This indicates that the micronutrient status of these children may be low, especially their vitamin A and iron status. The low mean levels of serum vitamin A, beta-carotene, iron and Hb in most groups of rural pre-school children confirmed the presence of vitamin A and iron deficiencies in black children (Vorster *et al*., 1997c:11).

The findings of the South African vitamin A Consultative Group (SAVACG) study indicated that 33,3 % of children in SA have a marginal vitamin A status and thus identified SA as having a serious public health problem of VAD. The most disadvantaged are the children in the 12-71 months age group, those living in informal type of housing and whose mothers are poorly educated. Only 1 % of children had serum vitamin A concentrations higher than 50 µg/dL (SAVACG, 1995:137). The study further showed that 3 to 3,9 year old children and those from Kwazulu Natal and Northern Province had the highest incidence of VAD (SAVACG, 1995).

With regard to iron deficiency an overall prevalence of 21 % with anaemia was present, with the lowest prevalence in Kwazulu Natal (10 %) and the highest in the Northern Province (34 %). No differences between rural versus urban, and between boys and girls were observed. On a national level, 9.8 % of the children had serum ferritin levels below 12 µg/L (reviewed by Vorster *et al*., 1997c:11).

2.2.2.3 Prevalence of micronutrient deficiencies in South African primary school children

The SAVACG study has shown the prevalence of anaemia is 21 % in SA children aged 6-71 months. The prevalence of moderate (7 %) and severe (0,2 %) anaemia is much lower. The SAVACG study showed that iron depletion or deficiency was present in 10 %, and iron deficiency anaemia in 5 % of children. These findings indicate that iron deficiency anaemia is not a serious problem in young children in SA, except in the 6-23 month old age group (SAVACG, 1995:177), where it is probably related to insufficient weaning practices.

In terms of iron status, 10 % of children in SA is iron depleted or deficient, one in twenty is severely iron depleted or deficient and one in twenty has iron deficiency anaemia. Anaemia and poor iron status are more prevalent in urban areas, and children in the 6-23 month age group are the most adversely affected (SAVACG, 1995:185).

Analysis of the data from a number of smaller studies done from 1976 to 1996 in which haemoglobin, red blood cell folate concentration, serum ferritin, vitamin E, alkaline phosphatase concentrations, calcium and retinol binding protein were measured, indicated the following:

- Iron and folate deficiencies existed in some white primary school children.
- Iron and folate deficiencies existed in many coloured primary school children.
- Iron deficiency is a problem in Indian pre-school children.
- Multiple micronutrient deficiencies existed in rural black pre-school children (vitamin A, iron, folate, vitamin E, vitamin B6).
- Athough rickets is rarely diagnosed in SA, the low serum calcium values in rural black children indicated a suboptimal calcium and vitamin D status (Vorster *et al.*, 1997c:12).

2.2.2.4 Prevalence of micronutrient deficiencies in South African adolescents

Little information and no recent studies on the biochemical profiles of white, coloured and Indian adolescents are available. The low mean concentrations of iron status variables of Indian children could be an indication of a possible iron deficiency in this group. The iron and folate deficiencies seen in younger black children are still present in black adolescents (Vorster *et al.*, 1997c:12).

2.2.2.5 Prevalence of micronutrient deficiencies in South African adults

Mean values of most nutritional status variables were found to be within normal ranges. However, a 23 % prevalence of low iron status was present in a small group of 20-year old white women. Iron deficiency anaemia is present in many adult South Africans (Vorster *et al.*, 1997c:12).

2.3 Vitamin A deficiency

2.3.1 Global patterns

Vitamin A status, on the basis of 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).

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 % (Humphrey, 1998:S2; Malanick, 1999:1).

VAD is a major problem in over 75 countries (CHU, 1998:3). In developing countries 125-190 million children of pre-school age are at risk of VAD, of which 5-10 million will develop xerophthalmia, and every year about 500 000 of these children lose their sight, the majority (70 %) within one year of developing VAD. These conditions can lead to 1-2,5 million child deaths annually (CHU, 1998:3; Humphrey, 1998:S2; Task Force Sight and Life, undated:1; West, 1998:9).

VAD is common among women in developing countries. Mean serum retinol concentrations of 1,05 µmol/L (300 µg/L) have been reported among diverse groups of pregnant south Asian women in comparison with values of 1,57 to 1,75 µmol/L (450-500 µg/L) in better nourished populations. Little attention has been paid to the effect of VAD on the health consequences for the women. The fact that VAD can predispose women to increased infectious morbidity and mortality is supported by evidence in children and animal models. Impaired barrier defences of epithelial tissues and compromised innate and acquired immunity could be some of the underlying mechanisms of VAD consequences in women (West *et al.*, 1999:570).

VAD as a public health problem occurs within an ambience of ecological, economical and social deprivations in the macro-environment of populations, as well as in the micro-environment of families. The relative influence of causal factors, illustrated in Figure 2.2, will vary among countries (WHO, 1995:5).

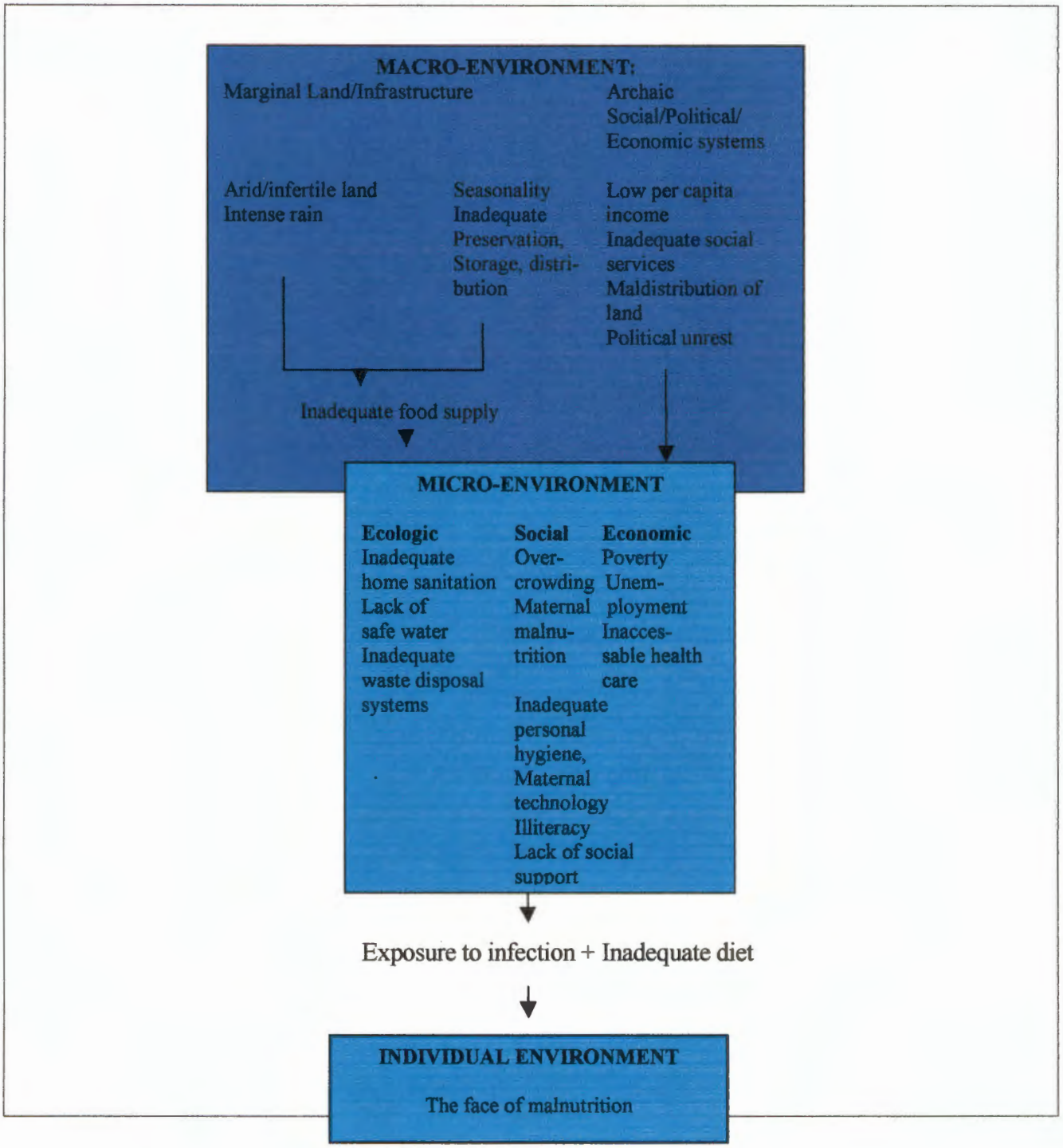

Figure 2.2 Illustration of the environmental causes of VAD (adapted from WHO, 1995:7)

Figure 2.3 is a summary of the prevalence of low serum retinol levels of the countries in which nationally representative studies have been undertaken since 1992 and Figure 2.4 shows the extent of the world-wide VAD problem.

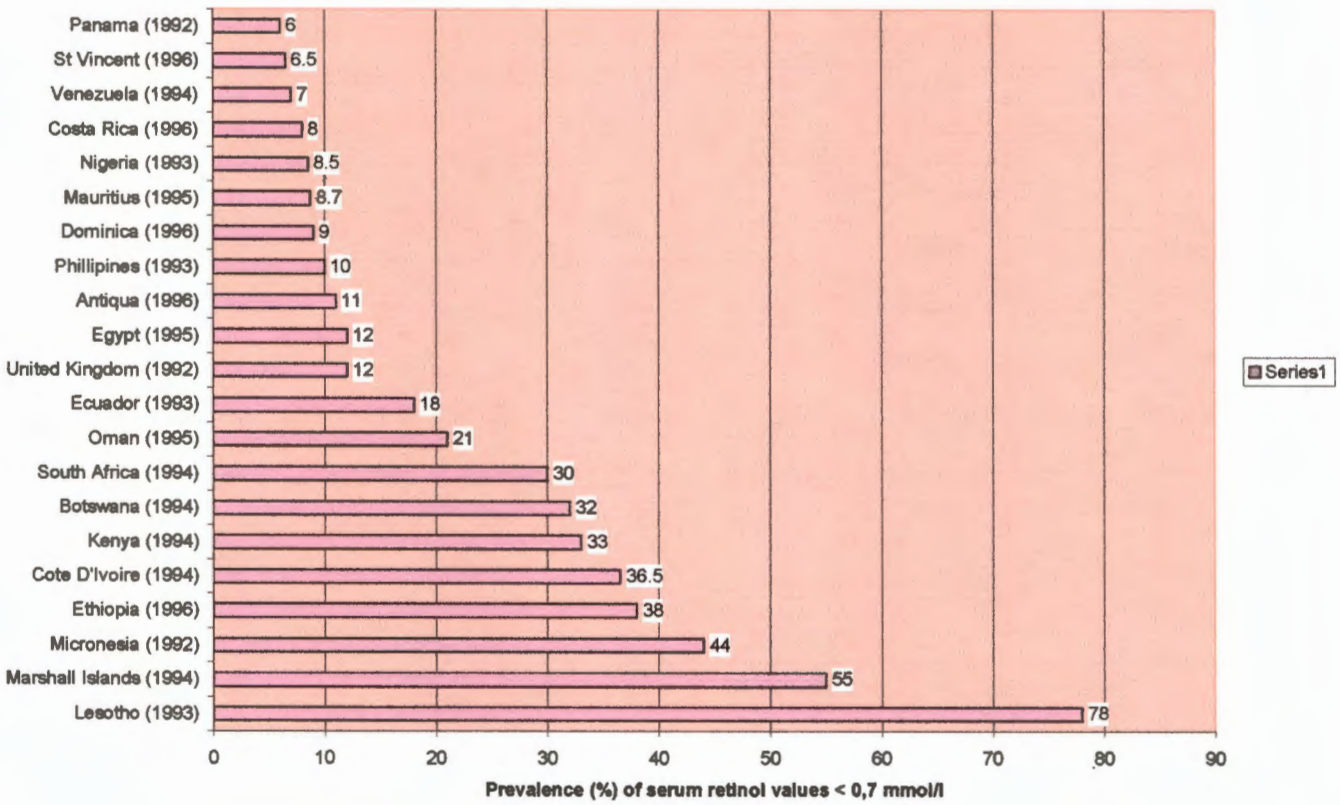

Figure 2.3 Prevalence of low serum retinol levels in some of the countries in which nationally representative surveys have been undertaken since 1992 (adapted from Alnwick, 1998: 141)

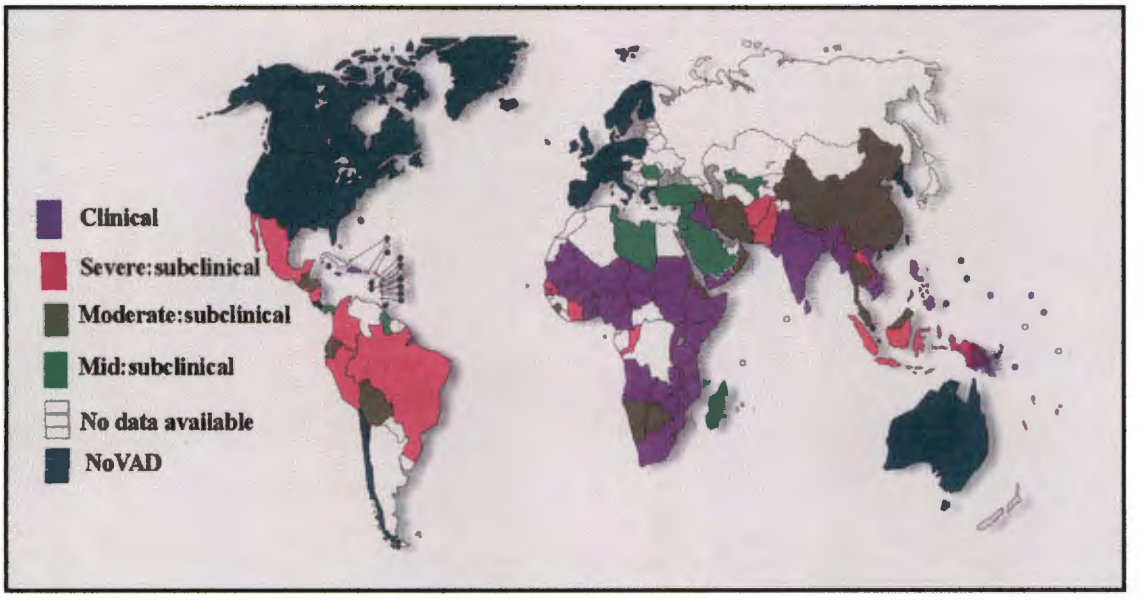

Figure 2.4 The extent of the world-wide VAD problem (WHO, 1998:2)

In India VAD is a significant public health problem. Surveys carried out in recent years indicated that the dietary vitamin A intakes of pre-school children and women were less than 40 % and 50 % of the recommended dietary allowances respectively (Nayak *et al.*, 1999:1).

In Nepal 27 % of postpartum women are vitamin A deficient, based on serum retinol levels of < 20 µg/dL. This is thus a significant public health problem. The diet in Nepal, especially in the southern region, is low in both animal and vegetable sources of vitamin A, and VAD is further exacerbated by cultural food taboos during pregnancy and lactation (Hollander *et al.*, 1999:1).

It was reported in 1999 that in Yemen micronutrient deficiencies, particularly iron, iodine and vitamin A are endemic. By regional standards, infant (8,3 %) and under five year old (11 %) mortality rates are still very high in Yemen (Zein & Al-Haithamy, 1999:1).

Recent evidence indicated that although the prevalence of clinical signs of VAD is decreasing world-wide, the prevalence of VAD in many so-called Third World countries is still high. Children are therefore at an increased risk of infections and mortality. Surveys in Africa indicated that SA was one of the African countries showing a serious public health problem due to VAD (Blaauw, 1999:30; SAVACG, 1995:142; Task Force Sight and Life, undated:1). In Africa, VAD is reported to affect 1,3 million children under five years of age (VAGI, 1997:3).

2.3.2 The South African situation

In Africa it is estimated that 31 million people suffer from VAD compared to 206 million suffering from iron deficiency (Verschuren, 1997:14). In 1994 the SAVACG study determined that one in three SA children under the age of six years had a marginal vitamin A status (serum vitamin A concentration below 20 µg/dL). The most disadvantaged children were those living in the rural areas and whose mothers were poorly educated. According to international criteria, the national prevalence of 33 % of marginal vitamin A status found, identified the country as having a serious public health problem due to VAD. Only 1 % of children had a serum vitamin A concentration higher than 50 µg/dL (Labadarios, 1999:5; CHU, 1998:3; SAVACG, 1995:4).

The prevalence of VAD in the provinces of SA mostly affected is as follows:

* 18 % in Northern Cape
* 31 % in Eastern Cape
* 32 % in North West
* 33 % in Mpumalanga
* 38 % in Kwazulu Natal
* 43 % Northern Province

(Labadarios, 1999:5; CHU, 1998:4; SAVACG, 1995:132).

Figure 2.5 shows the prevalence of VAD in each of the provinces in SA during 1994.

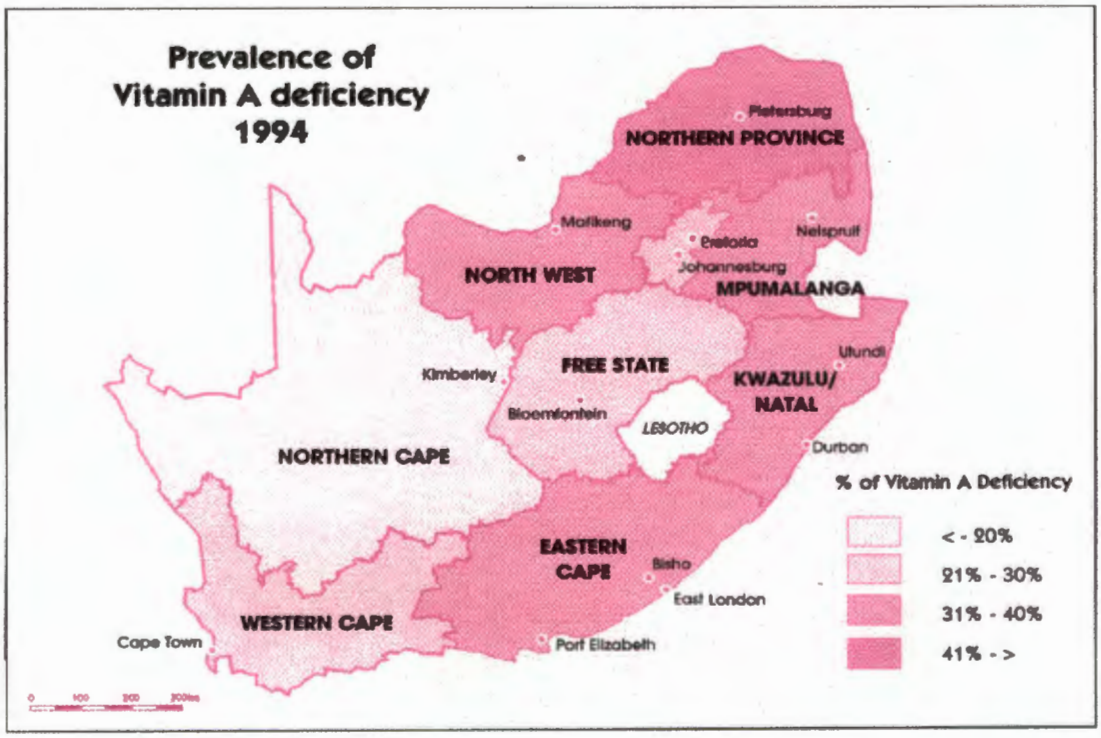

Figure 2.5 Prevalence of vitamin A deficiency in South Africa (DO H, 1998:4)

Other results from the SAVACG study are the following:

- Night blindness was reported to be affecting 12 % of children nationally, Bitot's spots were seen in 0,4-0,8 %, corneal xerosis in 0,2-0,7 % and keratomalacia or corneal scar in 0,1 % of children (SAVACG, 1995:130).
- The prevalence of xerophthalmia is low countrywide (< 0,06 %) and is mostly occurring in areas of the Northern Province, where vitamin supplementation has already been implemented to reduce the prevalence of VAD (SAVACG, 1995:39).
- No significant differences between men and women in mean serum vitamin A concentrations (SAVACG, 1995:132).
- Biochemical VAD was present in 3 % of the total population of children under six years and ranged from 1 % in Gauteng to 5 % in the Northern Province. The prevalence of biochemical VAD was double in the rural (4 %) compared to the urban (2 %) areas (SAVACG, 1995: 132).

Table 2.1 Mean vitamin A intakes of South Africans in RE *, comparison between the 24-hour recall and other methods with RDA# (Vorster *et al.*, 1997c:109)

Age group gender (years)	White		Urban Black		Coloured		Indian		Rural Black		RDA#
	24hr	other	24hr	other	24hr	other	24hr	other	24hr	other	
Children, 0-1,9		842		356		1003		534			375-400
Children, 2-5,9		1224	448,8	877	770	697		1243	694	641	400-500
Children, 6-10,9			567		604				607		700
Boys, 11-15,9									621		1000
Girls, 11-15,9									553		800
Men, 16-24,9	1172		373				661	1671			1000
Women, 16-24,9	763	1068	452				286	1498			800
Men, 25-64,9	2522	3427	640	2063			478			946	100
Women, 25-64,9	1048	3311	563	1267			782			1905	800

* RE = retinol equivalents; 1 RE = 1μg retinol = 6 μg beta-carotene
# Recommended dietary allowances of the USA (Food and Nutrition Board, 1989)
• Missing values : no data available

Table 2.2 is a meta-analysis of the mean vitamin A intakes of South Africans in RE. The mean vitamin A intakes measured by the 24-hour recall method was compared with those measured by other dietary intake methods (Vorster *et al.*, 1997c:109).

Table 2.2 Vitamin A intake in RE (data not included in Table 2.1) (Vorster *et al.*, 1997c:109)

Province	Date	Age	N	Mean intake
0-6 years old				
Free State (rural)	1996	0,5 - < 1	14	427,5
	1996	0,5 - < 1	12	403,6
	1996	1 - < 3	64	753,9
	1996	1 - < 3	59	750,4
	1996	3 - < 6	81	938,3
	1996	3 - < 6	93	1005
6-12 years old				
Northern Cape (rural)	1987	7 – 10	326	208
	1994	6 – 14	96	570
Adolescents				
Northern Cape (rural)	1987	11 – 14	114	140

All the subjects in the sample populations, shown in Table 2.2, in the Free State and Northern Cape were randomly selected and were rural black boys and girls. The methods used for measuring vitamin A intake were diet history and quantified food frequency questionnaire (QFFQ) in the Free State and 24-hour recall in the Northern Cape.

The mean intakes of some groups seem to reach the RDA. This does not support the high prevalence of VAD in SA. However, it should be kept in mind that in specific groups there might be subjects with high intakes and thus others with intakes below the RDA. Also, it is known that parasitic infections in SA children occur in many regions of SA, which may contribute to the lower observed vitamin A status.

Table 2.3 summarises the results on vitamin A intake during the 1999 National Food Consumption Survey (NFCS) done on children aged one to nine years old. These results showed that only children between one and six years old, living in urban areas and the Western Cape had sufficient vitamin A intakes according to the RDA's. At a national level 55-68 % of all children had a vitamin A intake of half the RDA, and 33,3 % of all children in all provinces, except the Western Cape, had a vitamin A intake of less than two-thirds of the RDA (Labadarios *et al.*, 2000:231,332).

Table 2.3 Mean vitamin A intake (RE/day) of SA children aged 1-9 years by province and area of residence (Labadarios *et al.*, 2000:451)

Province	Children aged 1-3 years		Children aged 4-6 years		Children 7-9 years	
	Mean intake ± SD	RDA	Mean intake ± SD	RDA	Mean intake ± SD	RDA
Eastern Cape	496 ± 535	400	459 ±481	500	554 ± 608	700
Free State	384 ± 445	400	494 ± 546	500	392 ± 421	700
Gauteng	660 ± 652	400	750 ±727	500	750 ±875	700
Kwazulu Natal	392 ± 652	400	420 ±764	500	500 ± 547	700
Mpumalanga	458 ± 820	400	509 ± 657	500	573 ± 742	700
Northern Cape	332 ± 290	400	338 ± 372	500	416 ± 329	700
Northern Province	531 ± 790	400	530 ± 579	500	665±1022	700
North West Province	428 ± 388	400	564 ± 485	500	451 ± 351	700
Western Cape	907 ± 860	400	840 ± 525	500	880 ± 573	700
Total SA	527 ± 664	400	549 ± 634	500	592 ± 680	700
Urban	671 ± 763	400	627 ± 572	500	680 ± 685	700
Rural	390 ± 503	400	480 ± 663	500	517 ± 671	700

2.3.3 Symptoms of vitamin A deficiency

* Ocular signs

One of the earliest signs of VAD is night blindness (XN) that occurs due to the body's decreased ability to generate rhodopsin, which is essential for vision in dim light. If untreated this may progress to conjunctival xerosis (dry eye), Bitot's spots to corneal xerosis and finally softening and melting of the cornea (CHU, 1998:14; Task Force Sight and Life; undated:2; WHO, 1995:1).

In its most severe form, VAD causes partial or total blindness, a condition called xerophthalmia, which is the main cause of blindness amongst young children (CHU, 1998: 14; Humphrey, 1998:S2; WHO, 1995:1). The criteria for xerophthalmia as a public health problem were developed to assist in the identification of a VAD problem of public health magnitude. The criteria apply to the vulnerable group, usually children up to six years old (Task Force Sight and Life, undated:2).

Table 2.4 WHO criteria of a public health problem of xerophthalmia (WHO, 1995:9)

WHO criteria of a public health problem of xerophtalmia		
Night blindness	(XN)	In > 1 %
Bitot's spots	(X1B)	In > 0,5 %
Corneal cirrhosis/ulceration/keratomalacia	(X2, C3A, X3B)	In > 0,01 %
Corneal scar	(XS)	In > 0,05 %
Plasma retinol of < 0,35 µmol/L (10 µg/dL)		In > 0,5 %

* Growth faltering

VAD is associated with reduced appetite, weight loss and failure to thrive. Malnourished children have a lower resistance to infection, and are more likely to become ill than well-nourished children. During serious infections such as measles and diarrhoea, a further weight loss is experienced (CHU, 1998:13).

* Increased number and severity of infections

Children with VAD are more prone to infections, especially gastrointestinal and respiratory infections. The severity of infections, particularly measles, is also greater amongst children with VAD (CHU, 1998:13).

2.4 Iron deficiency

2.4.1 Global patterns

Iron deficiency anaemia (IDA) is a major pubic health problem worldwide, with a total prevalence estimated at about 40 % of the world's population, that is over two billion people (Bruner, 1999:1; Davidsson & Stoltzfus, 2000:14; Gillespie, 1998:9; Lake, 1999:7; Venkatesh Mannar, 1999:23; West, 1996:789) with the highest prevalence in South Asia and Sub-Saharan Africa (Barasi, 1997:186; Ndossi *et al.*, 1999:26). If the assumption is true that for each case of anaemia there are 2,5 cases of iron deficiency, 5 billion people, or 80 % of the total world population, suffer from iron deficiency. These figures should, however, be treated cautiously as this is an estimate based on data collected in US national surveys and may not be true for other parts of the world (Davidsson & Stoltzfus, 2000:14).

The following scenarios increase risk of iron deficiency:

* Inadequate intake due to low income, poor food choice or vegetarian diets;
* low absorption rates due to interference from other dietary components, low stomach acidity or parasites;
* increased needs or losses due to growth, pregnancy, heavy menstrual losses or bleeding from other causes

(Barasi, 1997:186; Bruner, 1999:3; Gillespie, 1998:9; Graciano, 1999:2; SAVACG, 1995:183; Schumann *et al.*, 1998:129).

These factors tend to occur in infants (mainly between 9 and 18 months), children, teenagers and women in their reproductive years. These are the main vulnerable groups (Barasi, 1997:187; Bruner, 1999:1; SAVACG, 1995:160; Venkatesh Mannar, 1999:23). In developing countries, approximately 50 % of all children less than four years of age are anaemic, and the prevalence rates are very high for children less than 2-3 years old (Davidsson & Stoltzfus, 2000:13). The risk groups are mostly found in developing countries, where a combination of diets low in iron and poor living and/or working conditions encourage the prevalence of IDA (Graciano, 1999:1).

Prevalence of global iron-deficiency among various subgroups are estimated as follows:

- 48 % for pregnant women
- 26 % for school-children aged between 5 and 14 years old
- 48 % for infants and 1-2 year old children
- 35 % for non-pregnant women
- 39 % for pre-school children

(Davidsson & Stoltzfus, 2000:14; Gillespie, 1998:9).
The prevalence in the various subgroups may be three or four times higher in developing countries than in developed countries (Davidsson & Stoltzfus, 2000:14; Gillespie, 1998:9).

2.4.2 The South African situation

In eastern and southern Africa the iron deficiency prevalence ranges from 40-49 % in most countries, except for South Africa, Botswana, Namibia, Lesotho and Swaziland, which have prevalence of less than 40 %. Women of reproductive age, infants and children are most affected by anaemia (Ndossi *et al.*, 1999:26).

Data on iron status of SA children are scarce, but the data from the national survey by SAVACG has shown the prevalence of anaemia to be 21 % in children under six years of age. The prevalence of moderate (7 %) and severe (0,2 %) anaemia is much lower. Iron depletion or deficiency was present in 10 %, and IDA in 5 % of children. These findings indicate that IDA is not a serious problem in SA, except in the 6-23 month old age group (Labadarios, 1999:5; SAVACG, 1995:177).

In terms of iron status, 10 % of children in SA is iron depleted or deficient, 5 % is severely iron depleted or deficient and 5 % has IDA. Anaemia and poor iron status are more prevalent in urban areas and, as mentioned, children in the 6-23 month age group are the most adversely affected (SAVACG, 1995:185). The different iron status parameters for determining iron status and prevalence according to these parameters in SA children are shown in Table 2.5.

Table 2.5 Iron status in children 0,5-6 years of age (Labadarios, 1999:5)

Parameter	Prevalence (%)
Haemoglobin (Hb) (< 11g/dL)	21
Ferritin (F) (< 12 µg/l)	10
Mean corpuscular volume (MCV) (< 73 fl)	17
HB < 11; F > 12	16
Hb > 11; F < 12	5
Hb < 11; F < 12	5
Hb < 11;F < 12; MCV low	3

A number of studies in SA have measured iron intake of the different ethnic groups (reviewed by Vorster *et al.*, 1997c:92). Mean intakes are shown in Tables 2.6 to 2.8. These tables illustrate that low intakes in vulnerable groups may be a major cause of iron deficiency.

Table 2.6 Mean iron intakes (mg) of South Africans measured with the 24-hour recall method compared to RDA* (Vorster *et al.*, 1997c:92)

Age group gender (years)	White	Urban Black	Coloured	Indian	Rural Black	RDA*
Children, 2-5,9	8	5,3	8,8		11	10
Children, 6-10,9	9,3	8	12,9		13	10
Boys, 11-15,9	13,5	11	10		12,5	12
Girls, 11-15,9	9,5	10	8		10,6	15
Men, 16-24,9	16,1	9	12	12		10
Women, 16-24,9	9,5	7	10	9		15
Men, 25-64,9	14,5	10,5	11,4	10,1		10
Women, 25-64,9	9	7,5	7,4	7,5	20	15

* Recommended dietary allowances of the USA (Food and Nutrition Board, 1989)

Table 2.7 Mean iron intakes (mg) of South Africans measured with other methods (Vorster *et al.*, 1997c: 92)

Age group gender (years)	White	Urban Black	Coloured	Indian	Rural Black	RDA*
Children, 0-1,9	12	5,1	16,8	7,8		6-10
Children, 2-5,9	9	8	9	10	6	10
Children, 6-10,9						10
Boys, 11-15,9						12
Girls, 11-15,9						15
Men, 16-24,9	13,9			17,3		10
Women, 16-24,9	12			13		15
Men, 25-64,9	16,5	18,4	15,4		16,1	10
Women, 25-64,9	13,1	11,7	13,4		18,5	15

* Recommended dietary allowances of the USA (Food and Nutrition Board, 1989)

Table 2.8 Iron intake in mg (data not included in Tables 2.6 and 2.7) (Vorster *et al.*, 1997c:92)

Province	Date	Race	Age	N	Mean intake
0-6 years old					
Free State (rural)	1996	Black	0,5 - < 1	14	2,6
	1996	Black	0,5 - < 1	12	5,8
	1996	Black	1 - < 3	64	4,3
	1996	Black	1 - < 3	59	4,2
	1996	Black	3 - < 6	81	5,8
	1996	Black	3 - < 6	93	5,4
6-12 years old					
Northern Cape (rural)	1987	Black	7 – 10	326	7
Northern Province (rural)	1994	Black	6 – 14	96	13
Western Cape (rural)	1985	Black	11	104	9,0
Western Cape (urban)	1985	Black	11	50	10,5
Western Cape (rural)	1985	Coloured	11	156	8,
Western Cape (urban)	1985	Coloured	11	83	9,4
Western Cape (rural)	1985	White	11	145	10,9
Western Cape (urban)	1985	White	11	72	12,1
Adolescents					
Northern Cape (rural)	1987	Black	11 - 14	114	6

All the subjects in the sample populations in the Free State and Northern Cape were randomly selected and included boys and girls. The method used for measuring iron intake was diet history and QFFQ in the Free State and 24-hour recall in the Northern- and Western Cape and Northern Province.

The results of the 1999 NFCS (Table 2.9) showed that the mean iron intake of children between one and nine years was consistently low in the majority of provinces in SA. At a national level, 25-37 % of children had an iron intake of less than 50 % of RDA's with the lowest intakes reported in children living in the Free State, Eastern Cape and Northern Cape. The prevalence of children consuming less than 67 % of the RDA's ranged between 36 and 57 % nationally and 41-63 % had an iron intake of less than 50 % of RDA's. No significant gender differences were noted, but children of all the age groups living in urban areas had a significantly higher intake of iron than in the rural areas (Labadarios *et al.*, 2000:238,339).

Table 2.9 Mean iron intake (mg/day) of SA children aged 1-9 years by province and area of residence (Labadarios *et al.*, 2000:463)

Province	Children aged 1-3 years	Children aged 4-6 years	Children 7-9 years	RDA (mg/day)
	Mean intake ±SD	Mean intake ±SD	Mean intake ±SD	
Eastern Cape	7,2 ± 3,8	8,2 ± 3,6	9,3 ± 4,7	10
Free State	4,7 ± 2,9	5,1 ± 2,9	5,6 ± 4,9	10
Gauteng	8,0 ± 5,1	9,3 ± 4,6	10,2 ± 4,3	10
Kwazulu Natal	6,3 ± 3,4	7,7 ± 3,4	9,2 ± 4,4	10
Mpumalanga	5,7 ± 4,2	9,9 ± 5,7	9,2 ± 4,4	10
Northern Cape	4,3 ± 2,8	5,7 ± 3,9	5,8 ± 2,9	10
Northern Province	8,4 ± 6,3	9,1 ± 5,6	9,8 ± 7,5	10
North West Province	6,3 ± 3,5	6,7 ± 3,3	6,7 ± 3,0	10
Western Cape	8,3 ± 3,5	11,3 ± 4,4	12,3 ± 5,0	10
Total SA	6,9 ± 4,4	8,3 ± 4,5	9,2 ± 5,2	10
Urban	7,3 ± 4,2	8,7 ± 4,2	9,8 ± 4,8	10
Rural	6,5 ± 4,5	8,0 ± 4,7	8,8 ± 5,5	10

A cause of iron deficiency in SA is intestinal helminth infestation, affecting more than a quarter of the world's population at any one time (Gillespie & Johnston, 1998: 11; MI, 1998:55; Stoltzfus & Klemm, 1997). Helminth species, however, have different ways in which they obtain nutrition from the human host. Some are known to cause iron deficiency, others suppress appetite causing energy loss, and others cause inflammatory responses or pain. Research has shown that multiple infections are the rule rather than the exception in areas with poor sanitation. In regions of exceptional poverty in SA multiple parasitic infections often exist alongside nutritional deficits, as well as an increased stress-related violence against children, and poor education facilities (Kvalsig, 2000:94).

Hookworms and schistosomes are intestinal helminths contributing to anaemia. Hookworm infestations affect approximately 20 % of the world's population and have the most significant effect. Whipworm (*Trichuris trichiura*) infestation, especially the *Trichuris* dysentery syndrome caused by it, is associated with severe anaemia and may affect 4 % of some populations. Hookworms cause intestinal blood loss by feeding on blood in the intestinal mucosa and can thus significantly limit the effect of dietary interventions. The amount of blood lost is directly proportional to the number of worms infecting the host. A moderate infection of hookworm approximately doubles the iron losses of a child or menstruating woman. The prevalence and intensity of hookworm infection increases with age, and its effect is the greatest on the iron-status of school-going children, adolescents and adults (Gillespie & Johnston, 1998: 11; MI, 1998:55; Stoltzfus & Klemm, 1997). Schistosomiasis can also adversely affect iron status through gastric or intestinal ulceration and subsequent blood loss (Gillespie & Johnston, 1998: 11; MI, 1998:5).

Table 2.10 is a summary of the attributable fractions (%) of anaemia and severe anaemia with hookworm infection found in studies done in Zanzibar, Vietnam, Venezuela and Nepal (reviewed by Gillespie & Johnston, 1998:14). In a study done amongst primary school children (n = 12 000) in

Cape Town, SA, the prevalence of whipworm and roundworm (*Ascaris lumbricoides*) was estimated as 80,3 % and 69,7 % respectively (Haffejee et al., 2000:95).

Table 2.10 Attributable fractions of anaemia and severe anaemia with hookworm infection (adapted from Gillespie & Johnston, 1998:14)

Population (n)	Hookworm prevalence (%)	Anaemia prevalence (%)	Severe anaemia prevalence %)
Zanzibar:			
• Children: 4-29 months (263)	27	-	-
• Children: 30-71 months (327)	69	13	49
• School going children (582)	94	25	73
• Non-pregnant women (582)	91	33	65
• Men (495)	95	40	100
Vietnam:			
Non-pregnant women (5029)	37	15	
Venezuela:			
Children: 2-6 years (244)	55	17	
Children: 7-14 years (538)	78	25	
Women (222)	75	40	
Men (138)	84	20	
Nepal:			
Pregnant women (155)	77	4	46

2.5 Consequences of micronutrient deficiencies

2.5.1 Health consequences associated with micronutrient deficiency

In children three micronutrient deficiencies, namely vitamin A, iron and iodine, are considered to be a major health problem in developing countries. Globally these are presently receiving high priority. Communities that are affected 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 and psychomotor development and function and diminished working capacity in children and adults, as well as increased susceptibility and severity of infections, and mortality are the collective result of these micronutrient deficiencies (Davidsson & Stoltzfus, 2000:13; SAVACG, 1995:39; USAID, 1993:2; West, 1996: 789).

At the end of the 1970's less than 10 % of the children in the world were being immunised. Thus the nutritional status of the majority of children was further compromised by infectious diseases with an adverse effect on growth and development as a result of decreasing appetite, increasing energy requirements, inhibiting nutrient absorption and increasing nutrient losses through diarrhoea and vomiting. Most of these diseases can be prevented and a comprehensive immunisation programme would, therefore, have a positive impact on growth and development (SAVACG, 1995:40).

VAD occurs when the body stores are depleted to the extent that physiological functions are impaired even though clinical eye signs may not be present. The level of depletion at which physiological functions begin to be impaired is not entirely clear (WHO, 1995:1).

With increasing degrees of VAD there is a progression of subclinical changes, denoted first by the reduction of vitamin A stores in the liver. This is followed by a fall in serum retinol and changes in the structure of epithelial cells (metaplasia). The immune system is also compromised at this stage. The visual system is the last to be compromised and, with increasing deficiency, the signs become clinical. This leads to increased severity of some infections and risk of death (Task Force Sight and Life, undated; WHO, 1995:1). There is conclusive evidence that by improving the vitamin A status of young children with VAD the mortality can be reduced by 23 % (Alnwick, 1998:141).

The consequences of iron deficiency anaemia include congestive heart failure, increased susceptibility to infections, poor physical growth, increased fatigability, reduced work and mental performance, retardation of psychomotor development and reduced learning capacity (Graciano, 1999:3; SAVACG, 1995:177). Anaemia and iron deficiency can lead to impaired school achievement in school-children, and if left uncorrected, can lead to a huge drain on national development in the long term (Davidsson & Stoltzfus, 2000:13).

Severe anaemia during pregnancy can result in miscarriage and premature deliveries. IDA also increases susceptibility to lead intoxication. During pregnancy and breastfeeding in particular, the interaction between IDA and lead intoxication may have a negative effect on the health of both the mother and child (Graciano, 1999:3).

2.5.2 Economic costs of micronutrient deficiency

It is estimated that each 1 % decrease in Hb results in a 1,5 % decrease in work capacity and a 1-2 % decrease in work output can be expected. An enormous impact by anaemia on a national level can be estimated if the average productivity reduction is 20 % for an anaemic individual and if 50 % of all women and 20 % of all men are affected. The national economic output would then be reduced by 5-7 % (Davidsson & Stoltzfus, 2000:16).

The World Bank calculated that the economic losses attributed to malnutrition range from 6-12 % of the gross national product (GNP) of developing countries. If a figure of 6 % loss is applicable to SA, the costs of undernutrition alone could amount to at least four billion Rand, and may be higher if the cost of the undernourished chronically and acutely ill patients is taken into account (SAVACG, 1995:37).

In the development context, malnutrition is defined as a stagnant growth in almost all sectors of society. Individuals cannot fully benefit from educational and technological investments and are less able to compete effectively in the world economy (USAID, 1993: 2). Malnutrition and its treatment have invariably been seen as a costly drain on the national economy and more specifically on the health budget. In economic terms the prevention of malnutrition should be considered as a means of saving funds for national development (SAVACG, 1995:37). The economic consequences for individuals may be substantial as a one standard deviation (SD) rise in cognitive achievement can be estimated to be equivalent to a 7-12 % increase in earnings according to the World Bank (Davidsson & Stoltzfus, 2000: 16).

The prevention of malnutrition and its negative effects on the quality of individual and community life, together with the implications for national productivity and socio-economic development, therefore, is the strongest argument for any government to afford this the highest priority (SAVACG, 1995:37). Social and political development cannot occur without an adequately nourished, healthy and productive population (USAID, 1993:2).

2.6 Prevention and treatment of micronutrient deficiencies

2.6.1 General principles

There are many reasons why nearly 2 billion people fail to consume adequate micronutrients in their diets (USAID, 1993:5).

Vitamin A and iron are found in certain fruits, vegetables, and animal products that may be too expensive or seasonal. The food preparation method may further reduce their intake. Cultural taboos often exclude micronutrient-rich foods and may also contribute to the deficiencies. Iodine is usually found naturally in soil and seawater. In some parts of the world, however, soils have been depleted of iodine by glacial erosion or repeated floods. In these regions, external dietary sources are essential to provide adequate iodine intake (USAID, 1993:5).

A new concept is emerging in terms of micronutrient deficiencies. It is becoming increasingly clear that micronutrients may have unknown functions, such as in hormone or gene regulation, and that the requirements for maintaining optimal health may vary from those preventing conventional deficiency states. From a disease point of view micronutrient supplementation has led to significant health benefits. In China, the Linxian studies were the first to prove that micronutrient supplementation in an adult population with marginal micronutrient status, decreased total mortality as well as cancer incidence and mortality. The Nurses' Health Study (Liu *et al.*, 1999:412) and the Health Professionals' Follow-up Study in 1995, provided additional evidence that micronutrient supplementation of healthy adults with especially vitamin E, is associated with a significantly reduced risk of coronary heart disease (Rimm *et al.*, 1993:1454; Stampfer *et al.*, 1993:1447). Oakley (1993:1293) proved that major benefits are derived from folic acid supplementation in healthy adult populations in preventing neural tube defects in high-risk pregnancies. The association of folic acid status and CHD was a major motive for folic acid fortification of bread flour in the USA (Koehler *et al.*, 1997:170; Pfeiffer *et al.*, 1997:1395).

With the growing understanding of the extent and impact of micronutrient nutrition, a number of interventions have already demonstrated the feasibility and the benefits of correction and prevention. The impact of correcting micronutrient deficiencies has been reviewed by the Micronutrient Initiative (MI) (MI, 1997:6) to be the following:

- Preventing up to 40 % of child deaths;
- lowering the maternal deaths by 33 %;
- a 40 % increase in work capacity;
- improving population intelligence coefficient (IQ) by 10 to 15 points;
- raising the gross domestic product (GDP) by up to 5 %.

The MI was established in 1992. Their mission is to facilitate the achievement of the following goals related to the elimination of micronutrient malnutrition by supporting effective and sustainable programmes globally:

* virtual elimination of iodine deficiency disorders;
* virtual elimination of VAD and its consequences, including blindness;
* reduction of iron deficiency anaemia in women by one-third of the 1990 levels.

The MI recognises that solutions to overcome micronutrient deficiencies need to go well beyond health and nutrition systems. A combination of interventions must be emphasised and implemented, such as: promoting breastfeeding, diet modifications by improved food availability and increased food consumption, food fortification and supplementation (MI, 1997:5).

At a national level the constraints to making more vitamins and minerals available to the population can be largely addressed by implementing programmes designed to educate people in diversifying their diets. Commonly eaten foods can be fortified with the missing micronutrients or nutrient supplements can be provided through targeted distribution programmes (USAID, 1993:7). Supplementation, food fortification and diversifying to include more micronutrients in the diet can make an enormous difference. Programmes achieving results have been piloted and positive results evaluated. It is clear that micronutrient deficiencies are as preventable as it is devastating (MI, 1997:6).

The moderate to high prevalence of multiple micronutrient (vitamin A, iron and folate) deficiencies, coupled with low energy intakes in many black children suggests that single nutrient fortification or supplementation programmes would not be successful enough in addressing the problem. Increasing energy and micronutrient density of the total diet will be a more successful approach, especially in areas with inadequate sanitation and safe water (Vorster *et al.*, 1997c:11).

2.6.2 Diversifying the diet

Dietary modification is primarily a strategy to improve either the amount of food in the diet or its bioavailability (Venkatesh Mannar, 1999:24). Producing or purchasing a greater variety of affordable foods than those usually consumed is considered to be the safest and most sustainable long-term measure in the control of most micronutrient deficiencies (USAID, 1993:8). In a study done on the lactating mother-infant pair in Honduras, infant serum retinol and β-carotene and the mother's β-carotene levels were significantly increased by a higher intake of lical fruits and vegetables combined with β-carotene supplementation (Canfield *et al.*, 1999:43).

2.6.3 Micronutrient supplementation

Supplements for the prevention of deficiencies are usually given on a time-limited basis during immunisation days and family planning contacts. Supplementation is rapidly becoming part of treatment plans for measles and pneumonia and is cost-effective for reaching isolated areas with a high prevalence of deficiencies. Vitamin A, iron and iodine are available in concentrated or synthetic form at a relatively low cost and can be administered either orally or by injection (USAID, 1993:8).

Vitamin A supplementation is a low-cost, reliable and effective way to combat VAD and can be rapidly implemented as a programme strategy on a national scale. It should not necessarily be regarded as a

short-term measure, as most of the supplementation programmes implemented globally have a minimum of 5-10 year life. In many developing countries supplementation programmes have been running for decades (VAGI, 1997: 3).

Vitamin A supplementation programmes have already been proved effective in reducing mortality and morbidity of children and all children should initially be reached at the age of 6 months (VAGI, 1997:3). Supplementation can be integrated with existing programmes, for example with both routine and campaign-based immunisation programmes (VAGI, 1997:6).

In developing countries, a priority intervention is iron supplement distribution for women of reproductive age (USAID, 1993:23). The MI in partnership with UNICEF, the World Bank, International Life Sciences Institute (ILSI) and USAID is at present developing a communication strategy to promote effective interventions for iron (MI, 1999:1). Although many iron supplementation trials have been proven to be efficacious in carefully controlled circumstances, few have proven to be effective when broadly applied. Low compliance and lack of adequate supply systems have been suggested as reasons for this (Davidsson & Stoltzfus, 2000:13).

2.6.4 Fortifying food commodities or products

2.6.4.1 History

The food fortification concept was developed during the early part of the 20th century as a means of addressing micronutrient deficiencies that were prevalent in Europe and North America at the time. More than 80 years of experience in various developed and developing countries indicated that fortification of commonly consumed foods offers an opportunity to reduce, or even eliminate, the prevalence of these deficiencies safely and cost-effectively (Labadarios *et al.*, 2000:883).

In recent years, attention has increasingly turned to finding ways to fortify staple foods consumed by populations who are not only very poor, but also most vulnerable to micronutrient deficiency disorders. People in this group are usually found in rural areas and often consume mainly the foods that they grow (MI, 1999b:1). Since the early 20th century food fortification has been applied to reduce and eliminate micronutrient deficiencies in many countries as can be seen in Table 2.11. Today, food fortification is favoured more than ever (Sloan, 1995:24).

Food fortification cannot reach all populations deficient in essential micronutrients due to limited access to commercially or centrally processed foods. This can be a result of geography, poverty or cultural preference, public health and welfare approaches to deliver supplements or dietary education. For the large and expanding population that does, however, regularly purchase and consume commercially processed foods, fortification can make a difference (MI, 1997:7) and at the population level, food fortification is the best option when a suitable food vehicle can be identified (Davidsson & Stoltzfus, 2000:13).

Table 2.11 History of food fortification (MI, 1997:7; Murphy, 1996:69)

Country	Food fortification programme	Date
Switzerland	Salt iodisation	Early 1990's
Denmark	Vitamin A fortified margarine	1918
Canada	Milk fortified with vitamin A Flour fortified with iron	1945
Guatemala	Vitamin A fortified sugar	1970
Guatemala	Protein fortification of corn flour	1972-1976
USA	Vitamin A & D to fluid milk	1984
Philippines	Exploring of Vitamin A fortified monosodium glutamate (MSG) – not implemented	1985
Bangladesh	Vitamin A fortified wheat	1986
Argentina	Iron fortification of milk	1988
Pakistan	Iodine fortification of salt	1989
Venezuela	Iron fortification of pre-cooked corn flour	1991
Philippines	Vitamin A fortified wheat flour	1993
Andes of Peru	Fortified school breakfast	1993
Thailand	Instant noodles fortified with vitamin A, iron and iodine	1994
Mexico	Chocolate powder drink with iron, vitamin A and iodine	1994
Indonesia	Vitamin A fortified rice	1994-1996
South Africa	Salt fortified with iodine	1995
Ghana, Bangladesh & Guatemala	Double fortification of salt with iron and iodine	1996
Zambia	Maize meal	1998
Russia	Wheat flour with iron and iodine	1999
Japan	Rice with iron, vitamin A and B-vitamins	1999

Vitamin A food fortification is the addition of vitamin A to commonly used foods (CHU, 1998:17). The strategy to fortify selected staple foods with vitamin A holds great promise for more extensive use in the near future and to contribute significantly to the elimination of deficiency (Krause *et al.*, 1998:860; Task Force Sight and Life, undated:1; VAGI, 1997:3). Fortification is an effective and sustainable strategy to combat VAD and increasingly feasible in developing countries (VAGI, 1997:8).

Vitamin A fortification of commonly used foods has been employed in many countries. The success of fortification is dependent on a number of factors including: accessibility and affordability of the chosen food vehicle, and stability of the vitamin in the fortified food (Krause *et al.*, 1998:680; SAVACG, 1995:144).

Fortification of sugar is at present an approach in several countries, and in Central America it has been shown to be effective. Fortification of margarine, oil and products consumed by infants and young children has been the approach in most industrialised countries. It is estimated that 20-59 % of the vitamin A supply in Europe comes from fortified foods (Task Force Sight and Life, undated:1; VAGI, 1997:8).

A study in the Philippines in which 717 children participated, demonstrated that daily consumption of vitamin A fortified margarine for a period of 6 months dramatically reduced the prevalence of low serum retinol levels in children. Furthermore, as a whole, the average retinol levels increased despite high average levels at baseline among the children. The virtual elimination of new cases of xeropthtalmia in the experimental compared to the control children attests to the important contribution of fortified vitamin A foods to improving health among those who are vitamin A deficient (Solon *et al.*, 1996:722). A study from Guatemala during 1990 confirmed that fortified foods, particularly fortified sugar and margarine, made an important contribution to vitamin A intake (Krause *et al.*, 1998:862).

Fortification with beta-carotene is not at present recommended because of the uncertainties regarding its bioavailability, bioconversion to vitamin A and long-term safety (reviewed by SAVACG, 1995:144). A number of developing countries presented case studies at the XIX International Vitamin A Consultative Group (IVACG) meeting held in Durban in 1999 and confirmed that both synthetic retinol and beta-carotene are highly bioavailable in fortified foods. It was also reported that improvement in vitamin A status could be seen within a few months after consumption of fortified foods (Klemm & Ross, 1999: 6).

* Fortification in South Africa

In South Africa, the Directorate Nutrition within the Department of Health (DOH) spearheaded mandatory food fortification by establishing a food fortification task group (FFTG).

Each country has its own government body charged with setting food regulations. The control of micronutrient deficiencies, particularly VAD, iodine deficiency disorders (IDD) and iron deficiency is one of the focus areas of the Integrated Nutrition Programme (INP) of the Department of Health (DOH) in SA. The DOH (SA) aims to control micronutrient deficiencies through a combination of multi-pronged strategies, of which food fortification is a cost-effective medium to long-term intervention (De Hoop, 2000a:1).

Weigley *et al.* (1997:29) described **fortification** as the addition of nutrients at levels higher than those found or never found in the original food. In other words fortification is the process of adding vitamins and/or minerals to food in microgram or milligram quantities to increase its overall nutritional content (Klemm & Ross, 1999:52; Venkatesh Mannar, 1999:24; Lotfi, 1997:15; Giese, 1995:112). Brady (1996:12) described fortification as the addition of one or more nutrients to a food, whether or not it is normally contained in the food, for the purpose of preventing or correcting a demonstrated deficiency of one or more nutrients in the population or specific population groups.

Restoration is a general term for the replacement of nutrients lost during food processing to levels similar to the original levels. For example when "whole wheat" flour is processed to "white" flour, nutrients are unintentionally removed and thus important nutrients would be lost, unless they are

returned. Enrichment is the addition of nutrients to achieve concentrations as specified by the standards of identity. An example is when vitamins and minerals are returned to some processed grain products (Weigley et al., 1997:28-29). In SA a product may be called "enriched" with a specific nutrient when amounts of more than 15 % of the recommended daily allowances (RDA'S) for the specific nutrient are present in the product (SA, 1972:1214).

Since the 1960's enriched products were gradually introduced to the South African market. Examples include bread, breakfast cereals, maize and cold drinks. The first compulsory fortified product introduced to the South African market was salt in 1995, with a fortification level of 67 mg iodine per kg. The first step for collaboration on food fortification with vitamin A and iron commenced in 1996 at a consensus workshop attended by all the stakeholders. The FFTG, comprising of representatives from DOH, Industry, consumer organisations, professional associations and international organisations was established to assist the DOH with a food fortification programme for SA. The FFTG developed a framework with all the activities to be conducted for example situation analyses, feasibility studies and plan development. Subsequently, three food vehicles were identified as possible fortification vehicles in SA. These include sugar, maize meal and wheat flour (De Hoop, 2000a: 1). These three vehicles were identified as a result of the NFCS in 1999. The NFCS showed that the most commonly consumed items by children in SA are: fats and margarine, white sugar, brown- and white bread, whole milk and maize (Labadarios et al., 2000:888). The intakes by children aged 1-3 and 7-9 years old are summarised in Table 2.11.

Table 2.12 Average daily intake of children consuming potential food fortification vehicles based on the 24-hour recall during the NFCS (Labadarios et al., 2000:888)

Food	1-3 years	7-9 years	Not home grown	Maximum available for fortification	
	g/day	g/day	%	g/day	g/day
Fats/margarine	10	16	99,6	10	16
White sugar	19	23	99	19	23
White bread *	41	74	98	40	72,5
Brown bread *	52	86	98	51	84
Whole milk	176	145	93	164	135
Maize *	164	200	96	157	186

*Conversion factors were needed to convert consumption to actual flour or dry maize meal. Conversion figures reported in the NFCS survey are: dry maize to wet porridge at 250 %, white bread to flour at 64 % and brown bread to flour at 68 %.

It is, however, important to note that food fortification is not only for the benefit of the populations with a poor dietary intake, but will benefit the population at large. It is therefore increasingly realised that the dietary intake of micronutrients for the prevention of deficiencies may be entirely different from that necessary for the prevention of chronic degenerative diseases and diseases of lifestyle (Labadarios et al., 2000:886).

Agreement on cost implications, training and capacity-building of Industry, particularly small millers, and development of standards, regulations, the monitoring plan, and quality control and assurance

procedures are the next steps to be implemented in the fortification programme for SA (De Hoop, 2000a: 1).

2.6.4.2 The process of food fortification

Fortification is the process of adding vitamins and/or minerals to food in microgram and milligram quantities to increase its overall nutritional content (Giese, 1995:112; Klemm & Ross, 1999:52; MI, 1997:15; Venkatesh Mannar, 1999:24). Brady (1996:12) defines fortification as "the addition of one or more nutrients to a food, whether or not it is normally contained in the food, for the purpose of preventing or correcting a demonstrated deficiency of one or more nutrients in the population or specific population groups". The micronutrients added to food are diluted into the matrix of the macromolecule that gives food its structure and is thus theoretically invisible (MI, 1997:15). Fortification of food commodities or products already consumed by a large number of people is a growth industry for micronutrient programmes. Food fortification can be targeted to specific age groups or people in specific localities. For a country to succeed in food fortification, it needs food processing capacity and regulatory enforcement, and oversight plans for monitoring the quality and safety of the food (USAID, 1993:8). When imposed on existing food patterns, fortification may not necessitate changes in the customary diet of the population and does not call for individual compliance. It can often be dovetailed into existing food production and distribution systems, and thus implemented to yield results quickly. It can also be sustained over a long period of time (MI, 1997:7; Venkatesh Mannar, 1999:24).

In developing a fortification programme, the selection of an appropriate food vehicle is one of the most common constraints encountered. In order to develop a successful food fortification programme, the following steps must be followed:

- Select a cross-functional team, including food scientists, production engineers, market researchers, marketers, economic or financial analysts, political and consumer groups to be involved from the beginning.
- Determine the prevalence and distribution of the micronutrient deficiency.
- Select potential food vehicles. Alternative food items for fortification should be identified early on and be evaluated simultaneously if technical difficulties are experienced.
- Obtain consumption data for potential vehicles.
- Seek government and food industry support.
- Assess processing industry chain of potential vehicles.
- Choose fortificants and set levels of fortification, based on existing deficiencies and consumption patterns of vulnerable groups.
- Develop fortification technology. Relative humidity must be taken into account during development of any vitamin A fortificant.
- Determine the stability and bioavailability of the fortified food by means of field testing in the country of intended fortification use, and at several locations and humidity levels to confirm adequacy of the fortificant.
- Develop standards, legislation, and regulation for mandatory compliance. Any vitamin A fortificant must meet certain specifications, for example: stability for shelf life, appearance stability of more than six months in the field, bioavailability, and physical stability.
- Define final product and packaging and labelling requirements.

- Promote campaigns to improve consumer acceptance. Advertising of a fortified product should be allowed for food.

(Labadarios et al., 2000:887; Murphy, 1996:74; PATH, 1997:2).

Food fortification has the following strategic advantages:

- It engages new resources.
- A modest investment is needed.
- It is relatively cost effective.
- It builds on simple and familiar technology.
- It globalises the food industry.
- Demographic trends are capitalised.
- It assists with other public health strategies.
- It is sustainable (MI, 1997:7-8).

Basic criteria for food fortification include the following:

- There must be a demonstrated need for the nutrient.
- The vehicle must reach the population.
- The amount of fortificant added must supply adequate intake when the vehicle is consumed in normal amounts.
- The amount of fortificant added should not be harmful or toxic to individuals consuming high amounts of the fortified vehicle.
- The fortificant must be biologically available and stable.
- The fortificant should not cause any organoleptic changes to the food, for example flavour, shelf life, colour, texture or cooking properties of the vehicle food.
- Fortification should be technically feasible.
- There should be no significant change to the cost of the fortified food (Giese, 1995: 116; MI, 1997:16; Pankhurst, 1999:35).

Criteria for selecting food fortification vehicles as described by Klemm & Ross (1999:52) and the Programme for Appropriate Technology in Health (PATH) (1997:8) are indicated in Table 2.13.

Table 2.13 Criteria for selecting food fortification vehicles (Klemm & Ross, 1999:52; PATH, 1997:8)

Consumption	Processing/Storage	Marketing
• Consumed by a high proportion of the at-risk population • Regular consumption throughout the year in relatively constant amounts • Appropriate serving size to meet a significant part of the RDA's • Low potential for excessive intake • No change in consumer acceptability after fortification • No change in quality after fortification	• Centralised processing • Simple, low cost technology • Stability and bioavailability of added micronutrient • Minimum segregation of the fortificant and vehicle • Good stability during storage • No micronutrient interactions that compromise absorption	• Appropriate packaging to ensure stability and decrease degradation • Labelling according to prescribed standards • Adequate turnover rate at retail and household level • Affordable fortified food product

Unless micronutrients can be delivered in the quantities needed, it is difficult to expect a single food ingredient to serve as an ideal vehicle. A variety of food fortification vehicles, each fortified with a more modest proportion of recommended daily intake (RDI), may offer an effective option in many populations where a variety of culinary habits and inconsistent dietary intakes occurs (MI, 1997:9). The fortification of various food vehicles offers the following key strategic advantages:

- When a variety of food products is fortified with a lesser proportion of RDI, the possibility of consuming dangerous levels of a micronutrient is reduced.
- No single industry sector can resist on the grounds that it is being unfairly singled out (MI, 1997:10).

2.6.4.3 Legislation

A fortification programme requires the collaborative participation between various government sectors, food producers, private organisations and international agencies to be successful. The strongest expression of political commitment to eliminate VAD is legislative action to make a vitamin A fortification programme official. Such legislation must define the norms for implementing fortification, and include the responsibilities of all sectors involved. The regulations for vitamin A fortification must specify the type of vitamin A fortificant and the permitted range of retinyl palmitate in fortified products both at the refinery and the point of sale. All precautions and food safety conditions to be observed during production, storage and sale must be specified. Labelling must be enforced and true, accurate and essential information must be supplied as specified by the health authorities (OMNI *et al.*, 1996:1; Pankhurst, 1999:34).

Table 2.14 Status of legislation for sugar fortification in Latin America (OMNI *et al.*, 1996:2)

Countries enforcing legislation	Countries in process of legislating	Countries with interest (private or official)
Guatemala	Nicaragua	Brazil
Honduras	Ecuador	Dominican Republic
El Salvador		Bolivia, Colombia

The creation of a specific committee with representatives from different sectors is recommended to monitor the implementation of the fortification programme, analyse the information from various operating units and to ensure the compliance with legislation (OMNI *et al.*, 1996:1).

2.6.5 Sugar as vehicle

Sugar is an important energy source for many people globally. It is produced in more than 100 countries and production of sugar is increasing. Sugar processing and refining are carried out at only a few mills in sugar producing countries while sugar refining is done in some sugar importing countries. Fortifying sugar with micronutrients is thus both practical and feasible. Sugar is also regularly consumed by a vast majority of people, although consumption levels may vary. Fortification of sugar is thus an effective means to provide nutrients that are deficient in a population (OMNI *et al.*, 1996:1). In a study done in Bolivia in the early 1990's, the acceptability and consumption of vitamin A fortified sugar were measured by the National Secretariat of Health. The researchers recommended that all national sugar be fortified with vitamin A by the year 2000 (USAID, 1999:72). In Guatemala sugar has been shown to be an adequate vehicle for vitamin A fortification and it does not impair iron absorption (Viteri *et al.*, 1995:1153; Yip, 1995:1164).

In a recent report by the SA Bureau of Market Research, it was revealed that in both rural and urban areas, 88 % of the population-wide average for sugar is purchased by the poorest 20 % of the SA population. If it is assumed that those at risk of VAD are mostly drawn from the lowest 40 % income group, then a general assumption can be made that the at-risk population consumes about 92 % of the average (Venkatesh Mannar, 1999a:20). Because sugar is consumed by most South Africans, it is a potential vehicle for vitamin A fortification in SA. Tables 2.13 to 2.15 depict the mean daily sugar consumption in grams of the different ethnic and age groups of SA. Table 2.18 shows the results of the 1999 NFCS. These results show that the sugar consumption by urban children is slightly higher than in the rural areas and the consumption in Gauteng was similar to the national intake. The results further indicated that 99 % of households in SA used sugar which was purchased mostly from supermarkets (85 %) and small shops (14 %). The amounts of sugar bought varied amongst the provinces, but in Gauteng 2,5 – 5 kg packs were most frequently bought. Nationally, the most popular brand was Huletts, used by 59 % of all the households. Selati was the brand name mostly used in Gauteng (65 %), Mpumalanga (85 %) and Northern Province (88 %) (Labadarios *et al.*, 2000:499).

Table 2.15 Mean sugar intakes of South Africans in gram, measured with the 24-hour recall method (Vorster *et al.*, 1997c:90)

Age group gender (years)	White	Urban Black	Coloured	Indian	Rural Black
Children, 2-5,9	35,2	35,3	39,2		19
Children, 6-10,9					23
Boys, 11-15,9	82,1	52,4	54,5	68	32,3
Girls, 11-15,9	70,7	52,4	53,9	75	35
Men, 16-24,9	123,1	52	109	78,4	
Women, 16-24,9	73,3	51	96	68,2	
Men, 25-64,9	89,3	50,8	78,4	72,8	
Women, 25-64,9	51,8	45,8	62,8	48,4	61

Table 2.16 Mean sugar intakes (g) of South Africans measured with other methods (Vorster *et al.*, 1997c: 90)

Age group gender (years)	White	Urban Black	Coloured	Indian	Rural Black
Children, 0-1,9	23	16,1	32,2	18	
Children, 2-5,9	74,7	61,7	76,9	83,7	43,9
Children, 6-10,9					
Boys, 11-15,9					
Girls, 11-15,9					
Men, 16-24,9	159	118	114	103	77,5
Women, 16-24,9	88,7	125	119	79,6	81,7
Men, 25-64,9	93,6	123	155		33,2
Women, 25-64,9	51,1	94,2	126		44,6

Table 2.17 Sugar intake in gram (data not included in Tables 2.15 and 2.16) (Vorster *et al.*, 1997c: 90)

Province	Date	Race	Age	N	Mean intake
Northern Province (rural)	1994	Black	6 - 14	96	24

All the subjects in the sample population were randomly selected and included boys and girls. The method used for measuring sugar intake was the 24-hour recall method.

Table 2.18 Mean added sugar (g) intake of children by age and area of residence in SA and Gauteng as determined by NFCS (Labadarios *et al.*, 2000:263)

Age (years)	Gauteng Mean ± SD	RSA Mean ± SD	Urban Mean ± SD	Rural Mean ± SD	t-test p-value
Children 1-3 years	21 ± 19	22 ± 21	26 ± 23	18 ± 17	U/R 0,0001 Gender 0,56
Children 4-6 years	28 ± 23	29 ± 33	36 ± 30	24 ± 34	U/R 0,0001 Gender 0,95
Children 7-9 years	31 ± 23	31 ± 29	39 ± 33	24 ± 21	U/R 0,0001 Gender 0,58

U/R – Urban/Rural

In Table 2.19 the mean sugar intake of South Africans are compared to that in other developing countries.

Table 2.19 Per capita sugar consumption, and percentage of daily intake in selected countries (XVII IVACG Meeting, 1996)

Country (1994)	Consumption (g/person/day)	% of daily energy intake
Brazil	127	17
Peru	88	14
Guatemala	110	15
Honduras	85	12
India	42	5
Indonesia	42	5
Morocco	88	11
Mali	22	2
Egypt	80	10
Zambia	31	8
Cameroon	17	3
South Africa	100	15

2.6.6 Vitamin A fortification of sugar

2.6.6.1 Technology: the process

The objective of sugar fortification is to ensure that vitamin A needs for the population groups at risk of VAD are met without resulting in excessive intakes for individuals having a high sugar intake. The level of vitamin A to be added is determined by nutritional requirements and sugar consumption patterns. Socio-economic status and age groups must thus desegregate nationally representative data. Children younger than five years are most vulnerable to VAD and their recommended daily allowance

is 400 μg RE (1330 IU) per day compared to 600 μg RE (2000 IU) for pregnant women, the other group at high risk for VAD (Klemm & Ross, 1999:53; OMNI *et al.*, 1996:2).

Based on the NFCS (1999) results, it was found that sugar satisfied most of the defined criteria for a successful fortification vehicle in SA. Consumption is widespread nationally and fortification of sugar is industrially feasible with a centralised, modern and technically sophisticated industry. Certain challenges must, however, be borne in mind, namely:

- Approximately 30 % of all the SA sugar production is used by the food processing industry and the consumption of these foods are much lower than retail sugar amongst the low-income groups.
- Vitamin A is heat- and light sensitive and may be lost during processing into value-added foods.
- The presence of low cost sugar producers in neighbouring countries may continue to import their non-fortified, cheaper sugar into SA (Labadarios *et al.*, 2000:906).

A regional consultation of governments, sugar producers and international agencies was held during 1999 in Switzerland and a joint declaration issued recommending that a regional approach to fortification should be established with a view to implement a uniform regional fortification policy to overcome the challenges mentioned (Labadarios *et al.*, 2000:906).

Although the nutritional needs of vitamin A deficient children have been well recognised, the technical problems involved with food fortification to solve the deficiency are not as well recognised. Historically only one supplier of vitamin A, in the form of retinyl palmitate or acetate, existed for food fortification, namely Hoffmann-La Roche in Basel, Switzerland. Specific vitamin A formulations were developed for specific food processing applications (Murphy, 1996:69).

Formulation requires a significant investment in time and technical expertise by the vitamin A manufacturer. Unless a continuing demand exists for new vitamin A formulations, the development of these products may not be readily available (Murphy, 1996:69). Because the quantity of vitamin A added is very little, production of a homogeneous fortified product can be facilitated by diluting the retinyl palmitate (the form of vitamin A used in fortification) in a small amount of sugar to form a premix (OMNI *et al.*, 1996:2; Pankhurst, 1999: 36). Various types of commercial vitamin A fortification preparations are summarised in Table 2.20.

Table 2.20 Commercial vitamin A fortification preparations (Murphy, 1996:70)

Type	Ingredients	Food applications
250 CWS	Retinyl palmitate, acacia, sugar, modified food starch, BHT, BHA, sodium benzoate, a-tocopherol	Skimmed milk, dehydrated foods, dry cereals, beverage powders to be reconstituted before use
250 S	Retinyl palmitate, gelatine, sorbitol, modified food starch, sodium citrate, corn syrup, ascorbic acid, coconut oil, BHT, a-tocopherol, silicon dioxide, BHA	Dry mix and fluid milk products
250 SD	Retinyl palmitate, acacia, lactose, coconut oil, BHT, sodium benzoate, sorbic acid, silicon dioxide, BHA	Foods and baked products, dehydrated potato flakes, dried milk
500	Retinyl palmitate, gelatine, invert sugar, tricalcium phosphate, BHT, BHA, sodium benzoate, sorbic acid, sodium bisulfate	Dry mix and fluid milk products
Emulsified RP	Sucrose-retinyl palmitate emulsion in water	Tea leaves
Oil	Retinyl palmitate, BHA, BHT	None

The premix contains:

- regular sugar;
- cold water soluble vitamin A palmitate beadles containing 75 000 µg/g (250 000IU/g);
- a low peroxide, low in unsaturated fat vegetable oil which adheres the vitamin A beadlet to the sugar crystal. This prevents the separation of vitamin A from the sugar crystal and results in a homogeneously fortified product, without noticeable changes in the organoleptic properties of the sugar. Peanut or coconut oil is usually used in this process;
- an antioxidant blended from natural antioxidants, for example ascorbyl palmitate, DL-alpha tocopherol and lecithin. This prevents the oil from going rancid as rancid oil will result in the destabilisation of vitamin A. The antioxidants also prevent adverse sensory characteristics of sugar. Blending the oil and antioxidant in an inert, oxygen-free atmosphere prevents the oxidation of oil (OMNI *et al.*, 1996:2).

The premix is produced by mixing the sugar and vitamin A in a blender with a spraying device attached to it to allow the antioxidant mixture to be added during the mixing operation. After 10 to 20 minutes of mixing, the premix is packaged in 25 kilogram (kg) black polyethylene bags covered with polypropylene bags to minimise exposure to light, thereby preventing the destruction of retinol. The premix is manually or automatically added to sugar at a ratio of 1:1000. The best site for mixing the premix with the sugar is where low humidity and temperature are present. Fortified sugar must be packed in polyethylene bags (OMNI *et al.*, 1996:1; Pankhurst, 1999:36).

2.6.6.2 Stability

An industrially produced encapsulated vitamin A compound that is dry, solid and water soluble, facilitated the development of fortification technology. The capsule is still sensitive to air, light, moisture, heat and acids despite its excellent stability. Appropriate handling and storage conditions of the fortified sugar and the premix are thus a necessity (MI, 1997:11; OMNI *et al.*, 1996).

Table 2.21 Stability of retinol in fortified sugar (% retention in 56,25 kg bags) (Anon, 1997:3)

Location type	Months in storage		
	3	6	9
Cold – humid	90	77	66
Hot – dry	92	71	63
Temperature – humid	83	69	43
Hot – humid	80	62	40

Experimental data reported losses of 10-20 % during the processing of fortified sugar, and 20-40 % during storage after a one year period. To compensate for these losses, an appropriate overage of premix must be added to the sugar during the fortification process. Retinyl palmitate is susceptible to oxidation in the presence of light. Black polyethylene bags reduce the exposure to light and thus the degradation of vitamin A (OMNI *et al.*, 1996: 2).

Stability tests showed that fortified sugar packaged in polyethylene bags retains 50-70 % of the initial vitamin A level after a three-month storage period. Heat and moisture together are believed to be more detrimental to retinyl palmitate than either alone (OMNI *et al.*, 1996:2).

The vitamin A in fortified sugar remains stable in the preparation of foods at home, although moisture, heat and acidity reduce its activity. Vitamin A is sensitive to acids and losses can be expected. The stability of vitamin A in baked foods is good. The retention of vitamin A after baking is between 80 to 90 %. Micronutrient interactions with vitamin A in sugar are unlikely as sugar is a pure product with very small quantities of other compounds (OMNI *et al.*, 1996:2).

According to the NFCS data the vehicles suitable for fortification in SA include fats and margarine, white- and brown bread, sugar, whole milk and maize. Table 2.22 shows the projected impact on vitamin A intake of children consuming food vehicles should 100% of the consumed product be fortified.

Table 2.22 Projected impact on vitamin A intake of children consuming food vehicles (if 100% of the consumed product was fortified)* (Labadarios *et al.*, 2000:892)

Food vehicle	Reported consumption (g/day)	Addition levels (IU/g)	Stability (%)	% fortified	Added vitamin A (IU/day)	% of RDA provided
Children 1-3 years						
White bread	41	7,6	80	98	244	18
Fats/margarine	10	50	80	99	396	30
Brown bread	52	7,6	80	98	310	23
Whole milk	176	5	70	93	573	43
White sugar	19	50	50	99	470	35
Maize	164	9,4	60	96	888	67
Children 4-6 years						
Fats/margarine	15	50	80	99	594	36
White bread	69	7,6	80	98	411	25
Whole milk	167	5	70	93	544	33
Brown bread	78	7,6	80	98	465	28
White sugar	23	50	50	99	569	34
Maize	184	9,4	60	96	996	60
Children 7-9 years						
Fats/margarine	16	50	80	99	634	27
White bread	74	7,6	80	98	441	19
Whole milk	145	5	70	93	472	20
Brown bread	86	7,6	80	98	512	22
Maize	200	9,4	60	93	1049	45
White sugar	23	50	50	99	569	24

* The calculation is as follows:

1. Consumption for each vehicle in g/day X IU/g of fortification
2. This figure is then adjusted for vitamin A losses (stability %)
3. The figure is then adjusted for the proportion of the vehicle theoretically available for fortification (% fortified)
4. The result is the total vitamin A presumed to be available for intake in IU/day
5. This figure is then divided by RDA for the appropriate age group to reflect the proportion of RDA that might be provided by the vehicle.

2.6.6.3 Quality control

Quantitative methods are used in the quality control of the premix, whilst both semi-quantitative and quantitative methods are used for the fortified sugar. Quantitative methods include the use of high performance liquid chromatography (HPLC) or spectrophotometric methods. The HPLC method is based on the separation of vitamin A (retinol) from other substances that absorb radiant energy at an equal or similar wavelength to retinol. Detection of retinol in the HPLC column can be done using ultraviolet (UV) light or fluorescence. This method is accurate and does not destroy retinol. Another advantage is that only a small sample is needed for testing. The disadvantages of this method are that expensive equipment and highly trained personnel are needed. Furthermore only a few samples can be tested at once, and therefore makes this form of analysis expensive. The spectrophotometric method involves measuring the retinol absorbency in sugar after its selective destruction through exposure to UV light. This method is easy to use, less expensive than the HPLC method, and results can be obtained in a shorter period of time (OMNI *et al.*, 1996).

The semi-quantitative colorimetric method involves adding a chromogenic reagent to a volume of dissolved sugar to produce a blue colour. The intensity of the colour is proportional to the amount of retinol in the sample. This is measured against a set of standards. Semi-quantitative assays are done at 1-2 hour intervals during production to verify that the fortified sugar contains the amount of vitamin A stipulated in the range norms. Results are immediately available and permit adjustments to the amount of premix added to the sugar (OMNI *et al.*, 1996).

2.6.6.4 Costs

Political and public investment is essential whether national programmes call for dietary diversification, fortification or supplementation. Micronutrient interventions can be integrated into primary health care, family planning, agriculture, or basic education programmes and can be sustained with minimal long-term programme costs after initial investments have been made (USAID, 1993:9).

Health, nutrition and education are probably the most important factors in determining a nation's potential. Health care is a positive economic outcome contributing to national development, whereas nutrition intervention is economically productive and essential for economic growth. The appropriate and rational use of vitamin supplements or vitamin-fortified foods can substantially reduce health care costs that may result in reducing economic losses (Robertson, 1999:2).

Fortification is the most cost-effective and sustainable intervention option. The World Bank compared fortification with many other public health interventions in its World Development Report in 1993 and found that fortification was the most cost-effective intervention available. No examples were available where the cost of fortification increased the cost of a product by more than 1 %. In fact in many fortified products, the cost increase was 0,1 % or less (Popkin, 1996:10).

The costs of sugar fortification include capital investments (building and equipment costs) and recurrent costs (personnel, premix and fortified sugar production costs, monitoring and evaluation costs). The cost of a sugar fortification programme, however, is inexpensive when compared to the costs of VAD and other interventions (OMNI *et al.*, 1996). Based on costs from millers in Guatemala, it is estimated that a capital cost of R 3 million will be needed to fortify 150 000 tons of sugar. Based on standard procedure in the SA sugar industry, this equates to a return on capital of about R 14 per ton. This includes the 25 % after-tax return for the investment and 10 % residual value of R 3 million. The recurring operating cost of fortification at a level of 50 IU vitamin A per gram of sugar is estimated at R 67 per ton. With a capital cost of R 14 and operating costs of R 67 an incremental cost of R 81 per ton of sugar is projected. This will result in a rise in the on-shelf price of R 0,10 to R 0,11 per kg sugar. Consumer sensitivity to sugar price fluctuations was measured to be as little as R 0,05. Vitamin A fortified sugar will not be able to compete against non-fortified sugar at R 0,10. The solution for SA would be to fortify all the sugar or the costs of fortification must be borne by someone other than the consumer (McKerchar & Wilkes, 1999:34-35).

2.6.6.5 Successes of sugar fortification programmes

Guatemala was one the first countries to implement a sugar fortification programme to ensure adequate intake of vitamin A by the population with satisfactory results (OMNI *et al.*, 1996). The Philippines reported on a stability trial of fortified sugar at the XIX th IVACG meeting in 1999. Of the vitamin A added to highly refined sugar at 15 μg RE/ g sugar, 70 % was retained after 6 months storage at room

temperature, compared to 50 % in less refined sugar. When used in drinks, a good retention was experienced, namely 96 % in hot coffee and citrus juice, and 84 % retention in baking a cake. No reported changes in colour and taste of the fortified sugar were reported (Klemm & Ross, 1999:53).

Guatemala reported a better retention of vitamin A in refined sugar compared to an almost 0 % retention in unrefined sugar. These findings raise the issue that vitamin A retention is affected by the way the product is processed (Klemm & Ross, 1999:53).

National survey results from Guatemala and Honduras gave the first effectiveness data on large-scale vitamin A fortification programmes. These surveys reported large reductions in the proportion of children with low or deficient serum retinol concentrations. In 1966 the prevalence of children with low or deficient vitamin A levels ranging between 27 and 40 % (pre-fortification) compared to 14 to 16 % in 1996 (post-fortification). The question arose whether this was due to the consumption of vitamin A fortified sugar. Evidence from Guatemala showing higher average plasma retinol concentrations in the sugar consuming population groups (26 µg/dL) compared with the non-sugar consumers (17 µg/dL) of similar socio-economic status supports the hypothesis that part of the improved vitamin A status was due to the sugar fortification programme. The researchers also reported that at least 50 % of the RDA for vitamin A was met by sugar fortification, despite the retinol losses in many of the tested sugar samples (Klemm & Ross, 1999:54).

Table 2.23 indicates that at least five developing countries are fortifying sugar with vitamin A.

Table 2.23 Foods being fortified with vitamin A and iron globally

Country	Vitamin A	Iron
Bolivia *	Sugar	-
Botswana	Tsabana – special food for young children	-
Brazil – pilot programme	Sugar	-
Chile	-	Wheat flour
Egypt – some areas		Wheat flour
El Salvador	Sugar	Wheat flour
Grenada	-	Wheat flour
Guatemala	Sugar	Wheat flour
Honduras	Sugar	-
India – some areas	Cow's milk	-
Jamaica	-	Wheat flour
Kyrgyzstan	-	Wheat flour
Mexico – some areas	Chocolate drink mix	Chocolate drink mix
Pakistan	Cooking fat	-
Philippines	Margarine	-
St Vincent/Grenadines	-	Wheat flour
Sri Lanka	-	Wheat flour
Venezuela	Maize flour	Wheat flour Maize flour

2.7 Co-existence of under- and overnutrition

2.7.1 Introduction

There is a growing perception that in developing countries throughout the world, overnutrition-related chronic diseases such as obesity, diabetes mellitus, cardiovascular disease (CVD) and certain forms of cancer, often develop before the battle against undernutrition has been won (Shetty, 1997). In SA, the coexistence of undernutrition with overnutrition is evident from the already high prevalence of micronutrient deficiencies accompanied by very high prevalence of obesity in black women (Walker *et al.*, 2000:202), as well as hypertension (Kittner *et al.*, 1990:1269) and stroke (Vorster *et al.*, 1998:174) in urban Africans. High plasma fibrinogen level is now regarded as a major independent risk factor for stroke (Erikson *et al.*, 1992:115) and CHD (Wilhemsen *et al.*, 1984:5001). James *et al.* (2000:392) found in the THUSA study that in black South Africans high levels of plasma fibrinogen were associated with undernutrition in men and obesity (overnutrition) in women. Both the BRISK (Vorster *et al.*, 1998:173) and THUSA (James *et al.*, 2000:392) studies have shown that black South Africans aged 15-65 years had higher plasma fibrinogen levels than comparable groups of Europeans. The effect of vitamin A fortification on plasma fibrinogen, especially in the light of proven associations between fibrinogen and vitamin intake needs therefore to be examined in a clinical intervention study.

2.7.2 Fibrinogen as risk factor for cardiovascular disease

CVD, including coronary heart disease (CHD), angina pectoris and stroke, is one of the leading causes of mortality and morbidity globally and can be caused by a number of lifestyle or behavioural factors or other non-modifiable risk factors (Oosthuizen, 1999:149). The formation of a thrombus is often the key event leading to the clinical consequences (myocardial infarction or cerebral thrombosis). The key determinants of the process of thrombus formation are: slowing of blood flow, changes in blood composition (blood cells and clotting factors), and changes in blood interactions with the vessel wall. The process of thrombogenesis is also closely related to atherogenesis (Lip, 1995:155; Van der Bom *et al.*, 1998:621). The relationship between elevated fibrinogen, an acute-phase protein, and the risk of CHD was first reported in 1980 (Meade, 1996:6). The impact of haemostatic variables on CHD is well established and some clotting factors like fibrinogen and factor VII are consistently associated with an increased prevalence of CHD, independent of the well-known endogenous risk factors for CHD including high blood pressure, high serum cholesterol levels, and glucose tolerance (Lip, 1995:156; Moller & Kristensen, 1991:344; Montalescot *et al.*, 1998:H14; Salomaa *et al.*, 1994:1293; Sanchez-Bayle, 1993:322). The role of fibrinogen in thrombus formation is illustrated in Figure 2.6.

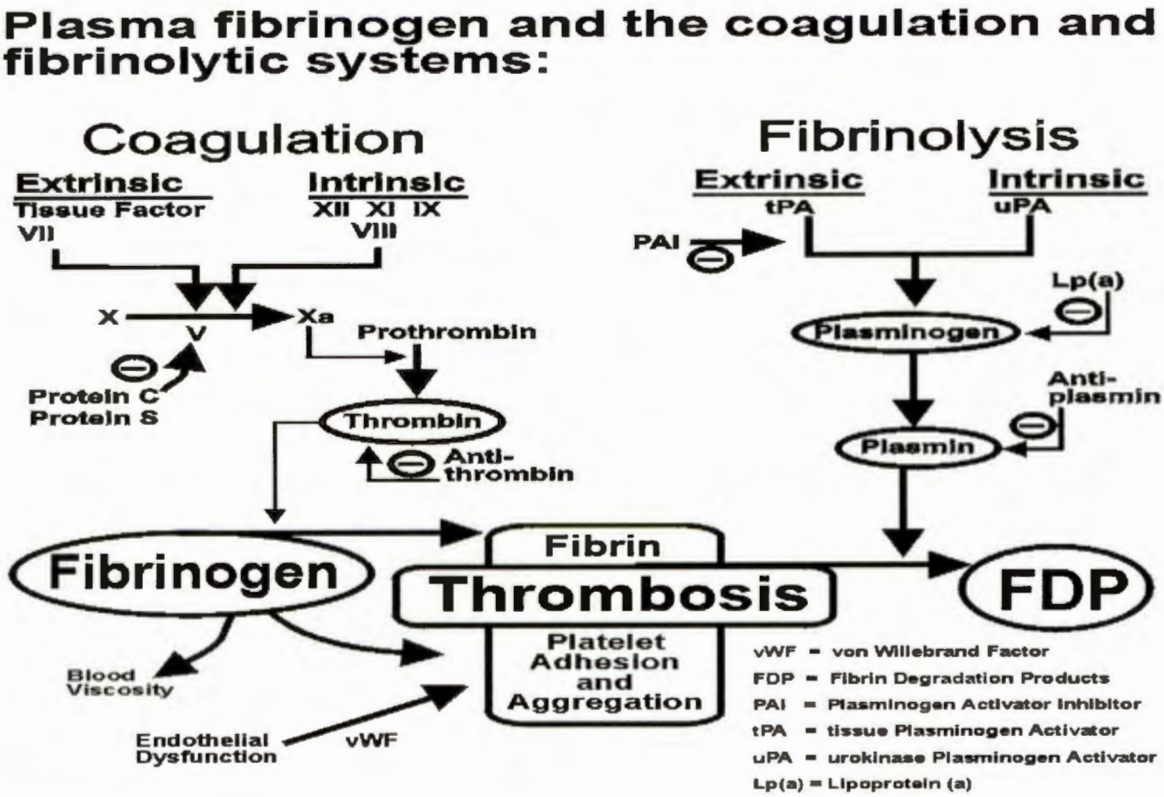

Figure 2.6 The relationship between formation (clotting) and dissolution (fibrinolysis) of fibrin networks (Vorster *et al.*, 1997a:672)

2.7.3 Factors influencing fibrinogen levels

Factors influencing plasma fibrinogen levels are: age, heredity, smoking, pregnancy, menopause, use of oral contraceptives containing oestrogen, obesity, elevated blood pressure, elevated serum cholesterol levels, elevated serum triglyceride levels, diabetes, social class, stress and infections (Folsom, 1995:21; Koenig, 1995:55; Lip, 1995:156; Meade, 1995:32; Montalescot *et al.*, 1998:H12). The normal plasma levels are between 1,5-4,5g/L although the minimum concentration necessary for haemostasis is 0,5-1 g/L (Lip, 1995:156).

- Effect of obesity

Increased BMI (> 30 kg/m^2) is associated with increased plasma fibrinogen levels. The distribution of body fat may be an important determinant of fibrinogen concentrations. A positive relationship between waist:hip ratio and fibrinogen in men and women has been proven in the MONICA study. High-energy intake and inactivity, factors causing obesity, have been associated with increased coagulation factors and decreased fibrinolytic capacities in the majority of studies. A decreased energy intake, weight loss and increased exercise are associated with an improvement in many haemostatic variables including fibrinogen (Vorster *et al.*, 1997a:673).

- Effect of alcohol

Alcohol forms an important part of the diet and social life of many people and provides energy, but is not regarded as a nutrient. Moderate alcohol consumption is known to be protective against heart disease, and this effect may be mediated in part through the effect on haemostasis. A possible explanation may be that acetate, the major metabolic product of alcohol, could influence fibrinogen synthesis, secretion or function, through lowering of free fatty acids (FFA). It has been proven that acetate influences the fibrin network in both in vitro as well as in vivo circumstances (Vorster *et al.*, 1997a:674).

- Effect of dietary fats and oils

Several long- and short-term studies have been reported where the effects of total fat intake and different fatty acids have been studied. This is mainly due to the fact that total fat and different fatty acids have profound effects on serum lipoproteins, and increased risk of CVD. Although total fat intake is associated with a change in haemostatic profiles, little evidence exists that total fat intake influences fibrinogen levels (Vorster *et al.*, 1997a:675).

With regard to the different types of fatty acids, it has been proven that the atherogenic or hyperlipidaemic saturated fatty acids are lauric, myristic and palmitic acids, and the thrombogenic fatty acids are myristic, palmitic and stearic acids. The long-chain unsaturated *n*-3 fatty acids from fish oils are both anti-atherogenic and anti-thrombogenic (Vorster *et al.*, 1997a:676).

- Effect of dietary carbohydrates

High-fibre diets have been proven to have beneficial effects on coagulation and fibrinolysis (Vorster *et al.*, 1997a:678).

- Effect of vitamins

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. The MONICA study has also shown that low plasma retinol levels were associated with lower fibrinogen levels and impaired fibrinolytic activity (reviewed by Vorster *et al.*, 1997a: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 poly-unsaturated 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.*, 1997a:678).

- Effect of specific foods

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

2.7.4 Measures to lower fibrinogen levels

If the hypothesis is accepted that elevated plasma fibrinogen levels are causally associated with atherogenesis and its thromboembolic complications, the lowering of fibrinogen levels will result in a reduced risk of CHD and stroke. This is of considerable clinical and public health interest. Unfortunately, no study has yet tested this hypothesis formally (Koenig, 1995:56) and the question of whether a decrease in fibrinogen levels will reduce the risk of CHD and stroke mortality and morbidity can not be answered as yet (Vorster, 1999:147). According to Lip (1995:165) "preventative measures against CHD risk from high plasma levels require the diagnostic determination of fibrinogen concentrations, with the aim of identifying and optimally treating high-risk patients". General measures include increased physical activity and smoking cessation. Therapeutic strategies may include drugs specifically developed to reduce plasma fibrinogen levels for example, ticlopidine, stanzolol, propanolol, nislodipine, oxypentifylline, calcium dobesilate and fibric acid derivatives. These drugs, however, are not practical therapeutic options as they have pharmacological effects other than lowering plasma fibrinogen levels (Koenig, 1995: 56; Lip, 1995:165).

Many dietary factors influence different variables of haemostasis, but not enough is known about the relationship between diet and haemostasis and the mechanisms involved to give detailed and specific advice to patients. The prudent low fat, high carbohydrate and fibre diet is known to protect against the development of chronic diseases of lifestyle and may have beneficial effects on plasma fibrinogen (Vorster *et al.*, 1997a:679). Various randomised trials have proved extensively that fibrinogen levels are not influenced by fat, carbohydrate and fibre intake in the short term. The only exception may be fish oil, rich in omega-3 fatty acids, which could lower fibrinogen levels although the evidence is equivocal (Lip, 1995: 165; Meade, 1996:6). Studies have shown that moderate alcohol consumption, increased garlic consumption, weight loss, regular exercise and better diabetic control have a favourable fibrinogen-lowering effect (Lip, 1995:165; Vorster, 1999:147). Until a safe and effective drug is available for reducing fibrinogen levels the emphasis should be on lifestyle changes, targeting the risk factors reducing the risk for both primary and secondary events (Vorster, 1999:147). Although associations between high vitamin A status and low fibrinogen levels have been found, the effect of increased vitamin A intake on fibrinogen level has not been examined in any clinical intervention trial.

2.8 The hypothesis

2.8.1 Hypothesis

The hypothesis tested in this study is 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 be vulnerable for iron deficiency, will benefit iron status, improve vitamin A nutritional status and decrease risk for future stroke by decreasing plasma fibrinogen, regardless of their initial vitamin A status.

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

2.8.2 Vitamin A – iron interactions

Since the first study by Findley and MacKenzie in 1922, several reports have suggested an interdependence between vitamin A and iron (reviewed by Bloem, 1995:501; Davidsson & Stoltzfus, 2000:15).

The known and putative interactions between vitamin A and iron were discussed in Chapter 1 (1.8). Table 2.24 summarises these possible interactions. This table shows there is evidence that for normal haemopoiesis, both vitamin A and iron are necessary, and that hypochromic anaemia is a feature of both VAD and iron deficiency. But the table also shows that vitamin A and iron may interact on other levels such as absorption and transport, and that a normal vitamin A status may be necessary for optimal utilisation of dietary and stored iron, and function of iron (also see 1.8). Although there is some evidence that Hb increases with vitamin A supplementation in pregnant women and children (see 1.8), it is not known if the iron deficiency often seen in SA blacks is related to VAD.

Table 2.24 Summary of vitamin A and iron interactions (see text in Chapter 1.8 for references)

Characteristic	Vitamin A	Interaction	Iron
Main dietary sources	• liver • dairy products • fish • yellow and dark green leafy vegetables	Share some sources	• haem iron in meat, poultry and fish • non-heam iron in dairy and plant foods
Absorption	• Adequate fat ↑ absorption • VA forms complex with Fe and inhibits phytates that decrease Fe absorption	VA ↑ Fe absorption	• Influenced by dietary iron content, bioavailability and rate of erythrocyte production. • Vitamin C ↑ absorption. • Anaemic persons absorb ↑ iron
Transport	• Retinol + RBP • RBP = acute-phase protein (↓ with infection)	• Both are negative APP • Correlations between serum retinol and Hb • Correlations between serum retinol and serum Fe, ferritin, TF	• Transported by TF • TF = acute-phase protein (↓ with infection) • Ferritin ↑ infection

Table 2.24 continued

Characteristic	Vitamin A	Interaction	Iron
Storage	• Retinol + RBP → liver	Both in liver	• Stored as ferritin and hemosiderin • Mostly in liver, RE and bone marrow
Main functions	• Vision • Growth and development → differentiation of cells: VA acts as hormone → regulate gene expression → essential for haematopoiesis	• Both necessary for normal heamopoiesis • VA necessary for haematopoiesis	• Synthesis of Hb (haemopoiesis) for transport of O_2 → tissues from lungs • Myoglobin in muscles for transportation of O_2 → metabolic needs • Cytochromes – respiration and energy metabolism • Enzymes
Deficiency effects	• Night blindness, xerophtalmia • Mycrocytic, hypochromic anaemia (megaloblastic, polycythemia aplastic) • High mortality rate • ↑ incidence of severe Infections • VAD → ↓ haematopoietic cells → megaloblastic anaemia • VAD → gelatinous degeneration of bone marrow • VAD → ↓ utilisation of endogenous iron stores • VAD → unresponsive to iron treatment • Lactating women most at risk	• Anaemia if both VAD and ID: normocytic, hypochromic • ↓ resistance to infections	• Microcytic, hypochromic anaemia • ↓ work capacity (fatigue) • ↓ psychomotor development (tension) • ↓ intellectual performance • ↓ capacity to control body temperature • ↓ resistance to infections • ↑ endothelial permeability • adverse pregnancy outcomes (LBW, premature babies, fetal death) • Females in reproductive years most at risk

Table 2.24 continued

Characteristic	Vitamin A	Interaction	Iron
Supplementation effects: VA and iron	• Anti-infective properties of VA → infection ↓, RBP & TF ↑ • Haemopoietic effects → ↓ anaemia • ↑ iron absorption by inhibiting phytates	• Improvement of Fe status with VA supplementation (↑ Hb) in pregnant women and children	• Infection ↓ → ↑ TF → ↑ Hb formation • Haemopoietic effects

RBP = retinol binding protein
TF = transferrin
LBW = low birth weight
Hb = haemoglobin
VA = vitamin A
RE = reticulo-endothelial
O_2 = oxygen
APP = acute phase proteins

2.8.3 Iron overload

Iron excess, known as siderosis, usually does not occur under normal circumstances since iron absorption is finely regulated by the body. Too much absorbed iron, however, can lead to large deposits of iron in the liver, lungs, pancreas, and other tissues. The major cause of iron overload or haemochromatosis is a genetic defect characterised by excessive iron absorption. This leads to organ damage, skin pigmentation, and liver cirrhosis (Mahan & Escott-Stump, 2000:131; Weigley, 1997: 202). The abnormal gene responsible for iron overload lies close to the histocompatibility locus antigen complex on chromosone 6. The prevalence is one in ten of European stock and is homozygous in one in 300 individuals (Eastwood, 1997:260). In sub-Saharan Africa, iron overload is related to a diet high in iron and affects more than 10 % of rural populations compared to 0,45 % in Western countries. The source of the excess dietary iron in Africa can be attributed to a traditional fermented beverage brewed in non-galvanised steel drums or pots (Gangaidzo *et al.*, 1999:278). The prevalence in black South Africans is estimated to be even higher.

A more common occurrence is secondary or acquired haemosiderosis. This condition is characterised by an abnormal accumulation of iron in the liver caused by long-term ingestion of large amounts of iron or frequent blood transfusions, although the heterozygous genetic defect resulting in excessive iron absorption may also play a role. Saturation of tissue apoferritin with iron is followed by the appearance of haemosiderin. Haemosiderin is similar to ferritin, but contains more iron and is very insoluble (Mahan & Escott-Stump, 2000:131). Haemosiderosis does not normally cause tissue damage. In rare cases, however, it is associated with tissue damage and the condition is called haemachromatosis (Mahan & Escott-Stump, 2000:131; Weigley, 1997: 202).

Dietary intake of iron or supplementation in excess of the RDA by adult men and postmenopausal women may contribute to an enriched oxidative environment in the body. This may favour oxidation of low-density lipoprotein cholesterol (LDLC), arterial vessel damage and other adverse effects associated with the cardiovascular system. Excessive iron, therefore, can contribute to generating excessive amounts of free radicals that attack cellular molecules, thereby increasing the number of potentially carcinogenic molecules within cells (Mahan & Escott-Stump, 2000:131). Sempos *et al.* (1997) summarised the evidence that excessive body iron increase risk of heart disease, and possibly also of cancer, as a result of oxidative activities. The strongest evidence came from the prospective Finnish Kuopio Ischaemic Heart Disease Risk Factor Study of 1931 randomly selected men free from CHD at baseline and who were followed for three years. The study showed that men with a serum ferritin level ≥ 200 μg/L had a greater than twofold risk of heart attack compared to those with lower serum ferritin. However, these results have not been confirmed in other studies. The authors recommended that more research in this area is needed before physicians can advise patients to lower iron status in order to prevent CHD.

Various studies have shown that a positive correlation between serum retinol and haemoglobin was found in children aged between five and 12 years old (Ahmed *et al.*, 1996:346; Lynch, 1997:106; Meija *et al.*, 1977:1175) and in pregnant women (Ahmed *et al.*, 1996:347; Lynch, 1997:106). In 1989 Bloem showed that serum retinol was significantly associated with packed cell volume, serum iron, ferritin, transferrin and saturation of transferrin (Bloem, 1995: 503). When considering iron overload and the evidence from studies that vitamin A supplementation has a positive effect on IDA, vitamin A supplementation or fortification may be the solution rather than iron fortification or supplementation in the treatment of IDA.

Against a background of a possible iron overload in SA blacks with dietary iron supplementation and a possible increase of CHD risk in those with already high risk for CHD (whites, Indians, coloureds), it seems important to examine if vitamin A supplementation may improve iron status. If so, additional iron fortification may not be necessary.

2.8.4 Vitamin A and fibrinogen

The relationship between nutrition and plasma coagulation factors, especially fibrinogen, is far from clear. In Table 2.25 the results of a few studies which examined these relationships are summarised.

Table 2.25 Relationship between vitamin A and coagulation factors as proved by research studies

Researchers	Sample population	Findings	Reference
Van Giezen, Boon, Jansen & Bouma	Rats	Retinoic acid increased fibrinolysis by selectively increasing tPA	Van Giezen *et al.*, 1993:384.
Van Bennekum, Emeis, Kooistra & Hendricks	Vitamin A deficient rats	Retinoic acid increased fibrinolysis	Van Bennekum *et al.*, 1993:R936.
Kruger, Vorster, Venter & Viljoen	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	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	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.

It was mentioned in 2.7.1 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 SA 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.

2.8.5 The hypothesis

The hypothesis developed for this study, namely that increased vitamin A intake through consumption of vitamin A fortified sugar will improve iron status and decrease plasma fibrinogen levels of a group of black South African women, is therefore based on the evidence that there is an interaction between vitamin A and iron absorption, transport and function, a possible interaction between plasma fibrinogen and vitamin A status, and therefore also a possible relationship between iron status and fibrinogen. Figure 2.7 illustrates these possible associations between vitamin A, iron and fibrinogen status and demonstrates the hypothesis that increased vitamin A status will benefit both iron status and plasma fibrinogen. The figure indicates that if iron deficiency is addressed by increasing vitamin A status, both micronutrient undernutrition and chronic disease risk will be addressed simultaneously.

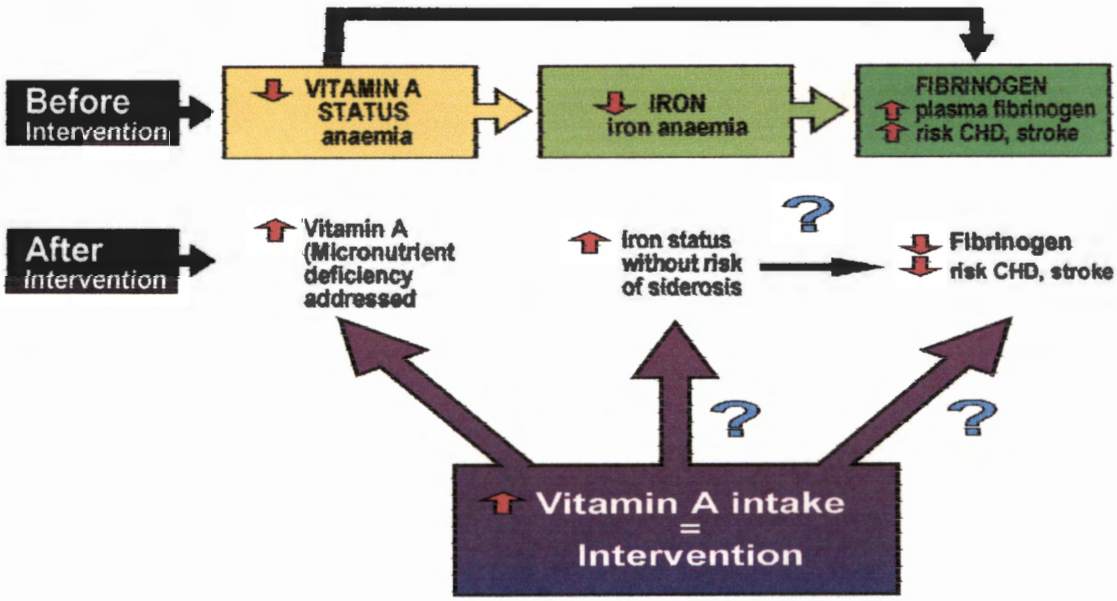

Figure 2.7 Associations between vitamin A, iron and fibrinogen status and suggested consequences of increased intakes of vitamin A

CHAPTER 3 : THE PILOT STUDY

TEA CONSUMPTION PATTERNS OF 13-25 YEAR OLDS IN THE VAAL TRIANGLE

3.1 Abstract

Objectives: This study formed part of the larger project in which food and beverage fortification as a way to address specific micronutrient deficiencies was evaluated in selected subjects in the Vaal Triangle. The objective of this study was to examine the suitability of the habit of tea drinking as a vehicle for fortification by determining the amount of tea consumed, the type of tea mostly consumed, milk and sugar additions, when tea is consumed, the reasons for tea consumption and whether people like tea.

Design: A combination of qualitative and quantitative research methods were used simultaneously and sequentially to collect data. Questionnaires were designed in which open questions addressing the objectives of the research were validated and sent out to collect the information.

Setting: Vaal Triangle, Gauteng, South Africa

Subjects: The study was conducted in a randomly selected sample of 500 male and female black South Africans, aged 13 to 25 years old.

Results: The results showed that most respondents (92,9 %) consumed at least one cup of tea daily, with rooibos tea selected as the most popular in this study (50 % of tea consumed). Sugar was chosen by 40,4 % and milk by 37,0 % of the sample to be the preferential ingredient added to tea. Respondents indicated that the preferred times for tea consumption were at breakfast, early morning and evening. According to the preference scales of these respondents, tea was the third most consumed beverage in summer and the first most consumed beverage in winter.

3.2 Introduction

Vitamins and minerals are micronutrients making headlines globally as evidence grows that they are not only necessary for normal growth, development and essential functions, but also assist in reducing the risk of heart disease, cancer and other chronic illnesses. Good nutrition is universally accepted as a basic human right, but it is estimated that globally more than 800 million people suffer from malnutrition and that in developing countries, more than 20 % of populations are hungry (reviewed by Vorster *et al.*, 1996:58).

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). In children, three micronutrient deficiencies, namely vitamin A, iron and iodine, are considered to be a major health problem in developing countries. Communities that are affected most are those in situations where poverty, unemployment, civil unrest, war and exploitation remain endemic (USAID, 1993:28; SAVACG, 1995: 1). Growth retardation, brain damage, diminished cognitive function and diminished

working capacity in children and adults, as well as increased susceptibility and severity of infections and mortality are the collective result of these micronutrient deficiencies (USAID, 1993:28; SAVACG, 1995: 15).

There are many reasons why nearly two billion people fail to consume adequate micronutrients in their diets (Malanick, 1999:1). Vitamin A and iron are found in certain fruits, vegetables, and animal products that may be too expensive or seasonal. The food preparation method may further reduce their intake. Cultural taboos often exclude micronutrient-rich foods and may also contribute to the deficiencies (USAID, 1993:28).

Micronutrient deficiencies can be addressed through supplementation, nutrition education or fortification programmes (Venkatesh Mannar, 1999:S23). Food supplementation is the provision of the micronutrient in capsule, tablet or elixir form. Dietary modification or diversification refers to either improving the type and amount of food, rich in these micronutients, ingested or its bioavailability. Food fortification is the adding of vitamins or minerals to food to increase its nutrient content (Venkatesh Mannar, 1999:S24).

Due to limited access to commercially or centrally processed foods, food fortification cannot reach all populations deficient in essential micronutrients. This can be a result of geography, poverty or cultural preference, public health and welfare approaches to deliver supplements or lack of dietary education. For the large and expanding population that does, however, regularly purchase and consume commercially processed foods, fortification can make a difference. The micronutrients added to food are diluted into the matrix of the macro-molecule that gives food its structure and is thus theoretically invisible (MI, 1997:113). Fortification of food commodities or products already consumed by a large number of people is a growth industry for micronutrient programmes. Food fortification can be targeted to specific age groups or people in specific localities. For a country to succeed in food fortification, it needs food processing capacity and regulatory enforcement, and oversight plans for monitoring the quality and safety of the food (USAID, 1993:28). When imposed on existing food patterns, fortification may not necessitate changes in the customary diet of the population and does not call for individual compliance. It can often be dovetailed into existing food production and distribution systems, and thus implemented to yield results quickly. It can also be sustained over a long period of time (USAID, 1993:28; MI, 1997:113).

Food fortification has many strategic advantages: It engages new resources, a modest investment is needed, it is relatively cost effective, it builds on simple and familiar technology, it globalises the food industry, demographic trends are capitalised, it assists other public health strategies and it is sustainable (MI, 1997:113).

One of the most common problems in developing a food fortification programme, is the selection of an appropriate vehicle (MI, 1999:1).

The vehicle that is to be used for fortification should have the following characteristics:

- It should be a component of most meals.
- It should not need prolonged storage (especially under hot and humid conditions).
- If the vehicle is darker in colour or has a stronger taste, one could use more reactive compounds.
- Segregation of the fortificant should not occur during storage (Venkatesh Mannar, 1999:S25).
- It should be made available to the target population through an effective distribution system.
- It must be acceptable, affordable and frequently consumed.
- It must be technologically and economically fortifiable.
- The fortification process should not influence taste, texture, appearance or colour and the vehicle itself must have a minimal negative effect on iron absorption (MI, 1998: 17).

Tea, a beverage brewed from the dried, processed leaves of *Camelia sinensis* is apart from water, the most widely consumed drink world-wide (reviewed by Vorster et al., 1996:26). Furthermore, the Transition and Health during Urbanisation in Southern Africa (THUSA) study in the North West Province of South Africa indicated that except for maize products, tea was the dietary item that was consumed in the largest quantities by the population (MacIntyre, 1998:258). During the NFCS survey the following information regarding tea consumption in SA was determined:

- Tea ranked third in the most frequently consumed food items of children as measured by the 24-hour recall method and tenth when determined by quantified food frequency questionnaire (QFFQ). According to these data the daily tea consumption level is 196 g and 185 g respectively.
- Tea ranked fourth in the most frequently found food items in households in Gauteng, and thus the Vaal Triangle as measured by a food procurement household inventory questionnaire. Tea was present in 80 % of all the households surveyed.
- Tea is mostly procured from supermarkets (83 %) and small shops (16 %) and 81 % of the households surveyed (n = 2184), bought tea on a monthly or fortnightly basis and 12 % weekly. Most households (39 %) procured ≥ 250 - ≤ 500 g of tea per month, whilst 25 % bought ≥ 500 - ≤ 750 g and 20 % bought ≥ 100 - ≤ 250 g. Only 9 % of these households procured less than 100 g per month and 7 % procured more than 750 g per month (Labadarios et al., 2000:548, 549, 587, 592).

When taken into consideration that a food fortification vehicle must be consumed regularly in relatively constant amounts, tea seems to be a suitable vehicle for fortification. The processing factors, for example the stability and bioavailability of the added micronutrient, as well as no interactions to compromise absorption, must, however, also be taken into consideration. Unfortunately the tannins in tea binds with the added micronutrients resulting in a low bioavailability of the fortificant.

A possible solution is to target "rooibos tea". Rooibos tea, South Africa's own indigenous herbal tea, could be used as an alternative for tea fortification as it is increasingly becoming more popular due to its alleged health benefits. Rooibos tea is, besides being enjoyed as a refreshing drink, claimed to assist in improving appetite and to cure insomnia, allergies and nervous reactions. It is also used in a natural Japanese medicinal product for its *in vitro* anti-carcinogenic and anti-mutagenic properties. It is rich in minerals, and one cup (3 g/200 ml) of rooibos tea, without milk or

sugar, will provide 0,07 mg iron, 7,12 mg potassium, 0,04 mg zinc, 0,04 mg manganese, 0,07 mg copper, 1,08 mg calcium, 6,18 mg sodium, 1,57 mg magnesium and 0,22 mg fluoride. The lower tannin levels (1 % compared to 12 % in ordinary leaf tea) and average consumption of six cups per day by South Africans may add to the fact that rooibos tea may be a more suitable vehicle for fortification than ordinary leaf tea (Joubert & Ferreira, 1996:79).

An alternative to tea as fortification vehicle could be the sugar or milk consumed with the tea in order to increase vitamin A- and iron intake in vulnerable groups. Therefore, a second possible solution could be to target the habit of tea drinking by fortifying the additions (milk and sugar) to tea.

The main focus of this study was to determine tea consumption patterns of 13 - 25 year olds in the Vaal Triangle as this age group is often affected by micronutrient deficiencies. The aim of this study was to examine the tea consumption patterns, especially when and how tea is consumed.

The objectives were, therefore, to determine the amount of tea consumed, the type of tea mostly consumed, how the tea is drunk (milk, sugar, lemon, etc), when the tea is drunk and whether people like tea (preference).

Seasonal differences were also investigated. Information was gathered from black South African participants and participating households did not include other ethnic groups (whites, Indians, coloureds).

3.3 Methods

3.3.1 Sample selection

Young black men and women volunteers, aged 13 to 25 years, in the Vaal Triangle were included. Five hundred high school pupils in the Vaal Triangle area and students at the Vaal Triangle Technikon formed an availability sample of learners drawn from randomly selected high schools and departments at the Vaal Triangle Technikon. The Vaal Triangle is situated in Gauteng, one of the nine provinces of SA, and is known as one of the most densely populated areas (see Figure 3.1). It is situated ± 70 km south of Johannesburg. The respondents can therefore be described as urban black South Africans.

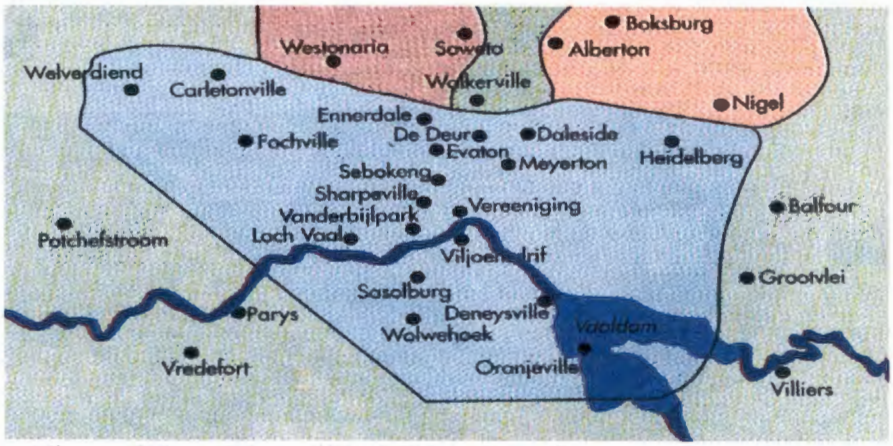

Figure 3.1 Map of Vaal Triangle in Gauteng

3.3.2 Questionnaires

A combination of qualitative and quantitative research methods were used simultaneously and sequentially to collect data. The measuring instrument was a tea consumption questionnaire (Appendix 1). Questions on information about the most commonly consumed tea (type), how the tea was prepared, what was added to the tea and how often it was consumed were included in the questionnaire. A preference list of various beverages was also included.

The reliability of the questionnaires was tested by having ten students at the Technikon complete one questionnaire each week for four weeks and comparing the answers. Validity was tested by having ten other respondents, answering and completing the questionnaires verbally and in writing. The results of the questionnaire were accepted to be reliable and valid as good correlation was found in both groups ($r = 0,873$; $P = 0,05$).

3.3.3 Data collection

Vaal Triangle Technikon food and beverage management students were trained as field workers who circulated 250 of the questionnaires at randomly selected high schools in the Vaal Triangle. The remaining 250 questionnaires were circulated in randomly selected classes in two departments by lecturers at the Vaal Triangle Technikon. Completed questionnaires circulated by the field workers were collected and sent to the researcher. Completed questionnaires from the Technikon were sent to the Department of Food for collection by the researcher. A 100 % response rate was obtained, because all 500 questionnaires handed out were completed.

3.3.4 Analysis of data

Descriptive statistics (frequencies, means, standard deviations, and confidence intervals) were determined using the SPSS for Windows (version 8,0) programme.

3.4 Results

3.4.1 Sample description

The sample comprised 500 participants. The numbers per sex and age are given in Table 3.1. A response rate of 100 % was obtained.

Table 3.1 The number per sex and age of participants

Age in years	Men (n)	% of total sample of men	Women (n)	% of total sample of women
13	17	3,4	17	3,4
14	18	3,6	19	3,8
15	19	3,8	20	4,0
16	18	3,6	23	4,6
17	17	3,4	15	3,0
18	16	3,2	19	3,8
19	15	3,0	19	3,8
20	16	3,2	19	3,8
21	21	4,2	20	4,0
22	25	5,0	23	4,6
23	18	3,6	26	5,2
24	20	4,0	25	5,0
25	16	3,2	19	3,8
	236	100	264	100

3.4.2 Tea consumption habits

As shown in figure 3.2, the majority of respondents (55,1 %) consumed more than 2 cups of tea per day with 28,5 % consuming 3-4 cups of tea per day. Only 7,9 % of the respondents reported that they do not drink tea.

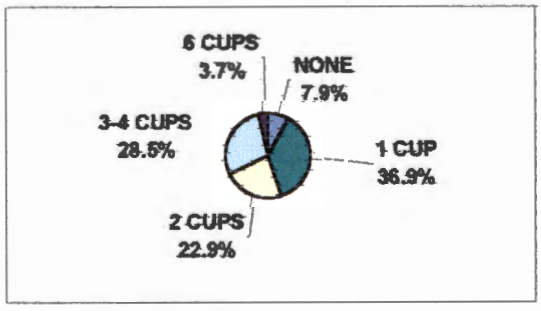

Figure 3.2 Quantity of daily tea consumption

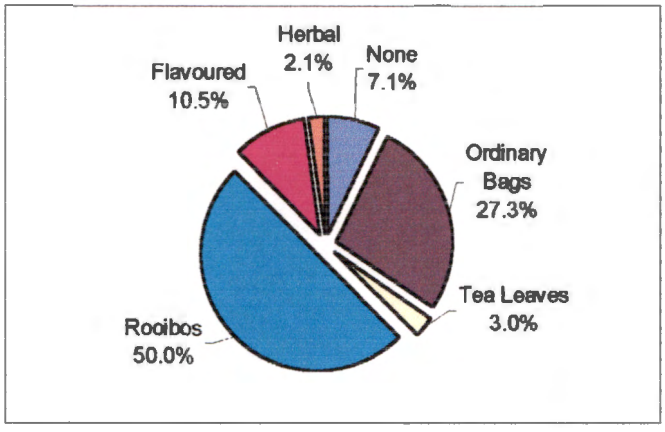

Figure 3.3 Type of tea consumed

The results from the question on favoured types of teas displayed in figure 3.3 showed that most respondents (50%) preferred rooibos tea. A total of 30,3 % consumed ordinary tea (black Indian tea brewed from bags or tea leaves).

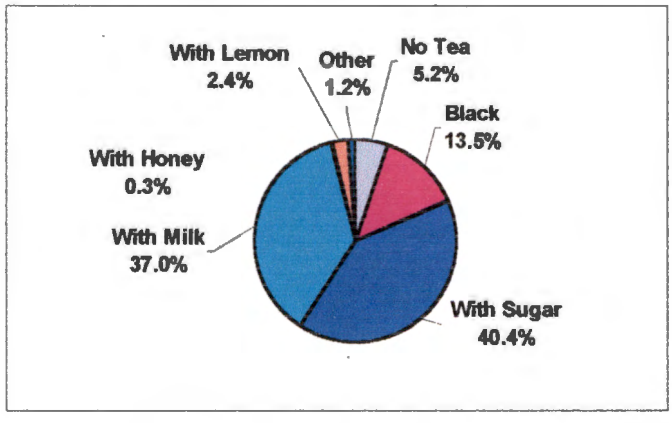

Figure 3.4 The way in which tea is mostly consumed

Figure 3.4 shows that 37,0 % of the respondents indicated that they usually add milk to tea, 40,4 % that they add sugar, while small percentages add honey and lemon.

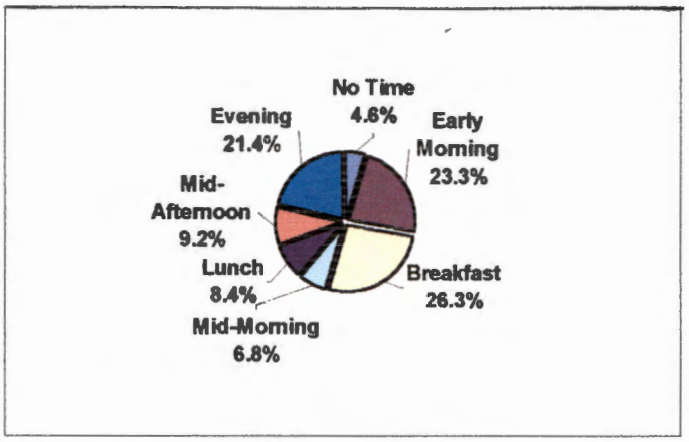

Figure 3.5 Time that tea is mostly consumed

The most popular times of tea consumption are displayed in figure 3.5. In response to the question on when the respondents prefer to drink their tea, it was indicated that the most popular times of consumption are breakfast (26,3%), early morning (23,3%) and evening (21,4%).

One of the questions asked in the questionnaire was the reason as to why respondents consume a specific beverage. Respondents who selected tea as their favourite beverage did so for various reasons. These included: the taste of tea is preferable to other beverages (57 %), tea is healthy (71 %), in winter it makes the subjects feel warm (9 %), and it quenches thirst (82 %).

A preference scale was included in the questionnaire to determine exactly how popular or unpopular tea might be, compared to other beverages, amongst this specific age and cultural group. Preferences were determined for both summer and winter seasons.

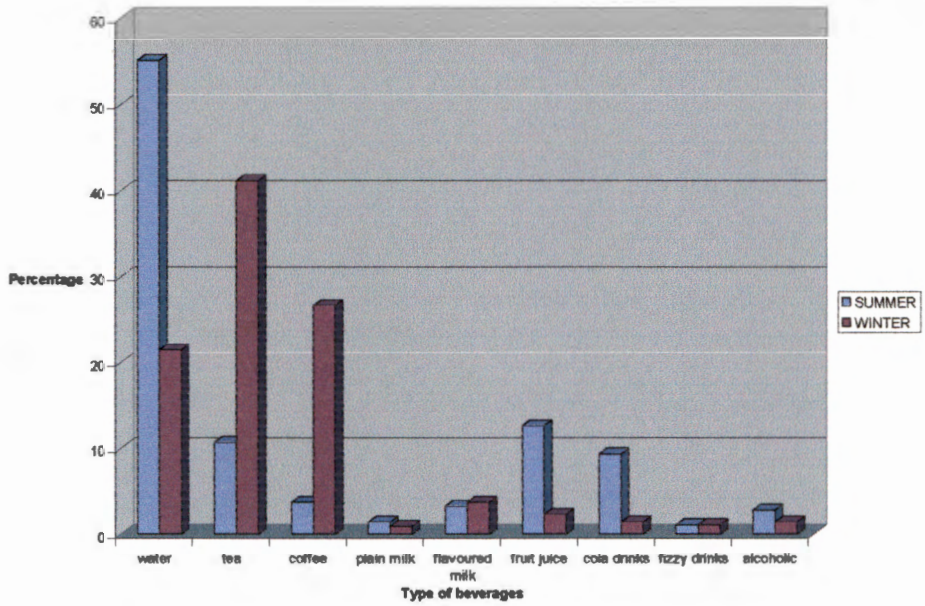

Figure 3.6 Seasonal beverage consumption

Figure 3.6 demonstrates that, as can be expected, hot beverages are consumed more in winter and cold ones in summer. Tea, however, is a popular beverage both in summer and winter. In winter most of the subjects consumed tea as a first choice (41,1 %) and in summer as a third choice (10,7 %) following water (55,1 %) and fruit juice (12,6 %). Of all the warm beverages, tea was the first choice in summer.

3.5 Discussion and conclusions

3.5.1 Discussion

The preliminary study was conducted to determine tea consumption patterns of young black South Africans living in an urban area. To achieve this, a quantitative and qualitative questionnaire was given to respondents in the sample population to complete, and afterwards it was statistically analysed. The results showed that tea is the second most consumed beverage in summer and the most consumed beverage in winter. The majority of the sample consumed two cups or more daily. Therefore, a large group of people can possibly be reached within the fortification target population with tea as vehicle for fortification. Considering the rate of consumption of tea amongst the respondents, prolonged storage of the tea should not be necessary. Tea is also readily and easily available to the majority of the population. MacIntyre (1998:103) showed that tea was the most popular hot drink consumed in the THUSA study and it was consumed on a daily basis. The NFCS (Labadarios et al., 2000:548) showed that children aged 1-9 years of age also consume tea regularly. Both these studies confirmed the results found in this study.

In this study it was found that 40,4 % of the sample population consumed tea with sugar. At the International Vitamin A Consultative Group (IVACG) meeting in 1996, it was reported that an average of 100 gram (g) of sugar per person per day is consumed in South Africa. This contributes to 15 % of the daily energy intake (Anon, 1997:1;Anon, 1996;89). The THUSA study reported an average sugar

intake of 35-60 g per adult person per day (MacIntyre, 1998:258). From the literature it is clear that sugar is an important energy source for many people globally, and also in SA. It is produced in more than 100 countries and production of sugar is increasing. Sugar processing and refining are carried out at only a few mills in sugar producing countries while sugar refining is done in some sugar importing countries. Fortifying sugar with micronutrients is thus both practical and feasible. Sugar is also consumed by a vast majority of people regularly, although consumption levels may vary. Fortification of sugar may thus be an effective means to provide nutrients that are deficient in a population (USAID, 1999:100).

Guatemala was one of the first countries to implement a sugar fortification programme to ensure adequate intake of vitamin A by the population with satisfactory results (OMNI *et al.*, 1996). The Philippines reported on a stability trial of fortified sugar at the XIX IVACG meeting in 1999. Of the vitamin A added to highly refined sugar at 15 µg RE/ g sugar, 70 % was retained after 6 months storage at room temperature, compared to 50 % in less refined sugar. When used in drinks, a good retention was experienced, namely 96 % in hot coffee and citrus juice, and 84 % retention in baking a cake. No changes in colour and taste of the fortified sugar were reported (Klemm & Ross, 1999:195). Guatemala reported a better retention of vitamin A in refined sugar compared to an almost 0 % retention in unrefined sugar. These findings raise the issue that Vitamin A retention is affected by the way the product is processed (Klemm & Ross, 1999:195).

National survey results from Guatemala and Honduras are some of the first effectiveness data on large-scale vitamin A fortification programmes. These surveys reported large reductions in the proportion of children with low or deficient serum retinol concentrations. In 1966 the prevalence of children with low or deficient vitamin A levels ranged between 27 and 40 % (pre-fortification) compared to 14 to 16 % in 1996 (post-fortification). The question arises whether this was due to the consumption of vitamin A fortified sugar. Evidence from Guatemala showing higher average plasma retinol concentrations in the sugar consuming population groups (26 µg/dL) compared to the non-sugar consumers (17 µg/dL) of similar socio-economic status supports the hypothesis that part of the improved vitamin A status was due to the sugar fortification programme. The researchers also reported that at least 50 % of the RDA for vitamin A was met by sugar fortification, despite the retinol losses in many of the tested sugar samples (Klemm & Ross, 1999:195).

Hendricks (1999:8) reported at the XIX th IVACG meeting that the sugar industry in South Africa is both geographically and economically concentrated and it is thus an attractive fortification vehicle from a monitoring perspective. Six of the seven sugar refining mills in South Africa are found in Kwazulu Natal where 90 % of the total amount of sugar is produced nationally. The other sugar mill is found in the Mpumalanga province. Based on the above-mentioned facts it can be concluded that sugar may be a suitable vehicle for vitamin A fortification.

3.5.2 Conclusion

The results of this study confirmed that tea is one of the most frequently consumed beverages nation-wide and a large group of people can possibly be reached with tea as vehicle for fortification within the fortification target population. Considering the rate of consumption of tea amongst the respondents, prolonged storage should not be necessary. Tea is also readily and easily available to the majority of the population. When taken into consideration that a food fortification vehicle must be consumed regularly in relatively constant amounts, tea seems to be a suitable vehicle for fortification. The processing factors, for example the stability and bioavailability of the added micronutrient, as well as having no interactions to compromise absorption, must, however, also be taken into consideration. Unfortunately the tannins in tea binds with the added micronutrients resulting in a low bioavailability of the fortificant. The technology for a successful tea fortification process must still be developed in SA.

An alternative to tea as fortification vehicle could be to target the habit of tea drinking by fortifying the additions (milk and sugar) to tea. The data from sugar fortification programmes in Guatemala (OMNI *et al.*, 1996) and the Philippines (Klemm & Ross, 1999:195) showed satisfactory results with regard to the stability of vitamin A and reported no changes in sensory properties of the fortified sugar. Sugar is used as a source of energy in SA and is not only added to tea, but also to vegetables and other beverages, and is used in baking. For these reasons, it was decided by the researchers to concentrate on sugar as fortification vehicle for the clinical intervention trial, because sugar was consumed on a daily basis by the target population.

CHAPTER 4 : METHODS

STUDY DESIGN AND METHODS OF THE CLINICAL INTERVENTION TRIAL

4.1 Introduction

This project was a team effort that was planned, co-ordinated and administered by the researcher. The researcher was also responsible for the ethical considerations, training of field workers, making arrangements with the schools and clinics, random sampling, drawing up and arranging statistical analyses of all questionnaires, co-ordinating biochemical analyses with the laboratories, as well as financial administration and catering and transport arrangements.

A pilot study done during May and June 1999 to determine the suitability of tea as a fortification vehicle (described in chapter 3), on a sample of 500 young black men and women between the ages of 13 and 25 years old, indicated that 56 % of the sample population consumed more than 2 cups of tea per day and that 40,4 % habitually use sugar in their tea.

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 iron status. 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.

4.2 Fortification of sugar

4.2.1 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. Another reason was that if the experimental and control subjects compared sugar it would be the same.

4.2.2 Fortification level

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:

- the basal daily vitamin A requirement of subjects in the target group;
- the maximum safe intake of supplementary pre-formed retinol, indicated by WHO/UNICEF which is 3000 RE or 10 000 IU per day for pregnant women; and
- habitual sugar consumption in grams per person per day for the target group (Arroyave & Dary, 1996a:11; PATH, 1997:19-20).

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 overage 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). At the time of manufacture the fortified sugar contained different levels of retinol palmitate depending on the batch. Vitamin A retention of the fortified sugar was not determined in this study since the duration of the intervention trial was short (12 weeks).

4.2.3 Fortification technology

The technology for vitamin A sugar fortification includes two processes, namely:

- the preparation of the premix, and
- the addition of the premix to sugar at the refinery (Arroyave & Dary, 1996a:13).

Only a small quantity of vitamin A is used in the production of a homogeneously fortified product. The process is facilitated by diluting retinyl palmitate in a small amount of sugar to form a premix. Retinyl palmitate is the form of vitamin A used in fortification of sugar (Anon., 1997:1; Arroyave & Dary, 1996a:6).

The premix contained:

- regular sugar,
- retinyl palmitate beadlets containing 75 000 µg/g (250 000 IU/g),
- a vegetable oil, low in peroxides and low in unsaturated fat, which adheres the vitamin A beadlet to the sugar crystal (Figure 4.1). (This prevents the separation of vitamin A from the sugar crystal and results in a homogeneously fortified product, without noticeable changes in the organoleptic properties of the sugar. Peanut or coconut oil is usually used in this process.),
- 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). This prevents the oil from rancidness which will result in the destabilisation of vitamin A. The antioxidant also prevents the development of adverse sensory characteristics of sugar. Blending the oil and antioxidant in an inert, oxygen-free atmosphere prevents the oxidation of oil (Anon., 1997:1-2; Arroyave & Dary, 1996a:16).

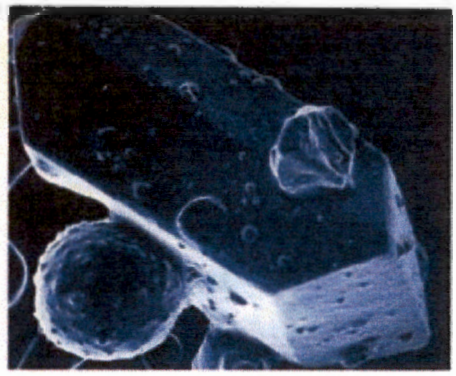

Figure 4.1 Electronmicrograph indicating vitamin A beadlets adhered to sugar crystal (Anon., 1997:2; Mora & Dary, 1995:1)

Table 4.1 shows the recommended formula for the premix. If a different retinol level is required in fortified sugar, the proportion of the premix to be added to the sugar should be adjusted rather than the composition of the premix.

Table 4.1 Premix composition (Anon., 1997:2; Arroyave & Dary, 1996a:13)

Ingredients	% Weight	Quantity
Sugar	76,35 %	86,63kg
Vitamin A 250 CWS	22,03 %	25,00kg
Peanut oil	2 %	1,82l
Antioxidant	0,008 %	0,009kg

The premix is produced by mixing the sugar and vitamin A in a V-type blender (Figure 4.2) with a spraying device attached to it to allow the antioxidant mixture to be added during the mixing operation.

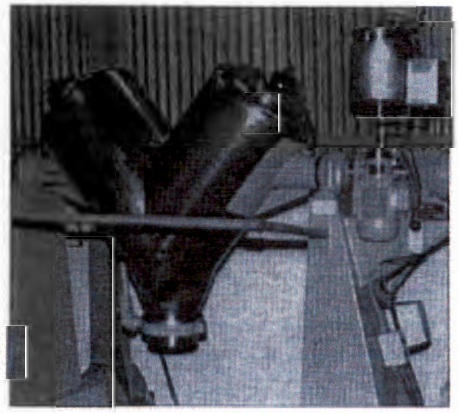

Figure 4.2 V-type mixer and oil deposit (Anon., 1997:2; Arroyave & Dary, 1996b:13)

After 10 to 20 minutes of mixing, the premix is packaged in 25 kg black polyethylene bags covered with polypropylene bags to minimise exposure to light, thereby preventing the destruction of retinol. The premix is manually or automatically added to sugar at a ratio of 1:1000. The best site for mixing the premix with the sugar is where the humidity and the temperature are low. The final fortified product must also be packed in polyethylene bags (Pankhurst, 1999:36; Anon., 1997:2; Arroyave & Dary, 1996b:14). The addition of the premix to sugar at the refinery is shown in figure 4.3. The fortification was done in three batches of 500 kg each. A total of 1500 kg of sugar was fortified for this project.

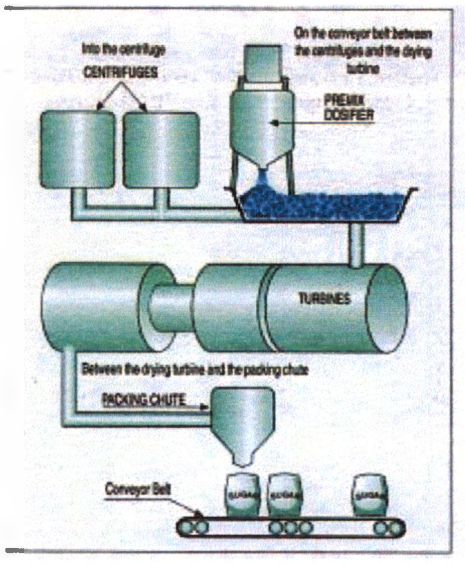

Figure 4.3 Possible points for premix addition during sugar production (Anon., 1997:3)

4.2.4 Packaging of fortified sugar

The fortified sugar was packed in plastic bags, whereafter it was placed into strong carton boxes. The label on the boxes included the manufacturing date and weight (25 kg) of each batch. It was not necessary to determine the expiry date of the sugar because the duration of the intervention trial was very short (12 weeks).

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. The reasons for distributing the sugar every two weeks were to:

- prevent participants from selling the sugar or giving it away;
- make it easy to carry home;
- remind the respondents to use the sugar frequently;
- prevent loss of vitamin A due to improper storage conditions.

4.2.5 Sensory analysis

No differences in the colour and flavour of the fortified sugar when compared to non-fortified sugar were initially noted. Both products initially given to the study population were identical with respect to colour, smell, taste and external packaging except for the codes written on the plastic bags. The sample population's reaction to sensory qualities of both the fortified and non-fortified sugar, were tested in the sugar compliance survey (Annexure 2).

4.2.6 Quality control

One of the most important attributes of an intervention trial is the quality of the product. The vitamin A levels of the different batches of fortified sugar were analysed and verified by Roche Products (Pty.) Ltd. (Annexure 3). Peroxide levels in the oil usually measured by the volumetric method, were not determined for this intervention trial. Other quality measures included in the study were:

- proper storage of fortified sugar at all times,
- using the same brand throughout the intervention trial,
- packing the sugar in smaller quantities two days in advance to prevent vitamin A losses,
- training the subjects in the correct storage methods for the sugar,
- distributing sugar in small quantities to respondents, and
- using bags which protected the sugar from UV rays.

4.2.7 Instructions

Written instructions were compiled by the researcher 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 programme and to encourage other household members to use the given sugar.

4.3 Objectives

4.3.1 Main objective

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

4.3.2 Subsidiary objectives

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

- Is sugar a suitable vehicle for vitamin A fortification in terms of dietary intake, bio-availability and consumer acceptance? (Chapter 5)
- What effect does vitamin A fortification have on the iron status of young females? (Chapter 6)
- What effect does vitamin A fortification have on the vitamin A status of

young females? (Chapter 6)

- What effect does vitamin A fortification have on plasma fibrinogen levels of young females? (Chapter 7)

4.4 Ethical considerations

The study was approved by both the Ethics Committees of Potchefstroom University for Christian Higher Education (CHE) and the Vaal Triangle Technikon. The protocol was submitted in accordance with the existing policy for research in both institutions (Annexure 5). 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 also signed the voluntary informed consent form prior to the inclusion of the subject in the study. The consent form drawn up included information explaining the purpose of the study as well as the procedures to be followed during the study (Annexure 6).

4.5 Sample strategy

4.5.1 Sample selection

A preliminary study was performed before the clinical intervention trial with the objective to select the sample population. The procedures followed included the sample procedure, completing demographic- and health and medication questionnaires and baseline measurements in order to select subjects meeting the inclusion criteria.

Power calculations are necessary to determine the likeness to detect an effect for a given sample size, effect size and level of significance (Florey, 1993:1182). Two variables were used in the power calculation namely serum transferrin receptor (sTfR) and fibrinogen because the objectives of this study included the effect of dietary vitamin A fortification on both iron status and fibrinogen levels. It was decided to use sTfR in the power calculation, as sTfR is a receptor protein that binds holotransferrin (transferrin and iron) during cellular uptake. When cellular iron levels decrease, synthesis of sTfR is increased. The amount of this protein on the surface of the cells is reflected by sTfR and an increase in serum levels correlates with iron deficiency. It thus appears that sTfR effectively monitors iron status in the normal state, during inflammatory conditions and in iron overload and is used in confirming iron deficiency (Ziegler & Filer, 1996:389).

To compare the means of continuously distributed variables such as sTfR or fibrinogen, the 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 calculated.

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 sTrF levels by 0,5 mg/L.

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

$$n = 2\,(1{,}96+1{,}28)^2\ 1{,}018^2/0{,}5^2$$
$$= 88$$

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

H_A : Vitamin A fortified sugar (80 IU per gram) consumption will decrease fibrinogen levels by 0,5 g/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 in an experimental study. A scaling-up factor was built into the sampling procedure to provide for drop-outs and 50 subjects per treatment group were selected to participate in the study.

Usually an experiment should be just long enough to allow the effect of exposure change to achieve the result in the hypothesised change in outcome (Margetts & Nelson, 2000:427). The half-life of ferritin is 30 hours and for fibrinogen 4 days, and the trial could thus have been conducted over a short period of time. For the study to be worthwhile, however, sufficient outcomes are needed and the longer the observation period, the more events might occur 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.

The sample consisted of 100 female volunteers aged 13-25 years resident in the Vaal Triangle. The age group chosen for this research project was the adolescent African female known to be a vulnerable group for iron deficiency (Vorster *et al.*, 1997c:36). Furthermore, this is a period of rapid changes in physical growth and maturation, and in psychosocial development. This period is characterised by low prevalence of most infectious and chronic diseases, but high health risks associated malnutrition, substance abuse, sexually transmitted diseases, pregnancy and accidental and intentional injuries. Adolescents are defined by the WHO as individuals 10-24 years old and by the United Nations (UN) as 0-19 years old (WHO, 1995:263). A regional probability sample was obtained implying that each female in a township would have a chance to be selected and that the probability of being selected would be known. Since the population size per township varied considerably, the sample was disproportionately stratified by township, namely a similar number of subjects was selected in each township. This implied that subjects had a greater or lesser probability to be selected depending on the population size of a given township. The survey was conducted between February and May 2000.

Sampling procedure

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

All the 13 to 18 year old females in the schools completed a form with the inclusion and exclusion criteria needed for participating in the project. Fifty of the names of those pupils who met the inclusion criteria were randomly selected as described above. The same technique was followed at the tertiary institutions and clinics to choose the other 50 subjects aged 19 to 25 years old.

The inclusion criteria were the following:

- Females
- Age between 13 and 25 years
- Non-pregnant and lactating mothers
- "Apparently" healthy
- Daily sugar consumption.

The exclusion criteria were the following:

- Males
- Pregnant and lactating women
- Age younger than 13 years and older than 25 years
- Females with diagnosed diseases like diabetes mellitus
- Females on any chronic medication.

All the subjects were eligible to participate in the study and have blood drawn if the following criteria were met:

- The informed consent form had to be signed by the parent or legal guardian for children under 21 years of age or by the subjects themselves when older than 21 years;
- normal body temperature;
- normal blood pressure.

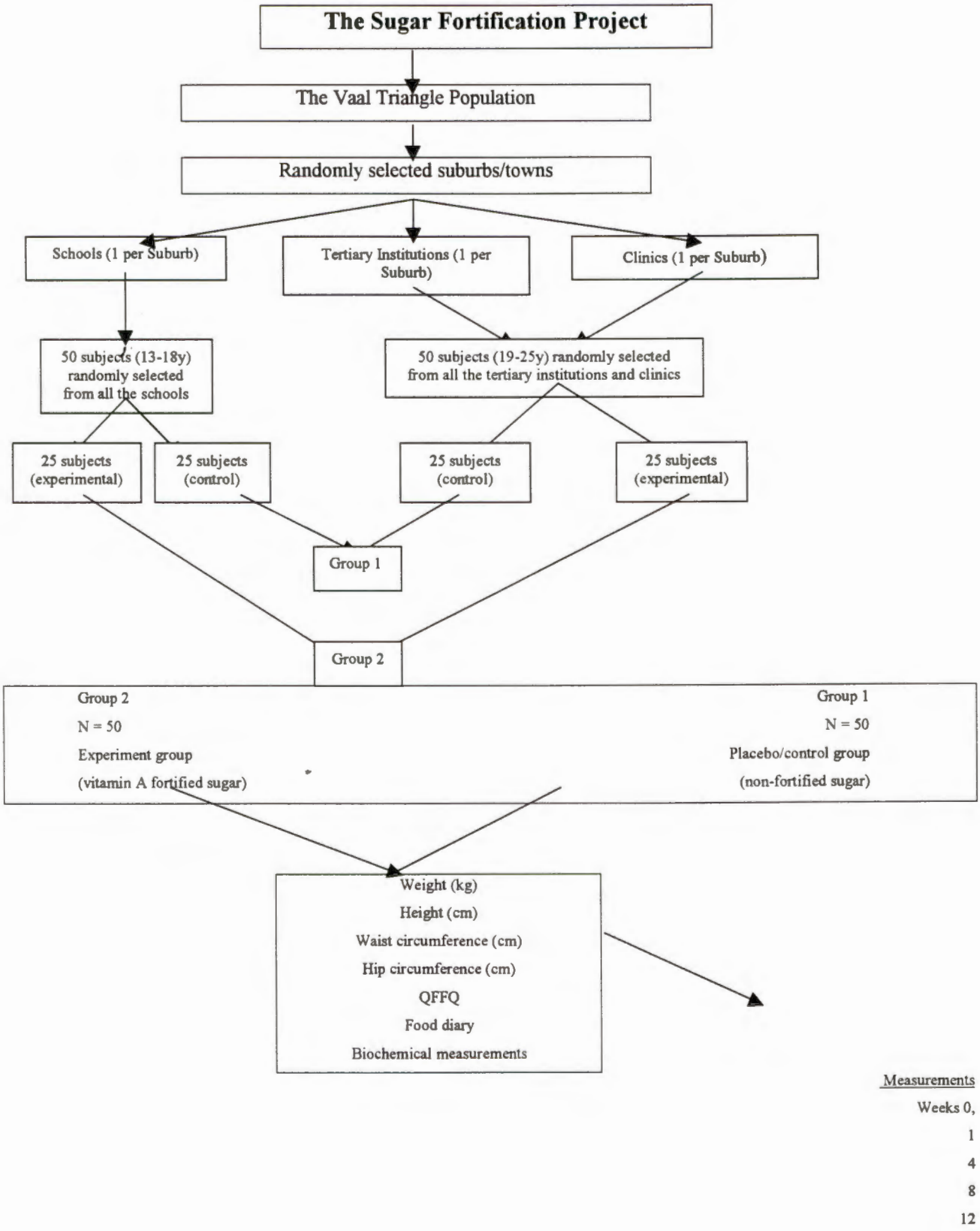

Figure 4.4 Study design

4.5.2 Baseline biochemical measurements

A double baseline measurement was done one week apart before commencing with the trial. The following baseline measurements were done:

- Vitamin A status by measuring serum retinol levels.
- Iron status by measuring blood Hct, Hb, serum iron, ferritin and transferrin.
- Plasma fibrinogen levels.

Mean values of the two baseline measurements were calculated and used as the baseline.

4.5.3 Questionnaires

Demographic questionnaire

The demographic questionnaire included questions on age, home language, the number of residents and rooms in the household, the residence setting, the responsible person for preparing meals in the household, whether respondents consumed alcohol on a regular basis, whether respondents smoked, the prevalence of high blood pressure, diabetes mellitus, stroke and obesity in the family, and sugar consumption patterns (Annexure 7). The reliability and reproducibility of the questionnaires was tested by having ten students at the Technikon complete one questionnaire each week for a period of four weeks and comparing the answers. Based on the results the questionnaire was accepted to be reliable and reproducible as a high correlation was found ($r = 0,523$, $p < 0,05$).

Health and medication questionnaire

The validated health questionnaire of the Gauteng Provincial Administration (GPA) was used (Annexure 8). A complementary medication questionnaire was drawn up and included questions on types, brand names and dosages of medication taken regularly (Annexure 9).

4.5.4 Characteristics of the sample population

Demographic and health profile of the sample population

The results obtained in the demographic, health and medication questionnaires are summarised in Tables 4.2 and 4.3. Only the subjects for whom data at both weeks 0 and 1 was available were included in these tables (n=93) as a drop-out rate of 7 % was experienced between weeks 0 and 1. The purpose of these questionnaires was to determine the suitability of the randomly selected subjects to be part of the clinical intervention trial. These results confirmed that the subjects chosen for the study were suitable.

Table 4.2 Demographic data of the subjects

Demographic variable	N	%
Age distribution (years)	**93**	**100**
13 years	7	7,5
14 years	7	7,5
15 years	7	7,5
16 years	9	9,75
17 years	7	7,5
18 years	6	6,5
19 years	6	6,5
20 years	7	7,5
21 years	7	7,5
22 years	7	7,5
23 years	7	7,5
24 years	9	9,75
25 years	7	7,5
Home language	**93**	**100**
South-Sotho	37	39,8
Zulu	15	16,1
Tswana	14	15,1
English	4	4,3
North-Sotho	4	4,3
Xhosa	2	2,1
Afrikaans	1	1,1
Other	16	17,2
Residence	**93**	**100**
Rural	13	14,0
Township	78	83,9
Informal settlement	2	2,2
Number of people in the household	**92**	**100**
1 – 2	12	13,0
3 – 4	17	18,5
5 – 6	47	51,1
7 – 8	10	10,9
9 – 10	6	6,6
Number of rooms per house	**93**	**100**
1-3	16	17,2
4 +	77	82,8
Responsible person for cooking	**93**	**100**
Self	48	51,7
Mother	36	38,7
Sister	7	7,5
Grandmother	2	2,1
Family history of chronic diseases	**93**	**100**
Hypertension	2	2,1
Diabetes mellitus	1	1,1
Stroke	1	1,1
Obesity	1	1,1
None	88	94,6
Subjects smoking	0	0

Table 4.2 Continued		
Demographic variable	**N**	**%**
Subjects partaking alcohol on a regular basis	8	8,6
Chronic medication	**93**	**100**
Asthma	3	3,2
Eye problems	1	1,1
None	89	95,7
Prevalence of eye problems	**16**	**17,1**
Chronic eye infections	4	4,3
Night blindness	4	4,3
Sensitivity to light	3	3,2
Watery eyes	3	3,2
Dry eyes	2	2,1

The results from the questionnaires showed that 95 % of the subjects were healthy, with no family history of hypertension, stroke or diabetes mellitus. The majority of subjects (69 %) had a normal weight (BMI 18,5-25). Night blindness was reported by 4,3 % of the subjects, which could be indicative of a vitamin A deficiency. The prevalence of VAD in the sample population was 5,3 % and 18,3 % of the subjects were iron depleted, 21,5 % suffered from iron deficiency erythropoiesis and 22,6 % were iron deficiency anaemic.

These results indicated that the inclusion criteria were met for all the subjects selected randomly for the sample population, as they were all females aged between 13 and 25 years old. None of the subjects indicated that they were pregnant or lactating when asked in an interview. All the subjects were apparently healthy with no prevalence of chronic illnesses. Only 4 % took chronic medication for asthma (3 %) and eye problems (1 %). These conditions are not life threatening diseases and thus were not taken into consideration when determining the health profile of the subjects.

Table 4.3 Baseline biochemical and anthropometric measurements

Variable	N	Percentage (%)
BMI (kg/m^2)	**88**	**100**
• < 18,5 (underweight)	13	14,8
• 18,5 – 25 (normal weight)	59	67,0
• 25 – 30 (overweight)	13	14,8
• > 30 (obese)	3	3,4
Waist:Hip Ratio	**88**	**100**
• <= 0,8 (normal weight)	81	92
• > 0,8 (overweight)	7	8
Systolic blood pressure (mm Hg)	**93**	**100**
• <= 80 (normal)	89	95,7
• > 80 (abnormal)	4	4,3
Diastolic blood pressure (mm Hg)	**93**	**100**
• <= 120 (normal)	91	97,8
• > 120 (abnormal)	2	2,2
Temperature (° Celcius):	**93**	**100**
• Normal (<= 37)	93	100
• High (> 37)	0	0
Iron status:	**93**	**100**
• Normal	35	37,6
• Iron depleted	17	18,3
• Iron deficient erythropoiesis	20	21,5
• Iron deficiency anaemia	21	22,6
Vitamin A status:	**93**	**100**
• VAD (< 1,05 µmol/L)	5	5.3
• Adequate (> 1,05µmol/L)	88	94.7
Normal glucose levels (4,2-6,4 mmol/L)	**93**	**100**

All the subjects randomly selected (n=93) were included in the sample population for the clinical intervention trial.

4.6 Methodology of the clinical intervention trial

4.6.1 Experimental

A double blind, matched "placebo"-controlled, parallel group, clinical intervention trial of 12 weeks in these subject volunteers took place from February to May 2000.

The sample consisted of 100 randomly selected females aged between 13 and 25 years old in the Vaal Triangle, randomly divided into two groups of equal size. After the baseline measurements were taken, seven subjects dropped out, leaving 93 subjects participating in the trial. The one group consisted of 47 subjects forming the experimental group. The experimental group consumed vitamin A fortified sugar for a period of 12 weeks. The second group consisted of 46 subjects forming the control group who consumed non-fortified sugar for a period of 12 weeks.

All the subjects were "blinded" to the treatment and received anonymous intervention in that 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 were packed identically in quantities of 5 kg black bags. All the field workers were blinded to the study, as only the researcher knew which sugar the subjects received.

4.6.2 Blood sampling and biochemical measurements

The same biochemical measurements for baseline were repeated during the 4th, 8th, and 12th week of the trial. A total of 472 (baseline X 100 subjects + week 1, 4, 8 and 12 X 93 subjects) blood samples were drawn for the determination of serum vitamin A, haemoglobin, Hct, SRBP, iron, ferritin, transferrin, total iron binding capacity and percentage transferrin saturation, and plasma fibrinogen, with methods summarised in Table 4.4.

The subjects were required to fast overnight (12 hours). Venous blood samples were collected using a 21-guage scalp vein infusion set. All the blood samples were drawn with minimal stasis and between 07H00 and 10H00 to avoid effects of diurnal variation. The following samples were collected from each subject:

- 5 ml EDTA (whole blood) for full blood counts and measurement of haematological markers: Hct, mean cell volume (MCV), red blood cell count (RBC), Hb and white blood cell count (WBC);
- 20 ml in silicone-coated tubes for preparation serum for the analysis of retinol, iron, ferritin and transferrin;
- 4,5 ml venous blood in a tube containing 0,5 ml sodium citrate (0,11 mol/L) for the preparation of plasma to measure fibrinogen levels.

Table 4.4 Methods employed for measurement of biochemical variables

Variable	Sample	Method
Full blood count (FBC)	EDTA-blood	Coulter counter
Haematocrit (Hct)	EDTA-blood	Numeric integration
Haemoglobin (Hb)	EDTA-blood	Cyanomethaemoglobin-colorimetric method
Mean cell volume (MCV)	EDTA-blood	Impulse generating
Red blood cell count (RBC)	EDTA-blood	Cell counting – autoanalyser
White blood cell count (WBC)	EDTA-blood	Cell counting – autoanalyser
Iron	Serum	Colorometric
Ferritin	Serum	Immunoturbidity
Transferrin	Serum	Immunoturbidity
s-Transferrin receptor	Serum	Immunoturbidity
Retinol	Serum	High Performance Liquid Chromatography (HPLC)
Fibrinogen	Plasma	Modified Clauss method

All the blood samples were collected and handled by a haematologist under controlled, standardised conditions. One of the most important attributes of the project was the importance placed on the quality of the data. For each round of data collection, a monitoring haematologist visited the blood collection points to check and calibrate the equipment in use, as well as supervise 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 of the full blood count used standard techniques according to existing routine procedures.

4.6.3 Training of field workers

Ten field workers 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 field workers received detailed instructions regarding anthropometric measurements and administering the qualitative food frequency questionnaire. Emphasis was placed on ensuring that the field workers were aware of the objectives and importance of the project.

In addition to the initial training sessions, field worker manuals were prepared and printed in English (Annexure 10). The instruction manuals were used by all field workers throughout the clinical intervention trial. The purpose of the manual was to ensure standardisation and uniform procedures.

A pilot study, including five volunteer Vaal Triangle staff members, was done in January 2000 to familiarise the field workers with the methodology, to test comprehension and to solve any possible problems that might occur. Aspects addressed included the suitability of the questionnaires, the use and standardisation of all instruments, the willingness of subjects and parents/guardians to allow the drawing of blood, the logistics of the transportation of blood to the analytical laboratory in Vanderbijlpark, and the time needed to complete the study per person. Discussion and solution of the problems encountered during this period equipped the field workers to trouble-shoot any possible problems they might experience during the study.

The group of researchers developed a field work administration form (Annexure 11). The purpose of the form was to ensure that the subjects completed the activities at each of the stations, and to indicate the different activities that took place each time during the intervention trial. The activities at different stations in order 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

4.6.4 Questionnaires

Various questionnaires were compiled and used in the study.

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 12). The questionnaire consisted of two components namely a list of the foods and a set of frequency-of-use response categories. An extensive list of defined foods was included with the aim of estimating total food intake, and thus dietary diversity. To verify intake, all the subjects completed QFFQ's in individual interviews with the assistance of field workers during weeks 0, 1 and 12 of the clinical trial. Food models were used simultaneously to determine portion sizes and to explain the food item to subjects.

The relative validity of the QFFQ was tested in the THUSA study using 74 volunteers. The reference measurement was a seven-day weighed record and an attempt was made to validate nitrogen intakes against nitrogen excretion in 24-hour urine collections. An additional measure of relative validity was the ratio of reported energy intake to estimated basal metabolic rate (MacIntyre, 1998:268). The QFFQ developed for the assessment of dietary intakes of the African population of North West province appeared to give relatively validated results for energy, the macronutrients, calcium, vitamin A and vitamin C (MacIntyre, 1998:347).

The reproducibility of the QFFQ was tested on a subsample 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). Alcohol and vitamin A showed the best reproducibility by performing well on all measures. Energy and carbohydrate showed satisfactory reproducibility, although the percentages of subjects classified in one quintile were lower. For all the other nutrients reproducibility was satisfactory on at least two of the analyses. Reproducibility was consistent amongst all subgroups and it was, therefore, concluded that the QFFQ used in the THUSA study was a relatively reproducible measure of dietary intake (MacIntyre, 1998:267).

Since the QFFQ was mainly used in the clinical intervention trial to determine sugar, as well as vitamin A and iron intakes, it was assumed that satisfactory levels of relative validity and reproducibility would be achieved by using the QFFQ, without adaptation, in the present study.

Food diary

A food diary was drawn up and tested for reproducibility. 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. The food diary was drawn up for a period of four consecutive days including a weekend (Friday to Monday) so that it could give clear information on the food consumption patterns. The subjects had to complete a four-day food diary four times during the intervention period of 12 weeks. (Annexure 13).

An estimated food method was used as the food and beverage consumption was quantified by estimating portion sizes as most of the subjects did not own measuring utensils or scales. Household measures like cups and tablespoons, as well as food modes, were used.

Reproducibility

All the questionnaires, except the QFFQ, were tested on ten Vaal Triangle Technikon student volunteers for reproducibility. The purpose and content of each of the questionnaires were explained by the various field workers and then completed by the subjects. Repeat interviews took place for four consecutive weeks after the first interview. The subjects were randomly assigned to the field workers and were not necessarily interviewed by the same field workers. 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.

4.6.5 Clinical examination

The physical signs of malnutrition that were observed during the study were summarised in Table 4.5.

Table 4.5 Physical signs indicative of vitamin A-and/or iron deficiency (Gibson, 1990:580)

Normal appearance	Signs associated with deficiency
Eyes Bright, clear, no sores at corners of eyelids, healthy pink and moist membranes, no prominent blood vessels or sclera	Pale eye membranes, redness of membranes, Bitot's spots, dull appearance of cornea (xerosis), soft cornea (keratomalacia), scar on cornea
Tongue Deep red, not swollen or smooth	Swelling, bluish colour
Face Healthy appearance, not swollen	Skin colour loss, pale
Cardiovascular Normal blood pressure for age	Elevated blood pressure

4.6.6 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. Anthropometric indices are important in nutritional assessment because they have the following advantages:

* the procedures are simple, safe, non-invasive techniques;
* applicable to large sample sizes;
* equipment required is inexpensive, portable and durable;
* measurements can be taken by relatively unskilled personnel;
* the methods are precise and accurate when standardised procedures are used;
* the procedures can assist in the identification of mild to moderate malnutrition, as well as severe cases of malnutrition;
* information is generated on past long-term nutritional history;
* the methods may be used to evaluate changes in nutritional status over a period of time and from one generation to the next;
* screening tests can be devised to identify individuals at high risk (Gibson, 1990: 157).

WHO recommended height-for-age and BMI-for-age as anthropometric indicators for adolescents (WHO, 1995:271). For this reason weight, height, 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. It was also used in the subject education and advising.

Table 4.6 Recommended cut-off values for adolescents (WHO, 1995:271)

Indicator	Anthropometric variable	Cut-off value
Stunting or low height-for-age	Height-for-age	<3rd percentile or < -2 Z-scores
Thinness or low BMI-for-age	BMI-for-age	< 5th percentile
At risk of overweight	BMI-for-age	≥ 85th percentile

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. The height of the subjects at the schools was measured by sticking a measuring tape on a wall. Body mass index (BMI) was calculated using the formula weight (kg)/height (m^2).

4.6.7 Statistical analysis

The results of the blood analyses were computerised and statistically analysed by a qualified statistician. 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. 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. Correlation was considered to be present if r => 0 with significance level P = 0,001, 0,05 or 0,1.

A key component of the project was the timely reporting and disseminating of dietary data, and was dependent on appropriate mechanisms for the rapid processing and analysis of data. To facilitate data processing, a standardised data entry and management software programme was developed by Mr Oscar Scharf. All the data entry operations were undertaken by a trained research assistant for the project at the Vaal Triangle Technikon. Means and SD's were calculated for food and nutrient intake.

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.

CHAPTER 5 : RESULTS

CONSUMER ACCEPTABILITY OF VITAMIN A FORTIFIED SUGAR CONSUMPTION

5.1 Abstract

Background: Marginal vitamin A deficiency has been identified as a major health problem in South Africa. Identification of a suitable food(s) for fortification with vitamin A, as a means of correcting the dietary vitamin A deficiency in a population, is an important step in developing a food fortification programme. The fortification of sugar is a promising approach in about 20 countries around the world where vitamin A deficiency is a public health problem.
Objectives: The objective of this study was to determine if sugar is a suitable vehicle for vitamin A fortification. The compliance, acceptability, advantages and disadvantages of vitamin A fortified sugar were also determined. Sugar was chosen as fortification vehicle due to the following reasons: existing consumption patterns, centralised processing, long shelf life, no sensory interference and economic feasibility.
Design: The sugar was fortified to yield 80 IU vitamin A per gram of sugar. The experimental group of 50 subjects consumed the fortified sugar, whilst the control group of 50 subjects consumed unfortified sugar for a period of 12 weeks. Compliance and acceptability were measured by a questionnaire and by means of focus group discussions.
Setting: Vaal Triangle, Gauteng, South Africa
Results: The results show that no differences in the colour and taste of the fortified sugar were noted. A statistically significant difference was, however, noted in the smell of the sugar that is fortified to a level of 100 % of the recommended daily allowance of the sample population. With all the results kept in mind, it is concluded that sugar can be used as a vehicle for vitamin A fortification.

5.2 Introduction

5.2.1 The problem of compliance measurements

According to DiMatteo and DiNicola (1982:7) the dictionary definition of compliance is yielding to a wish, a request or command of another. Instead of compliance, terms such as adherence, obedience, co-operation, concordance, collaboration and therapeutic alliance have been used. Haynes (1979:1-2) defines compliance as the extent to which a person's behaviour in terms of medication, following diets or executing lifestyle changes coincides with medical or health advice. The trouble with prevention is that the threat is so rarely urgent and likely to occur. Even with their good intentions, health professionals face many problems when trying to engage their patient's co-operation to adhere to their advice (Niven, 1989:202).

In a clinical setting, there is interest in monitoring compliance as well as intervention effects. This can lead to the substitution of an alternate medical regimen or some form of behaviour modification to induce better compliance (Gordis, 1979:24). It is, however, a complex and

multidimensional task to measure compliance. Whenever possible, researchers should apply multiple measures for compliance (Sackett, 1979:323). The measurement of compliance to a specific diet is difficult because the subjects and the researcher may have different perceptions of the quantity of food eaten (Chan & Molassiotis, 1999:437; Krasnegor, 1993:2).

5.2.2 Methods for measuring compliance

* *Direct methods*

To measure the level of compliance with any degree of precision is difficult but essential (Gordis, 1979:26; Niven, 1989:203). Direct methods are also called direct biological measures and these include blood levels and/or urinary excretions of medication, fortification or a metabolite marker or tracer (Rudd, 1993:197). Both of these methods necessitate obtaining blood and/or urine specimens (Gordis, 1979:26,35). Variations in metabolism of individuals bring about complexities that further confound the effort. Biological assays give little information about the consistency of medication or fortificant taking. In summary, these measures often raise as many questions as they answer (Rudd, 1993:197).

* *Indirect methods*

Indirect methods are also referred to as indirect non-biological measures, and include: therapeutic outcome, impression of the observer, subject interview, self-monitoring diaries and the recall method (Rudd, 1993:198; Gordis, 1979:26,30-40).

Table 5.1 is a summary of the possible objective measurements that can be used in this type of research to measure subject compliance (compiled by the researcher from various literature sources).

Table 5.1 Objective methods for determining subject compliance

Treatment/Substance being measured	Method of measurement	Possible application in this research
Oral medications or fortificants (Vitamin A fortified sugar)	Pill counts Pharmacy records Medication monitor Direct observation	Sugar left over Research records Special containers Visit each home
Diet (Vitamin A fortified sugar)	Biochemical (serum) Biochemical (plasma) Biochemical (urine) Endpoints (weight)	Serum retinol levels Plasma retinol levels Tracers Clinical endpoints regarding iron metabolism, for example serum iron, haemoglobin, haematocrit, red blood cell count, transferrin, ferritin
Markers (Vitamin A fortified sugar)	Riboflavin, Bromide Phenol red, Radioisotope Quinine	Not used in this study

5.2.3 Non-compliance

Non-compliance is universal and should be recognised as normal behaviour. There are no reliable criteria for predicting any patient's level of compliance (Conway *et al.*, 1996:29). Non-compliance is an important issue, both because it jeopardises the subject's health and well-being and it places an unnecessary burden on the health care system. Non-compliance also poses a

threat to the accuracy of clinical research efforts, because the measurement of the effectiveness of a new drug or treatment is based upon the assumption that patients have followed the conditions of administration (DiMatteo & DiNicola, 1982:1-3).

Factors that may affect non-compliance include: misunderstanding the instructions, quality of the interaction, as well as family influence and social isolation (Chan & Molassiotis, 1999:237,432; Hulka et al., 1976:847; Niven, 1989:203-205).

5.2.4 Methods for compliance research

The rules for scientific evidence are universal even when converted into specific tactics for compliance research. The first task is to define compliance in a precise, unambiguous, and appropriate manner in relation to the research question as well as the research setting. The selection and description of the sample population demands considerable thought. The study design should be repeatable, if the results are to be applied in the community at large. The choice of compliance measures must be appropriate both in the individual investigation and to the wider goals of pooling and of comparison with the results obtained by other investigators in other settings (Sackett, 1979:286-293).

5.2.5 Sugar compliance

The appearance, texture, and aroma of fortified sugar have to be evaluated to assess consumer acceptability. People do not eat what is not appealing (Johnson, 1994:124). No research studies could be found in the literature where sensory evaluation and compliance to sugar fortified with only vitamin A. In one study by Viteri et al. (1995:1153) where sugar was fortified with iron plus vitamin A, compliance and sensory evaluation tests were done. The results indicated that the acceptability and organoleptic properties of commonly used food recipes were excellent. The appearance of tea and coffee did not change with the addition of milk. Taste, texture and smell of foods were not altered. The field acceptability in the control and experimental communities was excellent.

5.3 Objectives

The major objective of this part of the project was to assess compliance to the use of the vitamin A fortified sugar in the clinical intervention trial. The subsidiary objective was to establish if sugar is a suitable vehicle for vitamin A fortification.

The specific aims were to establish:

- whether vitamin A fortified sugar was acceptable to the consumers;
- whether the experimental group complied with the intake of the vitamin A fortified sugar;
- whether the control group complied with the intake of non-fortified sugar;
- the advantages and disadvantages of sugar fortified with vitamin A;
- to develop a nutrition education programme to suit the needs for a vitamin A fortified product.

The answers to these questions would help to determine whether sugar is a suitable vehicle for vitamin A fortification in this particular target group. The literature describes sugar fortification programmes with satisfactory results in many other populations (OMNI, 1996:2; USAID, 1999:72; VAGI, 1997:8; Viteri et al., 1995:1153; Yip, 1995:1164).

5.4 Methodology

The detailed methodology of the study is given in Chapter 4. The focus in this chapter is on compliance measurement.

5.4.1 Sample

The study population consisted of 100 females aged 13-25 years that were randomly selected from clinics, schools and tertiary institutions in the Vaal Triangle. A regional probability sample was obtained. The inclusion criteria were females, aged between 13 and 25 years, non-pregnant and lactating mothers, apparently healthy females without chronic diseases, not using chronic medication, and with daily sugar consumption. Written consent was obtained from the subjects or parents and guardians prior to the study. The study was approved by both the Ethics Committees of the Vaal Triangle Technikon and Potchefstroom University for CHE. The survey was conducted between February and May 2000. The sample was grouped according to age. Stratum one (n = 23) and two (n = 23) consisted of subjects aged 13 to 18 years. Strata three (n = 27) and four (n = 27) consisted of females aged 19 to 25 years of age.

For the main intervention study, the sample of 100 was randomly divided into two groups of equal size. The one group consisted of 50 subjects forming part of 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 part of the control group who consumed non-fortified sugar for a period of 12 weeks with a drop out rate of 14 % for the experimental group and 20 % for the control group.

5.4.2 Compliance measurements : focus group discussions (FGD)

After studying the literature on focus group research methodology, it was decided to use this qualitative method to determine the participants' knowledge regarding nutrition, to evaluate the nutrition education programme, to determine compliance and to gain information of problems subjects might experience during the intervention trial. FGD usually provides insights into the attitudes, perceptions, and opinions of the participants (Katzenellenbogen *et al.*, 1999:177).

The FGD's were designed according to the guidelines from Krueger's (1997:3) focus group kit series. Questions, prompts and essential facts were compiled according to the aims of the four different sessions planned for the focus groups. The questions, prompts and facts were clustered according to the FGD phases as identified by Krueger (1997:1-101).

A multistage sampling procedure was used to select the participants for the FGD out of the initial 100 subjects (refer Figure 5.1). FGD sessions one and two consisted of the same participants divided into four focus groups whilst for each of session three and four other participants were selected. Session three consisted of four groups and session four consisted of another four focus groups (refer Figure 5.1). Each focus group consisted of six to seven participants. One participant out of each of the applicable age groups was randomly selected for inclusion in the applicable focus group (refer Figure 5.1). The participants received notices indicating the dates of the FGD sessions and were reminded about the discussions three days before the meeting.

A B Tech student acted as facilitator who led the discussions, and another B Tech student acted as moderator who was responsible for operating the tape recorder and taking notes. The FGD sessions were arranged simultaneously with the intervention trial of the main study.

Refreshments were served at the beginning of the session because the participants of the FGD were fasting for the clinical intervention trial. The facilitator and moderator took this time to become familiar with the group and also to put the participants at ease. The venues chosen for the different FGD sessions were quiet and adequately lighted. The chairs were arranged in a circle with a table in the center. The moderator recorded the date, time and place of the FGD. The names and characteristics of the participants were also noted. The group dynamics were very different for each group and were also noted by the moderator. All of the participants spoke English and where Sotho terms were given, the moderator or facilitator were able to translate the terms given. The focus group sessions lasted for approximately one hour. A small reward in the form of tea and a snack was given to each participant at the end of the session.

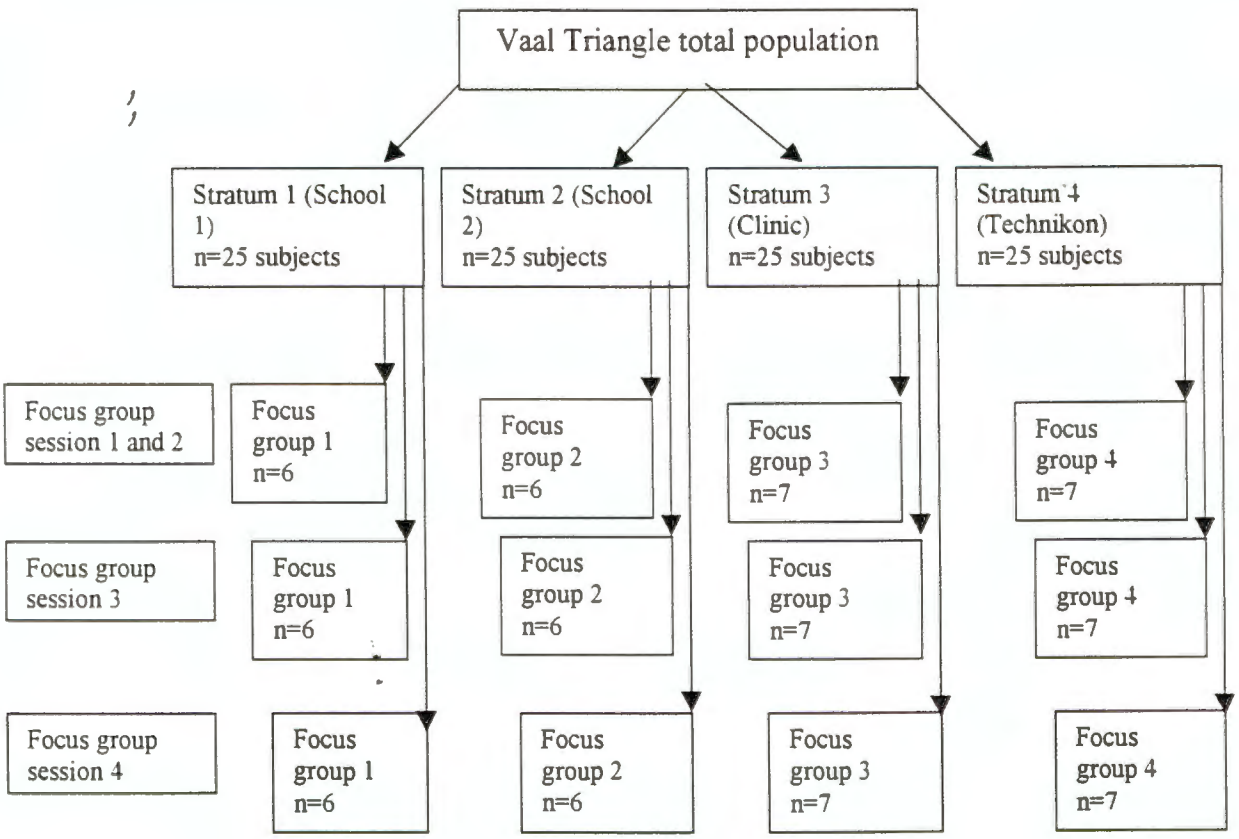

Figure 5.1 Selection of the sample population for focus group discussions

The researcher developed all four of the focus group questionnaires. The beginning phase consisted of a word of welcome. An overview of the purpose for the specific discussion and the ground rules for the discussion were given. This was followed by an opening first descriptive question. In each case it was a different general question. Introductory, descriptive, key and ending questions were included in each session followed by a final question. The subjects were encouraged to take part in the discussion unreservedly.

5.4.3 Compliance measurements : nutrition education programme (NEP)

The title of the nutrition education programme (NEP) was Foods and Nutrition. The nutrition education booklet (Annexure 18) was adopted and compiled by the researcher from the Food and Agriculture Organisation (FAO) document "Get the best from your food" (FAO, 1997:1-31). This document provided educational material for simple guidance on basic nutrition to the general public

and could easily be adopted for local use. Good eating habits, healthy foods and food preparation were the main focus areas of the NEP. Information was added to place more emphasis on vitamin A and dental caries and care of specific food items, namely sugar. The concept and messages of the NEP were formulated to be clear, positive, simple and easy to understand. Only one meeting per group was held. The duration of the lesson was one hour. The training aids consisted of an English booklet (Annexure 19) handed out to each subject to be kept for use at home.

Each of the B Tech students used for the training (n=2) was taught to evaluate the information gained by each individual by way of simple questions and discussions.

5.4.4 Compliance measurements : consumer acceptability

Adherence to prescribed dietary intake is one of the most important aspects of the success of any programme. As with all fortification and other nutrition-intervention programmes, a multiple systematic monitoring of compliance and of changes in nutritional status should be established (Chan & Molassiotis, 1999:432; Viteri *et al.*, 1995:1156). In this study, special care was taken to ensure consumption of the given sugar and to avoid replacement of part of the given sugar. Subjects were carefully informed about the purpose of the study and the importance of not changing their usual diet. This information was explained to the subjects by the researcher on administering the QFFQ's.

Compliance data were collected through direct and indirect methods. The indirect methods included a closed-end questionnaire comprising different sections including knowledge, compliance, behaviour and demographic data. Sugar consumption of all subjects was observed through QFFQ and food diaries. Both are also indirect methods. Possible changes in food consumption were checked during the baseline and the fourth, eighth and twelfth week of intervention. Blood analysis was the direct method used. Compliance could also be determined by collecting and analysing sugar samples taken from the participating households, but was not included in this study.

The compliance behaviour questionnaire that was used assessed the level of compliance of the different subjects. The design and the content of the questionnaire were developed on the basis of information gathered in the literature (Conway *et al.*, 1996:29-30; Chan & Molassiotis, 1999:433,435; Turrell, 1997:281; Viteri *et al.*, 1995:1154,1155,1157). Data were collected using a structured closed-ended question questionnaire handed out during the last week (12th) of the intervention trial. The structured interview was chosen to hasten completion of the questionnaire. The questionnaire was read to the subjects and the field workers filled out the answers for the subjects. The questionnaire was divided into two sections that focused on the acceptance, use and storage of sugar, and the amount of sugar used in certain foods or dietary compliance (Annexure 20).

5.4.5 Data analysis

Immediately after each of the FGD's, the facilitator and moderator met to review and complete the notes taken during the discussion. Any complaints or other comments received from the respondents were also noted. During these sessions the researcher further trained the field workers, and problems experienced were discussed so that the solutions could be implemented in the next FGD. The tapes and notes were analysed by the researcher, the facilitator and the moderator during the following week. The different FGD reports on the same topic were compared in table form by the researcher. The data were summarised in tables for each question. In the case of the evaluation of the nutrition education programme through focus groups, the pre- and post-FGD data were compared in the same table. Table 5.2 indicates how the focus group sessions were compiled.

Data were analysed using descriptive statistics to summarise the data. The SPSS® Version 6 was used for the analysis. Non-parametric and paired tests were done to compare for significant differences and a multivariate analysis was done to examine relationships between variables. Between group-comparisons were made by the chi-square technique thus comparing the subjects using fortified and unfortified sugar. For the purposes of this study, relationships at $p \leq 0{,}05$ were considered provisionally significant. The extent to which the subjects were complying in using the given sugar, was examined on the basis of simple frequency distributions. For incomplete questionnaires the applicable questions were indicated as missing data.

Table 5.2 Compilation of the different focus group sessions

Focus groups	**Focus group session 1**	**Focus group session 2**	**Focus group session 3**	**Focus group session 4**
Sample population	Four focus groups randomly selected. See Figure 5.1.	The same focus groups as for session 1.	Four focus groups randomly selected. See Figure 5.1.	Four focus groups randomly selected. See Figure 5.1.
Aims	To identify the participants' knowledge, attitude and behaviour of the following aspects: • How do they store their food? • Problems experienced with dental caries and obesity. • The use of sugar and vitamin A rich food in their diet.	To identify what new knowledge and attitudes the participants learned during the nutrition education programme regarding food storage, dental caries, obesity, and the use of sugar and vitamin A rich food in the diet.	To identify possible problems the group experienced with the use of the fortified and unfortified sugar.	To determine the compliance with and acceptance of the vitamin A fortified sugar.
Measuring instrument	See annexure 14	See annexure 15	See annexure 16	See annexure 17

5.4 Results and discussion

5.5.1 Focus group discussions

The participants of the four different focus groups, aged 13 to 18 years were very eager and spontaneous and participated fully in the discussions. These participants were friendly and trusting, giving frank and honest responses. The participants of the other six focus groups aged 19 to 25 were less spontaneous, but their attitude changed when their attention was drawn to the importance of the study. The analysis of the first two focus group discussions in each of the four sessions held produced most of the information. The subsequent focus group discussions gave very little new information but served to confirm the information already gained. After repeated analyses of both the written notes and taped conversations by the researcher, facilitator and moderator, the information was tabulated and broad conclusions could be drawn.

Results of sessions one and two

The results from the different questions asked during the FGD before and after the nutrition education programme will be discussed briefly. The order in which the data will be discussed is the same as used in the different FGD sessions. Each paragraph will discuss the response on the various questions asked.

- What is your favourite food?
  The subjects' favourite foods included porridge with meat and chakalaka or vegetables and gravy. The older age group had a stronger preference for chicken. Vegetables and fruit are, however, rated low on the list in the age group 13 to 18 years whilst the age group of 19 to 25 years did not even mention vegetables and fruits as favourite foods.

- In which way do you think the NEP changed the choice of your favourite food?
  Most of the subjects claimed that the NEP would not change their choice of favourite food, but they indicated in both cases that they knew more about balanced meals after the NEP.

- How will you store your sugar?
  Most of the subjects knew how to store sugar properly before the NEP. Sugar was either stored in its original bag or kept in a special container for the sugar.

- To which foods do you add sugar?
  The participants used sugar in cereals, tea, coffee, sour milk and soft porridge. Sugar was also used in vegetables like beetroot, pumpkin, sweet potatoes and home-made baked products.

- What do you think are the main problems when eating sugar?
  Even before the NEP the subjects knew the risks associated with eating too much sugar especially obesity, diabetes mellitus and tooth decay. Most of the subjects had an idea of the functions of vitamin A in the body, namely to build strong bones, and for good vision and to prevent night-blindness.

- What do you think is the biggest problem regarding the use of the fortified sugar with vitamin A?

The biggest problem regarding the use of the given sugar was that the subjects could not give the sugar away or lend it to their neighbours and friends.

Results of session three

The different questions asked during the third FGD will be discussed in table form. The questions of the FGD will be numbered from 3.1 to 3.10. The order in which the data is discussed is the same as used in the different FGD sessions. For each question the responses of the school children and the young adult group will be compared and tabulated in one table.

Table 5.3 Comparison of the type of food to which our sugar were added for school children and young adults

Question 3.1 To which foods do you add our sugar?

Age group 13-18	**Age group 19-25**
Pumpkin	Baked products, cookies
Desserts	Cereals
Cakes	Soft porridge
Drinks e.g. Six O	Tea, coffee, unsweetened juice e.g. Six O
Sweet potatoes	Custard and dessert
	Vegetables: pumpkin, home made beetroot

Table 5.4 Comparison of how sugar was being stored by school children and young adults

Question 3.2 How do you store the sugar we gave you?

Age group 13-18	**Age group 19-25**
In purple bag inside a stainless steel bin	In the given bag, in a cake tin
In bin out of purple bag	Tupperware container
Cupboard in purple bag	Stainless steel bin
Air tight container	Air tight container

Table 5.5 Comparison of the stage at which sugar was added during food preparation for school going children and young adults

Question 3.3 At what stage in the cooking process do you add the sugar we gave you?

Age group 13-18	**Age group 19-25**
Before cooking	Before cooking
While cooking	After cooking
After cooking	During cooking
Any time	Any time

Table 5.6 Comparison of how school children and young adults feel about the given sugar

Question 3.4 How do you feel about our sugar?

Age group 13-18	**Age group 19-25**
Proud to have received it	It is nice, makes happy
Gives energy	More sweet than natural sugar
Confused, not all members of family want to use the sugar	Gain weight and lose weight; rest stays the same
Discouraged, family thinks it is toxic	Confused, not able to share sugar with friends
	Sugar is nutritious

Table 5.7 Comparison of the type of beverages to which given sugar was added by school children and young adults

Question 3.5 In what beverages do you use our sugar?

Age group 13-18	**Age group 19-25**
Tea	Tea
Coffee	Coffee
Six O	Unsweetened juice e.g. Six O
Ginger-beer	Milo
Starch water	Milk
	Ginger-beer

Table 5.8 Comparison of the amount of sugar used by school children and young adults

Question 3.6 Does your participation in this project make a difference in the amount of sugar you and your family use?

Age group 13-18	**Age group 19-25**
Yes, saves money	Very sweet so reduces the amount
Yes, enough to use	No difference
	Nobody eats more sugar than normal

Table 5.9 Comparison of the difference between normal sugar and sugar used in the research project

Question 3.7 Do you think there is a difference in the sugar given to you and the sugar you normally use?

Age group 13-18	Age group 19-25
Tastes sweeter	No difference
Tastes the same	More sweet
Cake batter more runny	Tastier
	Gives appetite
	Less sweet

Table 5.10 Encouragement of other participants

Question 3.8 Suppose that you were trying to encourage a friend to participate in this research. What would you say?

Age group 13-18	Age group 19-25
Make them jealous	Sugar is healthy, nutritious
	Because vitamin A is added
	Because of the incentive received
	Nutritional guidance received in project

Table 5.11 The best qualities of the given sugar listed by the subjects.

Question 3.9 What do you think is the best quality of the sugar given to you?

Age group 13-18	Age group 19-25
The energy it gives	Gives energy
Provides vitamin A	Adds vitamin A in their bodies
Taste	Prevents tooth decay
	Builds strong bones
	Gives appetite
	Tastier

Table 5.12 Problems experienced with the given sugar

Question 3.10 What do you think is the biggest problem with the given sugar?

Age group 13-18	Age group 19-25
Less sugar should be given	If you have more than enough vitamin A in the body, it will cause obesity and other diseases
The packaging material should be improved	Not sharing the sugar with friends
The colour of sugar is changed	New way of storing the sugar
New way of storing the sugar	Sugar is sweeter
	Why a purple coloured bag?

Discussion

The subjects stored the sugar the correct way as learned in the NEP. Most of the participants kept the sugar in the UV protected purple bags, whilst the rest stored it in a closed container as can be seen in Table 5.2.

Sugar was added at any stage in the cooking process. The subjects tended to add sugar before and during the cooking process rather than after cooking the food (Table 5.4).

The subjects were confused because their family members believed the sugar was toxic, they were not allowed to give the sugar away and some of the family members did not want to use the sugar. According to Table 5.5 some of the subjects said they gained weight by using the sugar whilst others believed they lost weight.

Some of the participants claimed that they used more sugar than normally and that the sugar was less sweet than the sugar they normally used. Others used less of the sugar because it tasted sweeter to them. One subject believed that the sugar could change the consistency of cake batter as reported in Table 5.8.

One of the benefits of the intervention trial was that the participants received nutritional guidance. They thought the sugar to be nutritious because it contained vitamin A and that it prevented tooth decay, built strong bones and gave appetite (Table 5.10).

According to Table 5.11 the participants were concerned about the toxic effect of too much vitamin A and the unusual packaging material used.

Results of session four

The different questions asked during FGD session four are discussed in table form. The questions are numbered from 4.1 to 4.8. The order in which the data is discussed is the same as that used in the FGD sessions.

Table 5.13 Comparison of the acceptance of the given sugar between school children and young adults

Question 4.1 What would you tell a best friend of a family member about this product?

Age group 13-18	Age group 19-25
Sugar is special and designed for special persons	The product provides more nutrition value
Sugar contains vitamin A	Fortified with vitamin A
Designed to cure diseases e.g. high-blood pressure	The product is healthy
Sophisticated sugar	Gives more energy
	Tell her what I know about vitamin A

Table 5.14 Comparison of the dislike of the given sugar between school children and young adults

Question 4.2 If you could change one thing about this product, what would you change? What is the main reason for this change?

Age group 13-18	Age group 19-25
Change packaging for easy handling and transportation	Most subjects said nothing should be changed
Add flavour to attract consumers	Because the product is sugar, it can cause obesity
Change taste to moderate	The plastic bag

Table 5.15 Comparison of rating of the given sugar between school children and young adults

Question 4.3 What would it take for this product to get a gold star or a reward?

Age group 13-18	Age group 19-25
Taste and energy	Because of the nutrients and healthiness
Vitamin A added	Taste is good
The role it is playing in our bodies	Texture is fine
	Role vitamin A plays
	The energy it gives

Table 5.16 Comparison of the acceptance or rejection of the given sugar between school children and young adults

Question 4.4 What do you need to know about this product in order to accept or reject it?

Age group 13-18	Age group 19-25
Where does the sugar come from?	Is the quality of the sugar good?
How was vitamin A added to the sugar?	The quantity of vitamin A added to the sugar
Why only tested in females?	The price
Will it cost more or less?	Why purple bag?
	Nutrition information

Table 5.17 Comparison of the compliance to the given sugar between school children and young adults

Question 4.5 What was the greatest barrier to overcome in using the sugar?

Age group 13-18	Age group 19-25
Family members did not want to use the sugar of the participants	One respondent said that since eating the sugar the high blood pressure was reduced
Why a purple bag?	People don't understand process of fortification
	No barriers, no complaints

Table 5.18 Comparison of the solutions for non-compliance of the given sugar between school children and young adults

Question 4.6 What is needed to overcome these barriers?

Age group 13-18	Age group 19-25
Name of sugar company and expiry date	Not applicable
Nutritional information must be included on packaging	Advertisement
Give the NEP to all family members	Know the processes used

Table 5.19 Comparison of new advice given by the school children and young adults

Question 4.7 Do you have any advice for us when we introduce the new vitamin A fortified sugar to the public?

Age group 13-18	Age group 19-25
Role it plays in patients with HIV/Aids	Media: TV, radio, advertisements
Show the research to the media (TV)	
Invite all family members to nutrition education	

Discussion

The subjects realised that the sugar had a better nutritional value when fortified with vitamin A and could help to cure VAD. Some participants were concerned that sugar could cause obesity and that it was therefore not a suitable vehicle for vitamin A fortification, although they were of the opinion that it was better than ordinary sugar because it contained vitamin A (see Table 5.14).

It was important to fully inform the participants of the source of the sugar, how much vitamin A was included and whether a good quality of sugar was used (Table 5.16). The price of the fortified sugar raised concern with the participants. According to the participants, nutrition information as well as the expiry date must be provided on the packaging of the fortified sugar. The fact that the study was

only done with females raised some questions. The participants indicated that the total family/household should receive the NEP.

The media could play an important role to inform the consumers about fortified sugar as can be seen in Table 5.19. The participants were interested in the role of fortified sugar in patients with HIV/AIDS.

5.5.2 Sugar compliance and acceptability

The data obtained is presented in the same order as in the questionnaire with the various tables first and further illustrated with graphs, where necessary, followed by a brief analysis and discussion of the various aspects of the data. Frequency distribution was used to analyse most of the data. Between-group comparisons were done with Chi-square tests. In these cases the expected values of every cell were added to the cross-tabulation and the Pearson Chi-square test was performed on the cross-tabulation in order to determine any statistically significant differences of colour in the distribution between fortified and non-fortified sugar ($p \leq 0,05$). All the subjects (n=89) answered yes to the first question and thereby indicated that they liked the given sugar whether it was fortified or not.

Table 5.20 Sensory differences noticed in the fortified and non-fortified sugar

Variable	Fortified sugar		Non-fortified sugar	
	Yes (%)	No (%)	Yes (%)	No (%)
Difference in smell	9	91	36	64
Colour difference	30	70	18	82
Colour change in food when added	11	89	2	98

Of the subjects who received fortified sugar, 30 % (n=13) could detect a difference in the colour of the sugar whilst 18 % (n=8) of the subjects who received non-fortified sugar noticed a colour difference.

Table 5.21 Cross tabulation of difference in odour noticed in fortified and non-fortified sugar

			Does the given sugar smell different?		
			yes	no	Total
Type of sugar received	non fortified	Count	4	41	45
		% within Type of sugar received	8.9%	91.1%	100.0%
		% within Does the given sugar smell different?	20.0%	59.4%	50.6%
		% of Total	4.5%	46.1%	50.6%
	VitA fortified	Count	16	28	44
		% within Type of sugar received	36.4%	63.6%	100.0%
		% within Does the given sugar smell different?	80.0%	40.6%	49.4%
		% of Total	18.0%	31.5%	49.4%
Total		Count	20	69	89
		% within Type of sugar received	22.5%	77.5%	100.0%
		% within Does the given sugar smell different?	100.0%	100.0%	100.0%
		% of Total	22.5%	77.5%	100.0%

Hypothesis 1

H_0: There is no relationship between the type of sugar received and the colour of the sugar.
H_A: There is a relationship between the type of sugar received and the colour of the sugar.

There was no significant statistical evidence that there was a relationship between the type of sugar and the colour of the sugar (P=0,191) therefor H_0 is accepted in favour of H_A.

Nine percent of the subjects (n=4) receiving fortified sugar claimed detecting a difference in the smell of the sugar. Thirty six percent (n=16) detected a difference in smell of the sugar they received when compared to the sugar they normally use.

Hypothesis 2

H_0: There is no association between the type of sugar and the smell of the sugar.
H_A: There is an association between the type of sugar and the smell of the sugar.

According to the Chi-square test there was statistically significant evidence to conclude that there was an association between the type of sugar and the smell of the sugar (P=0,002) therefor H_0 is rejected in favour of H_A.

Only one subject (2 %) claimed to have noticed a change in the colour of the food when adding non-fortified sugar. Eleven percent (11 %) of the subjects receiving fortified sugar noticed a difference in the colour of their food.

Hypothesis 3

H_0: The type of sugar will not change the colour of the food it was used in.
H_A: The type of sugar will change the colour of the food it was used in.

The data do not support the hypothesis H_A that the type of sugar will change the colour of the food it was used in (P=0,086).

Table 5.23 Addition of sugar to food and the storage of sugar

Variable	Frequency		Percent	
	Yes	No	Yes	No
Addition of sugar while food is cooking	78	11	87,6	12,4
More sugar added after food is cooked	18	71	20,2	79,8
Containers used for storage	65	24	73	27
Handling of sugar during storage	86	3	96,6	3,4

The response to the question "Do you add sugar to the food while it is being cooked?" indicated that 87,6 % of the subjects added sugar during the cooking compared to 12,4 % who did not. Only 20 % of the subjects indicated that they added more sugar to their food after cooking. The rest (80 %) did not add more sugar to their food.

The subjects (n=89) stored sugar in different ways. There was a tendency to store sugar in the original packaging it was received (73 %). The results indicated that almost every subject (97 %) closed the container of sugar every time after use. Only three percent (3 %) of the respondents left the sugar container open after use.

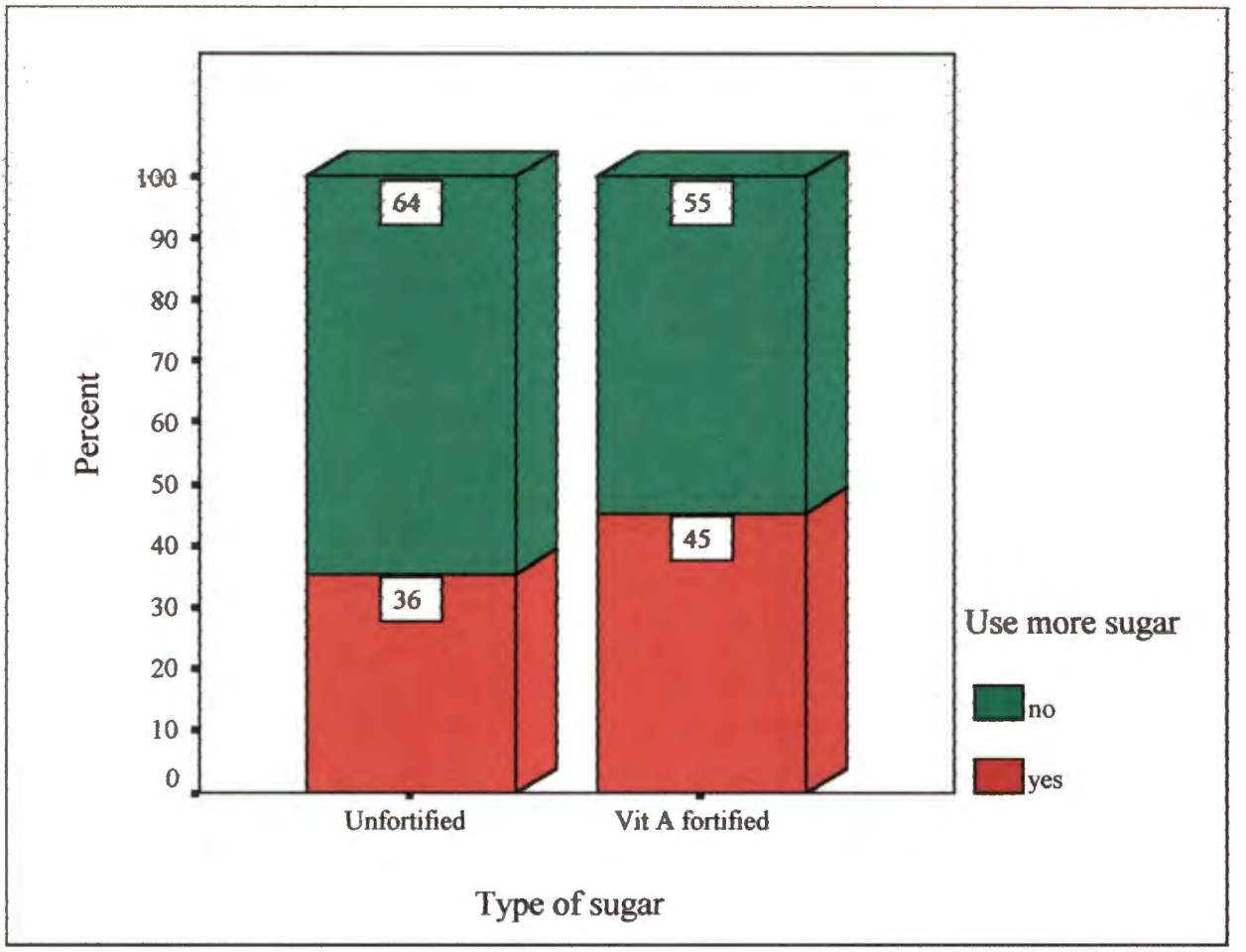

Figure 5.2 Type of sugar versus amount used during the intervention trial

Twenty of the subjects in the experimental group who received fortified sugar, and 16 of the subjects in the control group who received non-fortified sugar, indicated that they used more sugar than they normally would.

Hypothesis 4

H_0: There is no association between the type of sugar and the amount of sugar used during the intervention trial.
H_A: There is an association between the type of sugar and the amount of sugar used during the intervention trial.

There is no evidence to conclude that there is a statistically significant association between the type of sugar and the amount of sugar used (P=0,341). Thus H_0 is accepted in favour of H_A.

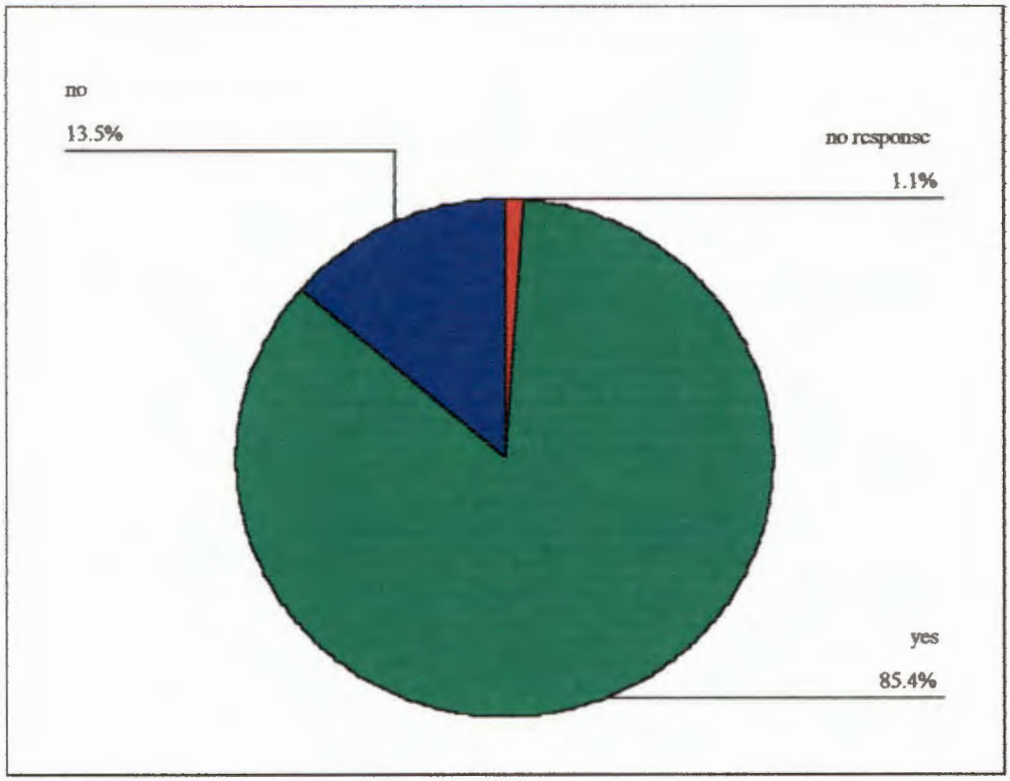

Figure 5.3 Are you sure of the reason why fortified sugar should be taken?

One subject did not respond to this question. Most of the subjects (86 %) knew the reason for using fortified sugar compared to 13 % who did not.

Table 5.23 Other roles of sugar in the diet

Variable	Frequency		Percent	
	Yes	No	Yes	No
Sugar cause illness	2	86	2,2	96,9
Forget to use the sugar	11	78	12,4	87,6
Like sweet foods	77	12	86,5	13,5
Teeth	20	69	22,5	77,5
Fat	26	63	29,2	70,8

It is clear from Table 5.23 that the majority of respondents (97 %) did not feel ill when using the given sugar. Only two respondents (2 %) indicated that the sugar made them feel ill. In one questionnaire no response was given. Of the 89 subjects participating in the intervention trial, 11 subjects forgot to use the sugar given to them. The majority of the subjects (n=78) remembered to use the sugar. Almost 90 % of the subjects liked sweet foods while 10% did not like sweet foods. Nearly three quarters (77 %) of the subjects thought that sugar was bad for their teeth. More than 70 % (n=63) of the subjects thought that sugar would make them gain weight whilst approximately 30 % (n=26) thought the opposite.

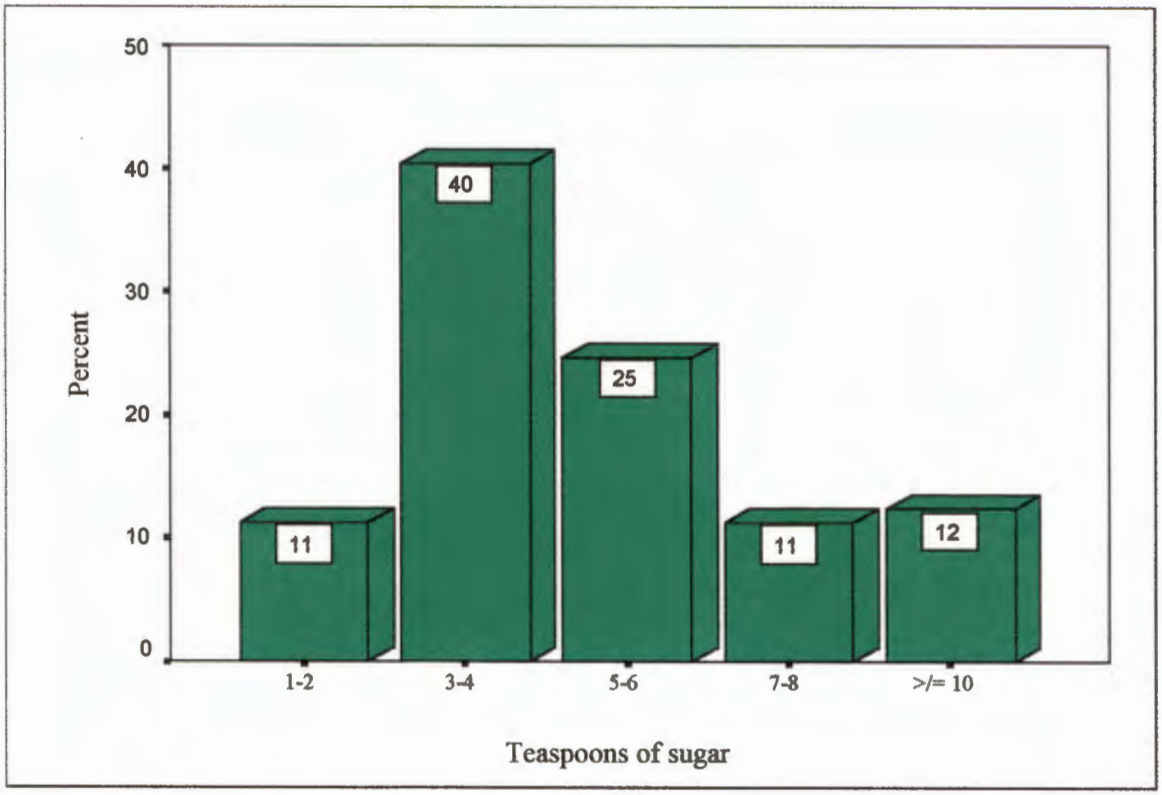

Figure 5.4 Amount of sugar used per day

The results showed that most of the subjects (40 %) used 3-4 teaspoons (15-20ml) of sugar per day whilst 25 % used 5-6 teaspoons (50-60ml) of sugar. Nearly 12 % used more than 10 teaspoons (50ml) of sugar per day.

Table 5.24 Amount of sugar measured in teaspoons used in coffee or tea (n=89)

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	1-2 teaspoons	34	38.2	38.2	38.2
	3-4 teaspoons	48	53.9	53.9	92.1
	5-6 teaspoons	3	3.4	3.4	95.5
	7-8 teaspoons	3	3.4	3.4	98.9
	>/= 10 teaspoons	1	1.1	1.1	100.0
	Total	89	100.0	100.0	

Discussion

From the data in Table 2.24 it is evident that most (54,9 %) of the subjects in this study used 2-4 teaspoons of sugar in their tea or coffee followed by 38,2 % using 1-2 teaspoons. Only one subject (1,1 %) used ten or more teaspoons of sugar.

When asked about sugar usage in specific food items, various answers were received. The results of the answers on this question are summarised in Table 5.25.

Table 5.25 Added sugar in various food items

Food item	No sugar added (% respondents)	1-2 teaspoons added (%)	3-4 teaspoons added (%)	5-6 teaspoons added (%)	> 6 teaspoons added (%)
Porridge	10		38	44	2
Pumpkin		30	33	13	19
Squash	21		70		9
Sweet potatoes	33	29	27		10
Carrots	30	47	22	2	
Tomatoes	25	62	11	1	1

The majority of the subjects (93 %) used white sugar. Only seven percent used brown sugar and the average consumption amounted to 31 gram per person per day. Ninety-three percent of the subjects would be prepared to pay more for sugar when fortified with vitamin A, whereas only 7 % indicated that they were not prepared to pay more for fortified sugar.

The final question asked in the sugar compliance survey 2000, was whether the subjects thought that sugar was "healthy". There was an overwhelming (100 %) positive reaction that sugar was indeed "healthy".

5.6. Conclusions and recommendations

5.6.1 Introduction

In conclusion, according to the results it seems as if the subjects used more sugar during the intervention trial although no statistically significant association between the use of fortified and non-fortified sugar could be drawn. Regarding the sensory qualities of the fortified and non-fortified sugar, only a difference in the smell of the sugar could be detected.

Most of the subjects stored the sugar the correct way and did not leave the containers open when not in use. The majority of the subjects remembered to use the given sugar during the intervention trial. Some of the subjects were not sure why fortified sugar had to be taken. The results indicated that nearly three quarters of the subjects thought sugar to be bad for teeth and weight.

With the exception of coffee or tea, porridge and pumpkin were the food items where most of the subjects used 2-4 teaspoons of sugar. Most of the subjects used 1-2 teaspoons of sugar in other foods where sugar was added regularly. The type of sugar used mostly by the subjects was white sugar

(93%). The subjects indicated that they would be prepared to pay more for sugar should it be fortified.

The sugar fortification study (SFS) provided the opportunity to determine the extent of compliance and acceptability of vitamin A fortified sugar in a community and individuals at risk of iron and vitamin A deficiencies. It was the first time that an intervention study with vitamin A fortified sugar was conducted in South Africa. The objective of this study was to determine if sugar was a suitable vehicle for vitamin A fortification. Through analysis of data gathered by means of focus groups discussions and compliance and acceptability questionnaires this objective was achieved. In the following section the findings of the SFS is summarised and recommendations for implementation are presented.

5.6.2 Summary of main findings

Part of the SFS attempted to determine the extent to which the study population (100 subjects), aged between 13 to 25, complied with the use of vitamin A fortified sugar. The subjects were informed carefully about the purpose of the study and that they would benefit from the vitamin A fortified sugar only if they did not change their diet. Close supervision of distribution and consumption of the sugar assured compliance. Only 11 % of the subjects said they forgot to use the sugar. More than 40 % of the subjects receiving fortified sugar indicated that they used more sugar than normally. This finding contradicts the finding of the FGD where the subjects indicated that they used even less sugar than normally. The reason may be because the subjects wanted to impress the facilitator. However, their commitment to the study is shown by the low dropout rate (17 %).

Taste, colour, odour and texture are concerns for both the consumer and the manufacturer. Vitamin A fortification can affect the sensory properties of the sugar. In this study, the subjects detected a statistically significant difference (P=0, 002) in the smell but not in the colour of the fortified sugar. This difference, however, was not mentioned during the FGD. The subjects used sugar with a variety of foods. According to the findings of this study, the fortified sugar did not change the colour of the foods to which it was added. The results of the FGD's indicated that all the subjects thought that fortified sugar is better than non-fortified sugar with regard to its nutritional content.

Toxicity of vitamin A is caused only with larger doses although the subjects recognised it as a matter of concern during the FGD's. Chronic toxicity is caused with intakes of 3-5 mg vitamin A per day and the most at risk group is pregnant women.

Ninety three percent (93 %) of the subjects indicated that they would be prepared to pay more for fortified sugar. Only 7 % of the subjects used brown sugar. The findings of this study are consistent with those of previous community trials in developing countries where staple food products have been fortified with vitamin A (Meija & Arroyave, 1982:91; Solon *et al.*, 1996:722; Viteri *et al.*, 1995:1161).

5.6.3 Problems experienced

The following concerns were raised during the FGD's:

- The **purple packaging material** used to distribute the sugar was a problem as it caused the subjects to be wary of the product as it was not the usual brown paper bag that sugar was usually purchased in.
- Another problem was the fact that the subjects **could not give or lend sugar** to their neighbours.
- Some family members believed that the sugar was **toxic** and did not use the sugar.
- Measurement of compliance is a complex multidimensional task. Compliance, should therefore, be measured by means of both direct and indirect methods. Only indirect methods were used in this study. The conclusions drawn from this research should be approached with care and cannot be **generalised** because only females aged 13-25 years participated in the project.

5.6.4 Recommendations

Recommendations for further research on measuring compliance and application of these results:

- The appearance, texture and aroma of fortified sugar must be further evaluated in order to assess **consumer acceptability** more widely.
- Determination of the loss of vitamin A during cooking and storage in the household environment must be done for estimating real intakes and the **level of the fortificant** to be used for fortifying the sugar.

Any intervention programme should be supported by nutrition education. In this study, the subjects received nutrition education in the form of focus group discussions. The subjects were informed about VAD and IDA as well as the importance of using the vitamin A fortified sugar. The subjects admitted that the NEP did not change their eating patterns, but that they were more informed about balanced meals after the NEP. They also indicated that the family members should form part of the NEP to ensure compliance. Regarding the implementation of a national vitamin A fortified sugar programme, NEPs should focus in particular on the developing communities in SA. The most needy people in the community should form the main target group of any intervention programme. The population must be informed of the importance of vitamin A for good health and nutrition. They should be informed of the risks of VAD, but it is important that this information does not imply that the consumption of sugar should be increased to control or eliminate VAD.

Non-compliance is universal and should be recognised as normal behaviour (Lask, 1994:27). There are no reliable criteria for predicting any respondent's level of compliance. As with all fortification and other nutrition intervention programmes, a systematic monitoring of compliance and of changes in nutritional status should be established. Measurement of compliance is a complex and multidimensional task in which measurement of the diet is a difficult component because of the different perceptions of the quantifying of food between the researcher and the subjects. In this study different types of measures were incorporated as measures of compliance: e.g. visits to the homes of the subjects, the completion of food diaries and blood tests. The data of compliance are extremely "soft". The degree of compliance is determined by the subject, the act of compliance usually occurs in circumstances where it cannot be observed directly by the researcher, and its appraisal depends on what the subject decides to do and on what he/she reports.

5.6.5 Conclusions

The goal for South Africa was to eliminate VAD as a public health problem by the year 2000. This resolution was adopted by the heads of states at the world summit for children in 1990. As indicated in the pilot study, no vitamin A interventions are currently undertaken in the Vaal Triangle region.

Ninety three percent (93 %) of the families in the NFCS procured sugar (Labadarios, 2000:24) which confirms the results of the SALDRU (SA Labour and Development Research Unit) household expenditure survey, where it was also found that 93% of households nation-wide consume sugar (Hendricks, 1999:4). Nearly the entire South African population thus consumes sugar, therefore making it an attractive vehicle for vitamin A fortification according to several surveys conducted nation-wide. Vitamin A fortification has a minor influence on the sensory properties of sugar, and it is technically feasible, thus making sugar a suitable vehicle for fortification. The results in this study showed that the subjects only detected a difference in smell. This could have been due to the high level of fortification (80 IU/g) in this study compared to the studies in Guatemala (50 IU/g). Although sugar offers specific opportunities, it would be better to fortify a variety of vehicles because it may offer an effective option to alleviate VAD in a diverse population.

Sugar fortification is one of the most promising long-term strategies for delivering vitamin A, largely because of proven efficacy, low recurrent costs to government, its sustainability and its low impact on household behaviours. However, it cannot be assumed that sugar will have a positive impact on all communities with VAD because not all people eat sufficient quantities of white sugar to make the intervention effective. A combination of fortification would, therefore, be more effective. Nevertheless, the results of this study indicated a positive attitude towards sugar consumption and fortification since 86 % of the subjects were aware of the advantages of fortification and 93 % indicated that they would be prepared to pay more for a fortified product because of the advantages it would offer. Although concerns were raised about the effect of sugar on obesity and bad teeth amongst the subjects, 100 % of the subjects was positive that sugar was healthy.

Fortunately, South Africa's sugar industry is concentrated, both geographically and economically, making sugar an attractive vehicle from a monitoring perspective. There are seven sugar-refining mills and 90% of the sugar produced nationally comes from KwaZulu-Natal.

These advantages show that this community based study can lead to the development of a better NEP and the improvement of the quality of life through participation in the NEP and the use of vitamin A fortified sugar. Compliance can be increased by improving the population's knowledge and awareness. In this study the subjects received nutrition and consumer education, but they recommended that all the members of the household should receive this education to assist with compliance. The findings of this study can thus provide data for the development of appropriate nutrition or consumer education messages, it provides a useful benchmark for future comparable studies and it improves future determination of compliance and acceptability of sugar fortified with vitamin A.

In the SFS 93 % of the subjects used white sugar on a daily basis, thus it may indicate that white sugar would be a suitable vehicle for fortification in the Vaal Triangle. According to the findings it can, therefore, be concluded that the subjects did comply with the vitamin A fortified sugar.

CHAPTER 6 : RESULTS OF THE INTERVENTION

EFFECT OF VITAMIN A FORTIFIED SUGAR ON VITAMIN A AND IRON STATUS

6.1 Abstract

Background: The role of vitamin A in iron metabolism has received increased attention during recent years. Studies have shown that vitamin A has protective effects during iron supplementation such as an increased mobilisation of iron. In the South African black population, where high prevalences of iron deficiency anaemia, as well as iron overload, has been described, it would benefit prevention and intervention programmes to know if vitamin A fortification/supplementation would improve iron status in vulnerable groups.

Aims: The major objective of this project was to examine the effect of vitamin A fortified sugar intake on iron status in a random sample of young females aged 13 to 25 years in a double blind parallel placebo-controlled clinical intervention trial. This is the first project in SA that used vitamin A fortified sugar in an intervention trial.

Methods: Anthropometric and baseline measurements were done twice during baseline on 100 randomly selected subject volunteers, randomised into two groups. A drop-out rate of 17 % was experienced after week 0. The sample population thus consisted of an experimental (n=43) group consuming fortified sugar (80 IU vitamin A/gram sugar) and a control group (n=40) consuming non-fortified sugar. Measurements were repeated at weeks 4, 8 and 12 and included anthropometry, blood pressure and blood biochemistry of variables related to iron and vitamin A status. Between and within-group comparisons at baseline and after 12 weeks are reported here. Validated questionnaires were used to determine demographic profiles, food consumption patterns and compliance with fortified sugar.

Results: The dietary intake data showed that these urban black women followed a western-type of diet with relatively low intakes (63,6 g per day) of maize porridge, the staple food in the traditional African diet. A mean daily intake of 31 g sugar was reported. Despite a relatively large dietary variety, with foods from all major food groups appearing in the list of top 22 foods consumed by weight, approximately 58 % of the subjects could be classified as either iron depleted or iron deficient at baseline, while 12 % of subjects had serum retinol levels below 30 µg/dL. Results from food diaries kept during the intervention showed that compliance was acceptable. Small but statistical significant increases in mean serum iron, ferritin, mean cell volume (MCV) and red blood cell count (RBC) and the serum transferrin receptor were observed in the experimental group at 4, 8 and 12 weeks compared to baseline. However, except for serum iron, these changes were also observed in the placebo group.

Significant positive correlations between serum retinol and haemoglobin (Hb), haematocrit (Hct), MCV, serum iron and ferritin were found at baseline. At the end of the study, serum retinol also correlated significantly with red blood cell count (positive) and transferrin (negative).

In the experimental group statistically significant improvements in serum iron variables when approximately 1446 µg RE vitamin A was consumed as fortified sugar, did not rectify the observed iron deficient status and were therefore not clinically significant.

Conclusion: It is concluded that 12 % of these urban black girls and women were vitamin A deficient and 58 % had low serum iron status. It is also concluded that vitamin A fortification alone could not clinically improve iron status, although small statistically significant effects on serum iron were observed. The study confirmed a relationship between serum retinol and iron status variables.

It is suggested that more research on the dietary amounts of vitamin A and iron to address vitamin A and iron deficiencies in this population should be done.

6.2 Introduction

Iron plays an essential role in many metabolic processes including oxygen transport, oxidative metabolism and cellular growth (Lynch, 1997:102). The 4-5 g of iron present in a normal adult can be divided into essential iron, transport iron and storage iron. The essential iron is found in the red blood cells as Hb (70 % of total iron or 30 mg/kg), in muscle as myoglobin (4 % or 4 mg/kg) and in enzymes such as cytochromes, catalases and peroxidases (less than 1 %). Transport iron is found in small amounts in blood, bound by transferrin (± 3 mg). Most of the storage iron is found in the liver (25 %) of which two-thirds consists of ferritin and haemosiderin (Gibson, 1990:350; Hallberg & Asp, 1996:4).

As mentioned above, the total body iron content of a normal adult varies from 3-5 g. Hb iron constitutes approximately 60-70 % of total body iron (1,5-3,0 g). Cells of the reticulo-endothelial system phagocytose aged or damaged red blood cells. Nearly all the iron derived from the breakdown of Hb is released into the circulation, and re-utilised by marrow erythroblasts for haemoglobin synthesis. The amount of storage iron varies from 1-2 g. Storage of iron occurs in two forms, namely ferritin and haemosiderin of which ferritin is predominant. Storage iron is divided between the reticulo-endothelial cells (mainly spleen, liver, and bone marrow) and skeletal muscle. Between 3-4 mg iron is present in the plasma, where it is bound to a specific protein, transferrin. The function of the transferrin is transporting iron (Hallberg & Asp, 1996:4-6).

A number of haematological and biochemical tests are used, alone or in combination, to assess iron status. Serum iron, total iron binding capacity (TIBC) and transferrin saturation are measured to assess iron supply to tissues. Reduced erythrocyte size, measured as mean corpuscular volume (MCV), and Hb levels can be measured as an indication of a significant iron deficiency. Red cell distribution width (RDW) is a measure reflecting the variability of erythrocyte size and this value is elevated in iron deficiency (Mahan & Escot-Stump, 2000:388; Ziegler & Filer, 1996:281).

It is important to distinguish between anaemia and iron deficiency anaemia (IDA). Anaemia is present when Hb production is reduced to such an extent that Hb concentration or Hct falls below 90-95 % of the normal range. Iron deficiency may not be the only cause of anaemia as a notable infection or mild inflammatory disease can cause anaemia. IDA is diagnosed when anaemia is accompanied by laboratory evidence of iron deficiency namely low serum ferritin (Ziegler & Filer, 1996:281).

In theory, iron depletion is categorised into three stages, ranging from mild to severe. The first stage, namely *level I* iron depletion, involves decreased iron stores as measured by decreased serum ferritin. No physiological consequences are present, but an increased vulnerability from long-term marginal iron balance may progress to a more severe deficiency. With low iron stores, a compensatory increase in iron absorption is present; preventing progression to the more severe stages (Ziegler & Filer, 1996:281).

The second stage, *level II* iron deficient erythropoiesis, is characterised by biochemical changes reflecting a lack of sufficient iron for normal Hb and other iron compound production. This stage is characterised by decreased transferrin saturation or increased erythrocyte

protoporphyrin, serum transferrin receptor (sTrF) or RDW. The Hb levels are still normal and this stage is often referred to as iron deficiency without anaemia (Ziegler & Filer, 1996:282).

The third stage of iron depletion, *level III,* is frank iron deficiency anaemia varying in severity according to how low the Hb concentration is. Iron deficiency can result in severe anaemia when the Hb concentration is <70 g/L (< 7,0 g/dL) defined by the WHO (Ziegler & Filer, 1996:282). The Hct is defined as the volume fraction of packed red cells. During iron deficiency the Hct falls only after Hb formation has become impaired. A marginally low Hb value may thus be associated with a near-normal Hct, but both are reduced in severe iron-deficiency anaemia (Gibson, 1990:354). Table 6.1 is a summary of the stages of iron status reflected by various haematological and biochemical tests (Ziegler & Fidler, 1996:282). Table 6.2 is an indication of the female haemoglobin and haematocrit levels below which anaemia is present (MI, 1998:8).

Table 6.1 Stages of iron status reflected by various haematological and biochemical tests (Ziegler & Filer, 1996:282).

Variable	Normal	Depleted stores	Iron deficiency	IDA
Serum transferrin	N	↓	↓	↓↓
Transferrin saturation	N	N	↓	↓
Erythrocyte protoporphyrin	N	N	↑	↑↑
MCV	N	N	N	↓
Hb	N	N	N	↓

Table 6.2 Female haemoglobin and haematocrit levels below which anaemia is present (MI, 1998:8)

	Critical level	
Age/physiological status	**Hb (g/dL)**	**Hc (%)**
Non-pregnant female	12,0	36,0
Pregnant female	11,0	33,0
Pregnant female – severe anaemia	7,0	-
Pregnant female – very severe (life threatening)	4,0	-

The role of vitamin A in iron metabolism has received increased attention in recent years (reviewed by Van Stuijvenberg et al., 1997:41). Several studies demonstrated an association between vitamin A status and iron or haematological status (refer Section 1.8). A direct correlation between serum retinol and Hb levels in women and children has been observed in a number of surveys. An association between VAD has also been demonstrated in that VAD affects iron transport and red blood cell production directly. Observations made in human and animal studies have shown that the iron deficiency anaemia associated with marginal VAD is characterised by a decrease in serum iron concentration, total iron-binding capacity, transferrin saturation and an increase in storage iron in the liver and spleen (Lynch, 1997:103). It has also been shown that vitamin A supplementation led to an improvement in iron status. In populations with low serum retinol levels vitamin A supplementation alone may result in increased Hb concentrations (Lynch, 1997:103; Van Stuijvenberg et al., 1997:41). This interaction between vitamin A and iron status may have implications for iron intervention programmes when

undertaken in areas where VAD is prevalent (Van Stuijvenberg et al., 1997:41). The same may be true for vitamin A intervention programmes where iron deficiency is prevalent.

It has been mentioned in the literature survey that, while there is a high prevalence of IDA in especially young African girls, there is also an increased susceptibility to iron overload – probably not only of increased intakes by men of beer brewed in iron containers, but also because of a genetic factor (Eastwood, 1997:260; Mahan & Escott-Stump, 2000:131; Weigley et al., 1997: 202). Therefore, fortification of staple foods such as maize or bread, which is eaten by both young girls and adult men, may address the problem of iron deficiency in the girls (and pregnant and lactating women) but at the same time may aggravate the problem of overload in adult men.

The hypothesis of this study, namely **that increased vitamin A intake through consumption of vitamin A fortified sugar will improve iron status and decrease plasma fibrinogen levels of a group of black South African women,** is therefore based on the evidence that there is an interaction between vitamin A and iron absorption, transport and function, a possible interaction between plasma fibrinogen and vitamin A status, and therefore also a possible relationship between iron status and fibrinogen (refer Figure 2.7). In this chapter the results of the effect of vitamin A fortification on vitamin A and iron status variables are reported.

This chapter will therefore attempt to prove that iron deficiency is addressed by increasing vitamin A status of young black South African women. If this is true it could mean that lower levels of iron fortification or supplementation may be necessary to address iron deficiency problems, without increasing the risk of an iron overload when genetically susceptible individuals consume iron fortified products.

Therefore, the major objective of this part of the project was to perform a clinical intervention trial under controlled conditions to examine the effect of dietary intake of sugar fortified with vitamin A on vitamin A- and iron status in young females aged between 13 and 25 years.

6.3 Methodology

6.3.1 Study design, subjects and intervention

The study population consisted of 100 females aged 13-25 years old that were randomly selected from clinics, schools and tertiary institutions in the Vaal Triangle. The inclusion criteria were female gender, ages between 13 and 25 years, non-pregnant and non-lactating, apparently healthy and a daily sugar consumption. Written informed consent was obtained from the subjects who were over 21 years old or parents and guardians of the younger ones prior to the study. The study was approved by both the Ethics Committees of the Vaal Triangle Technikon and Potchefstroom University for CHE. The survey was conducted between February and May 2000 (refer Chapter 4, Section 4.5). The sample population of 100 was randomly divided into two groups of equal size, an experimental and placebo group. The experimental group consumed vitamin A fortified sugar for a period of 12 weeks. The control group consumed non-fortified sugar for the same period of 12 weeks. All the subjects were "blinded" to the study and received anonymous intervention in that they knew that sugar was the intervention product, but did not know what it contained.

The sugar supplied to the subjects was fortified by Roche Vitamins and Fine Chemicals. One gram of sugar contained 80 IU vitamin A. The vitamin A fortified sugar was indistinguishable from the non-fortified sugar (placebo), were packed identically in quantities of 5 kg purple bags. The sugar was issued to the subjects every four weeks for a period of 12 weeks (refer Chapter 4, Section 4.2). None of the researchers who measured the subjects or analysed blood samples knew which subjects received the fortified sugar. This was therefore a double blind, placebo-controlled, parallel study lasting 12 weeks. Measurements were made twice at baseline and then after 4, 8 and 12 weeks during the intervention.

6.3.2 Laboratory methods

Time of measurements

The double baseline measurements, one week apart, as well as measurements at 4, 8 and 12 weeks, included measuring iron status by haematological parameters namely Hct, Hb, MCV and RBC, serum iron, ferritin, transferrin and also serum retinol. Week 0 values were mean values of each participant of two measured at baseline. Subjects were at least ten hours fasted for blood sampling.

Blood sampling

The blood collected was as follows:

- 5 ml EDTA (whole blood) for the full blood count and measurement of haematological markers: Hct, MCV, RBC, Hb and white blood cell count (WBC). These analyses were done directly after the sample was taken.
- 20 ml in silicone-coated tubes for preparation serum for the analysis of retinol, iron, ferritin and transferrin. Serum samples were frozen and kept at –80 ° C until analysis in one batch.

The method of blood sampling is described in Chapter 4 (Section 4.6.2).

Table 6.3 A summary of methods used to determine serum variables

Variable	Method	Laboratory	Coefficient of variation*
Full blood count (FBC)	Coulter counter; ABX $MICROS_{CT}$	Vaal Triangle Technikon	
Haematocrit (Hct)	Numeric integration	Vaal Triangle Technikon	24,96
Haemoglobin (Hb)	Cyanmethaemoglobin-colorimetric method	Vaal Triangle Technikon	22,33
Mean cell volume (MCV)	Impulse generating	Vaal Triangle Technikon	13,58
Red blood cell count (RBC)	Cell counting – autoanalyser	Vaal Triangle Technikon	29,46
White blood cell count (WBC)	Cell counting – autoanalyser	Vaal Triangle Technikon	68,09
Serum iron	Colorometric; Roche Unimate 5 Iron	Vaal Triangle Technikon	71,89
Serum ferritin	Immunoturbidity; Roche Unimate 3 FERR	Vaal Triangle Technikon	119,66
Transferrin	Immunoturbidity; Roche Unimate 3 TRSF	Vaal Triangle Technikon	42,85
s-Transferrin receptor	Orion Diagnostica Immunoturbidity assay at 600 nm	Vaal Triangle Technikon	5,10
Retinol	High Performance Liquid Chromatography (HPLC)	Medical Research Council (MRC), Tygerberg	64,28

* Coefficient of variation: $\frac{\text{standard deviation}}{\text{mean}} \times 100\ \%$ for reported measurements on control data

Researcher's contribution

The researcher's contribution was planning and co-ordinating the project by completing the ethical permission forms, making arrangements with the schools and clinics, random sampling, drawing up and arranging statistical analyses of all questionnaires, co-ordinating biochemical analyses with the laboratories, and training of field workers. Other responsibilities included financial administration and catering and transport arrangements.

Serum iron

The method used for serum iron measurement was the Roche Unimate 5 Iron (8X30 ml) art. 07 5181 2 Colorimetric test with Ferrozine ® or ascorbic acid. This is an in vitro diagnostic reagent system for quantitative determination of iron in serum. 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. The normal reference values for females are 8,8-27,0 µmol/L (49-151 µg/dL) and for males 9,5-29,9 µmol/L (53-167 µg/dL). This level is usually low during adolescence since most of the absorbed iron is needed for growth and the expanding red cell mass (Hallberg & Asp, 1996:5).

Serum ferritin

Serum ferritin levels fall during iron deficiency even before the characteristic changes in serum iron and total iron-binding capacity (Gibson, 1990:364). An in vitro diagnostic reagent system intended for use in the quantitative immunological determination of ferritin in serum was used, namely the Roche Unimate 3 FERR (1X20 ml) art 07 5182 0 Immunoturbidimetry assay at 600nm ®. The principle of this test is that human ferritin forms a precipitate with the suspended anti-ferritin coated latex particles. The normal reference values for females are 10-12 ng/mL and for males 20-30 ng/mL.

Serum transferrin

The Roche unimate 3 TRSF (4X1X5 mL) art 07 3708 8 Immunoturbidimetric assay at 340 nm ® was used to measure serum transferrin. This method also makes use of an in vitro diagnostic reagent system where human transferrin forms a precipate with a specific antiserum. The normal reference values are 2,0-3,6 g/L (200-360 mg/dL). Transferrin plays an important role in iron metabolism in that it maintains extracellular iron in a soluble form that is suitable for cellular uptake. It also regulates the supply of iron to cells by influencing its distribution within the body and the availability to individual cells. Normally the plasma of an adult contains of about 3 mg of transferrin-bound iron and the iron turnover rate is approximately 30-40 mg per day. Approximately 80 % of this iron is used for haemoglobin synthesis (Hallberg & Asp, 1996:4).

Blood cell counts, haemoglobin and haematocrit

Whole blood analyses were done on a haematology auto-analyser, the ABX MICROS$_{CT}$ within 6 hours of blood collection:

* The cell counting principle is based on an impedance variation penetrated by the passage of the 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. 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. Normal reference values are:

  - MCV 80-97 fl
  - RBC 3,80-5,80 x 10^6/mm^3
  - WCC 3,5-10,0 x 10^3/mm^3

* To determine the Hb, venous EDTA blood is mixed with a reagent which 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. Three different sets of criteria can be used for the interpretation of Hb data:

  - The Hb cut-off points derived from the NHANES II white population as 11,8 g/dL for females aged 11-14 years old, 11,7 g/dL for females aged 15-19 years old and 11,9 g/dL for females aged 20-44 years old.
  - The concentration of Hb below 12 g/dL where anaemia is likely to be present for females older than 14 years.
  - The criteria given as the upper limit of "moderate risk" of deficiency as 11,5 g/dL for females aged 13-16 years old and 12 g/dL for females older than 17 years old (Gibson, 1990:353).

* 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 (MCV). The normal range is 80-97 fl.

* The Hct is measured as a function of the numeric integration of the MCV and the normal range is 35-50 %.

6.3.3 Dietary intakes

A 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 (refer Section 4.6.4). The dietary intake of the randomly selected sample population was reference checked by means of completing a food diary four times during the intervention period of 12 weeks. These diaries consisted of a selection of foods rich in vitamin A and iron. The main purpose of measuring the intakes with

the food diaries was to measure compliance of the dietary intake of vitamin A fortified sugar (see Annexure 13).

6.3.4 Clinical examination

The physical signs that were assessed by specially trained nursing sisters during the study are summarised in Table 6.4.

Table 6.4 Physical signs indicative of vitamin A and/or iron deficiency (Gibson, 1990:580)

Normal appearance	Signs associated with deficiency
Eyes Bright, clear, no sores at corners of eyelids, healthy pink and moist membranes, no prominent blood vessels or sclera	Pale eye membranes, redness of membranes, Bitot's spots, dull appearance of cornea (xerosis), soft cornea (keratomalacia), scar on cornea
Tongue Deep red, not swollen or smooth	Swelling, bluish colour
Face Healthy appearance, not swollen	Skin colour loss, pale
Cardiovascular Normal blood pressure for age	Elevated blood pressure

6.3.5 Anthropometry

The WHO recommended height-for-age and BMI-for-age as anthropometric indicators for adolescents (WHO, 1995:271). For this reason weight, height, weight-for-height, and hip and waist circumference were recorded during weeks 1,4,8 and 12 of the trial. Body mass index (BMI) was calculated using the formula weight (kg)/height (m^2) (refer Section 4.6.6).

6.3.6 Statistical analysis

The data were computerised and the SPSS ® program, version 6.0 used to analyse data. Paired t-tests were done to compare for significant differences and a multivariate analysis was done to examine relationships between variables (refer Section 4.6.7).

6.4 Results

6.4.1 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 6.5.

Table 6.5 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.

6.4.2 Dietary patterns of the sample population

The QFFQ's were analysed to determine the food items most often consumed by the sample, and are summarised in Table 6.6. The data in Table 6.7 indicate the results of the food diaries.

Table 6.6 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 6.6 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. 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 specially rich source of iron (red meat, spinach) appeared in this list.

Table 6.7 Analysis of food diaries (P = 0,05) : daily mean intakes of the total group

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 RE)	1068±560	1201±959	1220±843	1349±954	1000*/600**
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.

Table 6.7 shows 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 13). The vitamin A added to the sugar, which the experimental subjects consumed, is not included in the analysis.

Table 6.7 indicates that 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 was reduced gradually until the end of the study. In the focus group discussions the subjects reported that more sugar was consumed because they received it for free (refer Section 5.5.1).

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) were 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.

6.4.3 Haematological variables

The haematological and biochemical data are summarised in Table 6.8. Only the subjects for whom data was available for weeks 0, 1, 4, 8 and 12 are included in this table as a further 10 subjects left the study between weeks 1 and 12 (n=83). The mean haematological measurements for the subjects at baseline were similar for both the experimental and placebo groups. A notable exception, however, was the significantly higher number of subjects in the experimental group with suspected IDA, which are shown in Table 6.9.

Table 6.8 shows that in the experimental group, most of the iron status variables were within the normal ranges at baseline, except for mean Hct which was slightly lower and the mean serum transferrin receptor which was increased. Serum iron was slightly, but statistically significant, higher at weeks 4, 8 and 12 in the experimental group, compared to baseline. In this group, serum ferritin increased significantly after 4 weeks but returned to baseline levels at weeks 8 and 12. Transferrin was significantly lower at weeks 4, 8 and 12, as were RBC and the transferrin receptor. No changes in Hb and Hct were observed. Mean serum retinol of the experimental subjects was slightly, but significantly, increased during weeks 8 and 12. Except for serum iron, the same significant changes were observed in the placebo group.

Table 6.8 Changes in serum variables indicative of iron and vitamin A status during experimental period of 12 weeks

		Experimental group (n=43)				Placebo group			
Variable	**Normal Range**	**Week 0/1**	**Week 4**	**Week 8**	**Week 12**	**Week 0/1**	**Week 4**	**Week 8**	**Week 12**
Serum iron (mean) ± SD	8,8-27,0 µmol/L	11,9 [a,b,c] ±6,0	12,9 [a] ±7,8	12,8 [b] ±7,2	12,2 [c] ±8,8	14,2 ±5,2	12,2 ±5,2	14,4 ±5,6	13,4 ±5,9
Ferritin (mean) ± SD	10-12 ng/mL	26,5 [a] ±28,4	27,4 [a] ±26,0	25,3 ±27,1	24,2 ±29,0	25,4 [a] ±17,4	26,2 ±18,0	31,5 [a] ±29,1	25,6 ±19,7
Transferrin (mean) ± SD	2,0-3,6 g/L	3,41 [a,b,c] ±0,5	3,11 [a] ±0,5	3,14 [b] ±0,5	3,24 [c] ±0,5	3,26 [a,b,c] ±0,5	2,98 [a] ±0,5	2,89 [b] ±0,4	3,10 [c] ±0,4
Red Blood Count (mean) ± SD	3,8-5,8 X 10^6	4,16 [a,b] ±0,3	4,04 [a] ±0,3	4,10 [b] ±0,3	4,05 [a] ±0,3	4,30 [a,b] ±0,3	4,20 [a] ±0,3	4,16 [b] ±0,3	4,20 [a] ±0,4
Haemoglobin (mean) ±SD	11-16,5 g/dL	12,9 ±1,7	12,2 ±1,9	12,8 ±2,0	12,6 ±2,0	13,4 ±1,1	12,9 ±1,1	13,2 ±1,1	13,2 ±1,1
Mean Cell Volume (mean) ± SD	80-97 fl	82,7 [a,b] ±8,8	82,3 ±8,9	83,3 [a] ±8,8	83,4 [b] ±8,9	83,6 [a,b] ±6,3	83,7 ±6,1	84,6 [a] ±5,9	84,6 [b] ±6,0
Haematocrit (mean) ± SD	35-50 %	34,4 ±4,1	33,2 ±4,6	34,2 ±4,8	33,8 ±4,5	35,9 ±2,6	34,6 ±2,7	35,1 ±2,8	35,4 ±2,8
S-Transferrin receptor (mean) ± SD	0,8-2,3 mg/L	2,57 [a,b,c] ±1,7	2,23 [a] ±1,5	2,15 [b] ±1,4	2,19 [c] ±1,5	1,96 [a,b] ±0,4	1,72 [a] ±0,4	1,67 [b] ±0,4	1,70 [a] ±0,4
Serum retinol ± SD	>= 30 µg/dL	47,0 [a,b] ± 17,2	43,9 ± 14,4	51,9 [a] ± 14,4	49,2 [b] ± 14,8	43,3 [a,b] ± 14,7	43,3 ± 12,0	49,5 [a] ± 14,0	47,6 [b] ± 15,5

Means with the same symbol differs significantly within groups. The same subjects have been used throughout the trial, and paired t-tests were used for comparison ($P < 0,05$).

The results in Table 6.9 indicate that 44 % of the experimental group and 40 % of the placebo group had a normal iron status at baseline indicating that 42,2 % of these subjects had a normal iron status. After twelve weeks of dietary intake of vitamin A fortified sugar, only 33 % of the experimental group could be classified as having a normal iron status whereas the prevalence in the placebo group stayed the same (40 %). Serum iron levels of the experimental group were significantly lower at baseline than that of the control group (11,8 versus 14,4 µmol/L). Only the serum iron levels were adjusted for the control group as shown in Table 6.10.

Table 6.9 therefore indicates that in the experimental group, more subjects (n = 29) could be classified as having abnormal iron status at 12 weeks compared to baseline (n = 27), while the number stayed the same in the placebo group (n = 16).

Table 6.9 Summary table of the iron status of the respondents

		Baseline		**Week 4**		**Week 8**		**Week 12**	
Iron status		**n**	**%**	**n**	**%**	**n**	**%**	**n**	**%**
Normal	E	19	44	16	37	13	30	14	33
	P	16	40	12	30	16	40	16	40
Iron depletion	E	6	14	1	2	5	12	4	9
	P	10	25	6	15	2	5	7	18
Iron deficient erythropoiesis	E	8	19	8	19	12	28	11	26
	P	9	23	12	30	11	28	7	18
Iron deficiency anaemia	E	10	23	18	42	13	30	14	33
	P	5	13	10	25	11	28	10	25
Missing values	E	5	10	5	10	5	10	5	10
	P	12	23	12	23	12	23	12	23

E = experimental group P = placebo group
n = number of subjects who completed the study
% = percentage of subjects who completed the study

Table 6.10 Statistical significance of adjusted serum iron values

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means					
		F	Sig.	t	df	Sig. (1-tailed)	Mean Difference	Std. Error Difference	95% Confiden Lower
Difference of week 12 - baseline	Equal variances assumed	8.973	.004	.698	81	.487	.9319	1.3350	-1.2895
	Equal variances not assumed			.713	63.940	.478	.9319	1.3071	-1.2497

Serum retinol values for all the subjects who completed the study (n=83) are given in Table 6.11 (missing subject numbers represent the drop-outs). Mahan and Escott-Stumpf (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 88 % (73/83) had normal serum retinol levels (≥ 30 µg/dL).

Table 6.11 shows that of the experimental group, 23 (53,5 %) of the subjects had an increase of more than 5 μg/dL serum retinol at week 12 compared to baseline, while 29 % showed no change and 25,6 % had lower levels. Comparative figures for the placebo group were 37,5 % who showed an increase, 40 % who had no change and 22,5 % who showed a decrease.

At baseline, 10 subjects, five in each group (11,6 and 12,5 %) had serum retinol levels below 30 μg/dL. After 12 weeks, three subjects in the experimental group (7,0 %) and seven in the placebo group (17,5 %) had serum retinol levels below 30 μg/dL. In the experimental group, in four of the five subjects, serum retinol increased to levels > 30 μg/dL, but two other subjects showed decreases to levels < 30 μg/dL after 12 weeks.

Table 6.11 Comparison of individual serum retinol levels (µg/dL) at baseline and after 12 weeks

No	Experimental group		Change	No	Placebo group		Change
	Baseline (µg/dL)	End (µg/dL)	(> 5 g/dL)		Baseline (µg/dL)	End (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	↓
77	37,0	37,4	↔	95	59,8	68,9	↑
78	56,9	39,4	↓	96	63,3	84,7	↑
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 %	
n<30 µg/dL	$5^{11,6\,\%}$	$3^{7,0\,\%}$		n<30 µg/dL	$5^{12,5\,\%}$	$7^{17,5\,\%}$	
n 30-40 µg/dL	9	8		n 30-40 µg/dL	15	12	

↑ increase, ↓ decrease, ↔ no change

Table 6.12 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.

Table 6.12 Correlations between serum retinol and other serum, blood and plasma variables 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)

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).

6.5 Discussion

6.5.1 Introduction

After the association between vitamin A and iron had first been mentioned in 1926, it took years before the subject became of interest. In the late 1970's several researchers, especially Hodges *et al.* and Meija *et al.* tried to elucidate this relationship (quoted by Bloem *et al.*, 1989:335). This study attempts to define the role of fortified vitamin A intake on iron status of females in their reproductive years. The value of the study is that it could indicate whether vitamin A fortification of sugar would have beneficial effects on iron metabolism and status, thereby reducing the need for iron fortification.

The interpretation and discussion of the observed changes in iron status variables of the experimental group should be done against the following background that 12 % of the subjects had low (< 30 µg/dL) serum retinol levels, 60 % could be classified as having low iron status in the total group, mean intakes of vitamin A exceeded the recommended intakes of 600-1000 µg RE, varying from 1068 to 1349 µg RE over the 12 week period. The results indicate that

approximately 60 g sugar was eaten daily, which provided, at a fortification level of 80 IU vitamin A per gram sugar, an additional 4800 IU or 1446 µg RE vitamin A per day to the experimental group. The results of the food diaries also indicated that although not 100 %, compliance of intake to the distributed sugar was acceptable.

The salient questions that should be addressed and discussed in this study are:

- Did the additional vitamin A intake by the experimental group influence their vitamin A status?
- Did the additional vitamin A intake by the experimental group influence their iron status, and if so, to what extent?
- What are the relationships between vitamin A and iron status?
- What are the practical implications of these results?

6.5.2 Effects of vitamin A fortified sugar on vitamin A status

Despite the higher mean intakes of vitamin A, 10 (12 %) of the subjects could be classified as VAD at baseline, having serum retinol levels < 30 µg/dL. Although this prevalence is lower than the 30 % found in pre-school children in the SAVACG study (Vorster *et al.*, 1997c:11), it does indicate that marginal VAD is present in urban black women aged 13-25 years. Mean serum retinol increased from 47,0 to 49,2 µg/dL after 12 weeks in the experimental group receiving fortified sugar. Individual data showed that 54 % of these subjects showed an increase of > 5 µg/dL in serum retinol over the 12 weeks, while 26 % showed a decrease and 21 % no change. In the placebo group the comparative figures were 38, 23 and 40 %.

These results suggest that more subjects in the experimental group showed an increase in serum retinol levels (54 % versus 38 %) while more subjects in the placebo group showed no change (40 % versus 21 %). This effect of the intake of vitamin A fortified sugar is also illustrated by the fact that whereas both groups had five subjects at baseline with levels below 30 µg/dL, after 12 weeks the experimental group had only three and the placebo group seven in this category. Serum retinol in healthy adults is maintained between 40 and 50 µg/dL. Excess retinol is stored in the liver (Mahan & Escott-Stumpff, 2000:70). In subjects with sufficient vitamin A intake, serum retinol is therefore not the best indicator of vitamin A status. However, although smaller than expected, probably because of mean serum retinol in the normal range and relatively high dietary intakes, it does seem that the fortified sugar improved vitamin A status in the experimental subjects.

6.5.3 Effects of vitamin A fortified sugar on iron status

The significant changes in serum iron of the experimental group, compared to no significant changes in the placebo group, suggest that the additional vitamin A consumed did influence iron metabolism. However, the high percentage (56 %) of experimental subjects with an abnormal iron status (depleted plus iron deficient erythropoiesis plus IDA) could not be lowered by the extra vitamin A intake. The pattern of changes in the iron status variables is intriguing, and is shown for the experimental group in Table 6.13.

Table 6.13 Patterns of changes* in iron status variables of the experimental group

Variable	Week 4	Week 8	Week 12
Serum iron	↑	↑	↑
Ferritin	↑	↔	↔
Transferrin	↓	↓	↓
Red blood cell count	↓	↓	↓
Haemoglobin	↔	↔	↔
Mean cell volume	↔	↑	↑
Haematocrit	↔	↔	↔
S-transferrin receptor	↓	↓	↓
Serum retinol	↔	↑	↑

* Statistically significant ($P \leq 0,05$) compared to baseline: ↑ increase
↓ decrease
↔ no change

The transferrin receptor is a key iron-related protein that regulates the influx of transferrin bound to iron to all body cells (Cook *et al.*, 1995:50). In this study the soluble form of the receptor reflects the total body mass of cellular transferrin receptor, and is accepted as a measure of total erythropoiesis and for detection of iron deficiency according to Cook *et al.* (1995:50). In over 90 % of patients with IDA there is a 3-5 fold elevation of the serum receptor levels. The receptor:ferritin ratio has been used to identify the development of iron deficiency in patients with ongoing infection or inflammation (Cook *et al.*, 1995:52). The increase in ferritin (at 4 weeks) and the decrease in the serum transferrin receptor, coupled to the increases in serum iron and decreases in transferrin, strongly suggest small but significant improvements in mean iron status of these subjects. However, except for serum iron (which was higher in the placebo group during baseline) these changes were also observed in the placebo group. It seems, therefore, that the changes in iron status variables found in the clinical trial, cannot be ascribed to the additional vitamin A intake. They were probably related to changes in dietary iron over the 12 week period.

Another important aspect to be taken into consideration is the possible relation to infection. Infections can lower the serum iron- and Hb levels (Meija & Chew, 1988:600). No conclusions of infections could be drawn, as no direct evidence on infections, including HIV/AIDS was gathered. Because serum ferritin is an acute phase reactant, the levels increase during infections, whereas transferrin, a reverse acute phase reactant, decreases during infections (Herbert, 1992:1506). The small but significant decreases in transferrin in both groups suggest that mild infections could have played a role in the observed changes in iron status variables.

6.5.4 Relationships between vitamin A and iron status

A summary of the correlation coefficients between serum retinol and some of the other biochemical variables are found in Table 6.12. The significant correlations observed between serum retinol and iron status variables in this homogenous group of subjects confirm a relationship between vitamin A and iron status. The results reported in Table 6.12 clearly indicate that serum retinol is related to both stored iron (ferritin) and circulating iron (serum iron, transferrin) as well as to iron uptake by tissues (serum transferrin receptor). These results

confirm observations from other populations (Bloem *et al.*, 1989:335; Fishman *et al.*, 2000:128-129; Suharno *et al.*, 1993:1327).

However, these results also confirm the role of vitamin A in haemopoiesis. Serum retinol showed sustained positive correlations with Hb, Hct and mean cell volume. The emergence of the significant correlation with red blood cell count after only 50 % of the subjects consumed vitamin A fortified sugar, further suggest that vitamin A plays an important role in haemopoiesis. The relationships with Hb and Hct were actually stronger at the end of the study period, supporting the role of vitamin A in haemopoiesis. These relationships were calculated for the total group to ensure meaningful relationships. It is suggested that in follow-up studies with larger numbers, vitamin A supplemented subjects could be divorced from non-supplemented subjects. This study was not designed to explore the mechanisms of these relationships and further research is suggested.

6.5.5 Practical implications of these results

The first important observation of this study was that of these randomly selected volunteer urban black girls and women aged 13 to 25 years of age, following a western-type diet, 12 % could be classified as being marginally vitamin A deficient (serum retinol levels < 30 µg/dL) and 58 % as having an abnormal iron status. Clearly, this population group, young women in their early reproductive years, needs attention, especially regarding their iron intakes and status. This study indicated that although additional vitamin A intake improved serum retinol and iron in some subjects, no clinical beneficial effect on iron status was observed. This suggests that in the design of suitable intervention programmes, not only vitamin A intakes, but also iron intakes need to be addressed. The results further suggest that increased vitamin A intakes could mean that less additional iron could be needed to rectify the iron deficiency. However, this will have to be tested in a suitable iron deficient group with graded amounts of additional iron and vitamin A.

6.6 Conclusions

It is concluded that 12 % of these randomly selected volunteer urban black girls and women were vitamin A deficient, while 58 % had low iron status. It is further concluded that although approximately 1446 µg RE additional vitamin A in the form of fortified sugar improved the serum iron significantly, these effects were not large enough to clinically improve iron status. The reported relationships between serum retinol and iron status variables were confirmed. It is suggested that an increased iron intake, either through fortification or increased intakes of iron-rich foods would be necessary to address the iron deficiency problem in these women. Further research to assess whether simultaneous fortification with vitamin A and iron would decrease the amount of iron needed to rectify iron deficiency in this population is necessary.

CHAPTER 7 : RESULTS

EFFECT OF VITAMIN A FORTIFIED SUGAR ON FIBRINOGEN LEVELS

7.1 Abstract

Background: Epidemiological studies indicated that vitamin A intake and status are associated with plasma fibrinogen levels. Plasma fibrinogen, a risk factor for coronary heart disease and stroke, is elevated in black South African women.
Aims: To examine the influence of vitamin A fortified sugar on plasma fibrinogen concentration of young urban black South African women in a double blind, placebo-controlled parallel trial.
Methods: A hundred randomly selected volunteers aged 13-25 years were randomly allocated to an experimental and placebo group. Of these, 83 completed the 12-week trial. Dietary intakes, anthropometric variables, serum retinol, white blood cell counts and plasma fibrinogen were measured twice at baseline and after that at 4, 8 and 12 weeks of the intervention. The experimental group received vitamin A fortified sugar (80 IU/g) and the placebo group non-fortified sugar. Both the subjects and researchers doing the measurements were blinded to the intervention.
Results: Weekly dietary diaries indicated that these subjects consumed approximately 60 g sugar daily, resulting in an additional vitamin A intake of approximately 1446 µg RE per day by the experimental group. Plasma fibrinogen levels were significantly lower in the experimental group after 4 and 8 weeks but not after 12 weeks, a period when the mean BMI was significantly increased. No significant changes in plasma fibrinogen of the placebo group were observed.
The results indicated that mean plasma fibrinogen of these subjects were relatively high and that the consumption of the additional vitamin A by the experimental subjects could have been responsible for the observed decreases in fibrinogen at weeks 4 and 8 of the study period. They also indicate that the increased BMI and/or infections could have resulted in the increased mean fibrinogen level seen at week 12 of the experimental group. No relationships between plasma fibrinogen and serum retinol were found.
Conclusion: It is concluded that despite no relationships between plasma fibrinogen and serum retinol, this study suggests that increased intake of vitamin A is associated with a decrease in plasma fibrinogen. However, more research is needed to confirm this effect.

7.2 Introduction

The evidence that coagulation factors may be important in the aetiology of atherosclerotic vascular disease was initially derived from case-controlled and angiographic studies in which elevated mean fibrinogen levels were manifested in men with clinical coronary heart disease (CHD). More evidence came from a number of prospective epidemiological studies indicating that plasma fibrinogen levels were definitely predictive of CHD, stroke and total mortality (Krobot *et al.*, 1992:780).

Fibrinogen is a mediator in chronic infection, inflammation and coronary heart disease (Jousilathi *et al.*, 1997:506). The clear association between high fibrinogen levels and the risk of CHD and stroke 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.*, 1986:1050). 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 CHD, stroke and lower extremity arterial disease. As evidence to implicate fibrinogen as a risk factor for cardiovascular disease (CVD) accumulates, 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 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 which 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 haemostasis factors (Rogers *et al.*, 1988:197).

In a study using a rat model, retinoic acid increased fibrinolysis by selectively increasing tissue plasminogen activator (tPA) without affecting plasminogen activator inhibitor (PAI-1), urokinase plasminogen activator (uPA), plasminogen or alpha-2-antiplasmin. These results were confirmed in a vitamin A deficient rat model. A study in healthy elderly women indicated that those who took micronutrient supplements and who had significant higher levels of serum vitamin A, retinol-binding protein, pyridoxal and pyridoxal phosphate also had significantly lower plasma fibrinogen levels. The Swedish MONICA study also found that high plasma retinol levels were associated with lower plasma fibrinogen levels, as well as with low tPA and high PAI-1 levels (reviewed by Vorster *et al.*, 1997b:120).

If the hypothesis is accepted that elevated plasma fibrinogen levels are causally linked to atherogenesis and to its thrombo-embolic complications, lowering fibrinogen levels should retard the atherosclerotic process and reduce cardiovascular events, which is of considerable clinical and public health interest. Socio-economic indices have an inverse association with the plasma fibrinogen levels (Markowe *et al.*, 1985:1313). 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 (Malanick, 1999:1). It is therefore possible, if the link between vitamin A status and plasma fibrinogen exists that these vitamin A deficient individuals may also have increased fibrinogen and elevated risk for CHD and stroke.

The major objective of this project was to perform a clinical intervention trial under controlled conditions to examine the effect of vitamin A fortified sugar on plasma fibrinogen of young females aged between 13 and 25 years old. The answers to the following questions would help to determine whether fortification has an effect on nutritional status:

a) Is there a correlation between vitamin A status and plasma fibrinogen levels?
b) What effect does fortified vitamin A have on the plasma fibrinogen levels of the experimental group?

7.3 Methodology

7.3.1 Study population and design

The study population consisted of 100 females aged 13-25 years old that were randomly selected from clinics, schools and tertiary institutions in the Vaal Triangle. The inclusion criteria were female gender, ages between 13 and 25 years, non-pregnant and lactating, "apparently healthy" females with a daily sugar consumption. Written consent was obtained from the subjects or parents and guardians prior to the study. The study was approved by both the Ethics Committees of the Vaal Triangle Technikon and Potchefstroom University for CHE. The survey was conducted between February and May 2000 (refer Chapter 4, Section 4.5).

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. The sugar supplied to the subjects was fortified by Roche Vitamins and Fine Chemicals. One gram of sugar contained 80 IU vitamin A. All the subjects were "blinded" and received anonymous intervention in that 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 identically in quantities of 5 kg purple bags. The sugar was issued to the participants every four weeks for a period of 12 weeks (refer Chapter 4, Section 4.2). The researchers who took measurements and analysed blood samples were also blinded to the intervention. This was therefore a double blind, placebo-controlled parallel study.

7.3.2 Laboratory methods

A double baseline measurement was done one week apart before commencing with the trial. Baseline measurements included measuring vitamin A status by measuring serum retinol. Plasma fibrinogen was measured at the same time. Follow-up measurements were done during weeks 4, 8 and 12 of the trial (refer Section 4.5.2).

Venous blood (4,5 ml) was collected in a tube containing 0,5 ml sodium citrate (0,11 mol/L). Blood was separated within 2 hours of blood collection, and citrated plasma stored at 15°C until assayed. Plasma fibrinogen levels were determined within 8 hours by 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 warmed test tube, incubated for 60 seconds, whereafter 200 µL Multifibren U reagent, pre-warmed to 37°C, is added and clotting time determined. Fibrinogen concentration (g/L) is obtained by using a reference curve prepared in the laboratory, using standard plasma.

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).

The reference curve was established using fibrinogen standards. These standards were tested as samples. Values obtained were plotted on a log-log format. Checking the values of control plasma after every 15 tests monitored accuracy of the procedure. Control values did not vary more than 2SD from the prescribed mean.

7.3.3 Anthropometry

The WHO recommended height-for-age and BMI-for-age as anthropometric indicators for adolescents (WHO, 1995:271). For this reason weight, height, weight-for-height, hip- and waist circumference were recorded during weeks 1, 4, 8 and 12 of the trial. Body mass index (BMI) was calculated using the formula weight (kg)/height (m^2) (refer Section 4.6.6).

7.3.4 Dietary intakes

Subjects kept specially designed dietary diaries to monitor compliance (see Chapters 5 and 6). Compliance was acceptable.

7.3.5 Statistical analysis

Data was computerised and the SPSS ® program, version 6.0 used to analyse data. Paired tests were done to compare for significant differences within groups and a multivariate analysis was done to examine relationships between variables (refer Section 4.6.7).

7.4 Results

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 43 subjects remained in the experimental group compared to 40 in the placebo group.

There was no significant difference in the numbers lost to the study amongst the two groups (see Chapter 6). Although the mean fibrinogen levels for the participants (2,99 ± 0,52 g/L) was higher than for the drop-outs (2,70 ± 0,95 g/L), there were no significant differences between the two groups. 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. A summary of the differences between the participants and drop-outs can be found in Table 6.5.

The results in Table 7.1 indicate that 13,64 % of the experimental group and 6,82 % of the control group presented with fibrinogen levels above the normal range (> 3,31 g/L).

At baseline 46 % of respondents had a fibrinogen level < 2,1 g/L, 43 % a level of 2,72 – 3,30 g/L and 10 % a level of > 3,30g/L.

Table 7.1 Summary table of distribution of fibrinogen (g/L) in the sample

		Baseline	**Week 4**	**Week 8**	**Week 12**
Percentage of respondents < 2,71 g/L	E P	38,7 54,6	56,8 54,6	60,5 55,0	36,4 40,9
Percentage of respondents ≥ 2,71 and ≤ 3,31 g/L	E P	47,7 38,6	34,1 18,2	16,3 35,0	45,5 45,5
Percentage of respondents > 3,31 g/L	E P	13,6 6,8	9,1 27,3	23,3 10,0	18,2 13,6
	E	n = 43	n = 43	n = 43	n = 43
	P	n = 40	n = 40	n = 40	n = 40

E = Experimental group P = Placebo group

Table 7.2 gives the changes in plasma fibrinogen observed during the 12 weeks, as well as changes in serum retinol, WBC and BMI. It is clear that no significant changes 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.

Table 7.2 Changes in plasma fibrinogen and related variables during vitamin A fortification

		Experimental group (n = 43)				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	2,57 ±0,54	2,86 ±0,58
White Blood Count (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
Body Mass Index (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 7.3 shows that in these subjects serum retinol was not related to plasma fibrinogen. 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 (r = 0,206; P = 0,062) at baseline, but was not observed after 12 weeks, and was probably not reflecting a real association.

Table 7.3 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)

7.5 Discussion

Increased plasma fibrinogen is a major risk factor for CHD (Folsom, 1995:21; Cook & Ubben, 1990:444) 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.

There is some controversy in the literature about a "normal" plasma fibrinogen level (Vorster, 1999) 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 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. 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.

In Chapter 6 it was shown that subjects receiving the fortified sugar consumed approximately 1446 µg RE vitamin A in addition to their already adequate vitamin A intakes from foods (1068-1349 µg RE). Despite these high mean intakes, 12 % of the subjects could be classified as being vitamin A deficient. The question is whether this additional amount if vitamin A (almost reaching the RDA of 600 µg RE) could have been responsible for the observed decreases in plasma fibrinogen levels of the experimental group.

Table 7.2 suggests that during ingestion of vitamin A fortified sugar in the experimental period, plasma fibrinogen levels did decrease but returned to baseline levels at 12 weeks. It is therefore not clear if this is a true effect of increased intakes of vitamin A, a normal variation, or whether other factors could have been responsible for this increase at 12 weeks.

It seems that it could not be a normal variation, since similar effects were not observed in the placebo group. From the literature (reviewed by Vorster, 1999) it seems that obesity and weight increases are associated with increases in plasma fibrinogen. The experimental subjects showed a significantly higher BMI at week 12 and it seems reasonable to conclude that this increase in BMI has been responsible for the increase in plasma fibrinogen.

Fibrinogen is an acute phase protein. The increased white blood cell count of the experimental group at weeks 8 and 12 could indicate some type of infection (colds) 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 positive relationship between plasma fibrinogen and BMI has been observed in many studies (reviewed by Vorster, 1999). It is of interest that this relationship was even stronger at the end of the study period. This suggests that the increase in plasma fibrinogen seen at 12 weeks (compared to the decrease at 8 weeks in the experimental group) was a result of the increase in BMI. Therefore, it seems reasonable to argue that the slight, but significant decrease in plasma fibrinogen from baseline to week 8 in the experimental group, was a result of the vitamin A intake. This then confirms the hypothesis that increased vitamin A intake will have a beneficial effect on plasma fibrinogen. Although this study was not designed to examine possible mechanisms, it could be that improved vitamin A status because of higher intakes, could lower susceptibility to inflammation and infections and therefore of acute phase proteins, such as fibrinogen.

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.

7.6 Conclusion

This study attempted to lower the fibrinogen levels of 13-25 year old black South African females by means of dietary intake of vitamin A fortified sugar. 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 of the experimental group, and possibly indirectly because of prevalence of infections. The results are therefore inconclusive and more research is indicated. It is suggested that a vitamin A supplementation study should be repeated in a VAD group and fibrinogen monitored in such a study.

CHAPTER 8

COMBINED DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

8.1 Discussion

8.1.1 Introduction

The major objectives of this study were to

- assess how much and what types of tea are consumed by young black South Africans in order to judge if tea with added sugar is a suitable vehicle for fortification;
- examine the feasibility to fortify sugar with vitamin A for this population group;
- examine the effects of the consumption of vitamin A fortified sugar on vitamin A status, iron 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.

The high prevalence of both undernutrition – especially of vitamin A and iron, and overnutrition - especially in black women, motivated this study.

8.1.2 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.

  Because one of the aims of this study is to provide data that can be used in micronutrient fortification programmes on a population level, it was thought that a random sample from a vulnerable population would be the best sample.

  However, baseline data indicated that although mean iron intakes of these subjects were low, vitamin A intakes were generally sufficient. The result was that 12 % of the subjects were marginally vitamin A deficient, and 60 % could be classified as having an abnormal iron status.

  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 power of the study. Calculations based on variations in receptor showed that 88 subjects were needed to obtain significant results. However, the statistically significant changes in serum retinol and serum iron suggest that the power of the study was sufficient, despite the fact that no clinical significant results were obtained.

- The third limitation may be the fact that only serum retinol was measured to determine vitamin A status. Krause and Escott-Stumpf (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.

8.1.3 Main findings

The salient findings of this study were:

- At least one cup of tea was consumed daily by 92,9 % of the 500 randomly selected participants in this study, with rooibos tea selected as the most popular in this study (50 % of tea consumed). Sugar was chosen by 40,4 % and milk by 37,0 % of the sample to be the preferential ingredient added to tea. According to the preference scales of these respondents, tea was the third most consumed beverage in summer and the first most consumed beverage in winter.

- Sugar fortified with vitamin A seemed to be acceptable, as no differences in the colour and taste of the fortified sugar were noted. A statistically significant difference was, however, noted in the smell of the sugar that was fortified to a level of 100 % of the recommended daily allowance of the sample population. Compliance to the intake of the fortified sugar was good as 93 % of the participants in this study used white sugar on a daily basis. This may indicate that sugar is a suitable vehicle for vitamin A fortification.

- 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 % who showed an increase, 40 % who had no change and 22,5 % who showed a decrease.

- The dietary intake of vitamin A fortified sugar resulted in significant changes in serum iron of the experimental group. This result suggests that the additional vitamin A consumed may have influenced iron metabolism. However, the high percentage (58 %) of experimental subjects with an abnormal iron status (depleted plus iron deficient erythropoiesis plus IDA) could not be changed by the extra vitamin A intake.

- 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.

- The significant correlation observed between serum retinol and iron status variables in this homogeneous group of subjects, confirms a relationship between vitamin A and iron status. 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 between 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).

The above results clearly indicate that vitamin A intakes influence iron status, but not to the extent that it could rectify iron deficiency at the levels of iron consumption of these subjects. More research is needed to examine how much vitamin A and dietary iron are needed to normalise vitamin A and iron status simultaneously. The results also indicated that sugar is a suitable vehicle for vitamin A fortification. The results further suggested that vitamin A may influence plasma fibrinogen, but more studies are needed to confirm this observation.

The study was not designed to elucidate the mechanisms by which vitamin A interacts with iron metabolism. However, the changes in serum iron and the significant relationships between serum retinol and iron status observed suggest that vitamin A may influence iron metabolism on several levels – either directly or indirectly, as illustrated in Figure 8.1.

Figure 8.1 Schematic diagram of possible influence of vitamin A on iron metabolism in adults

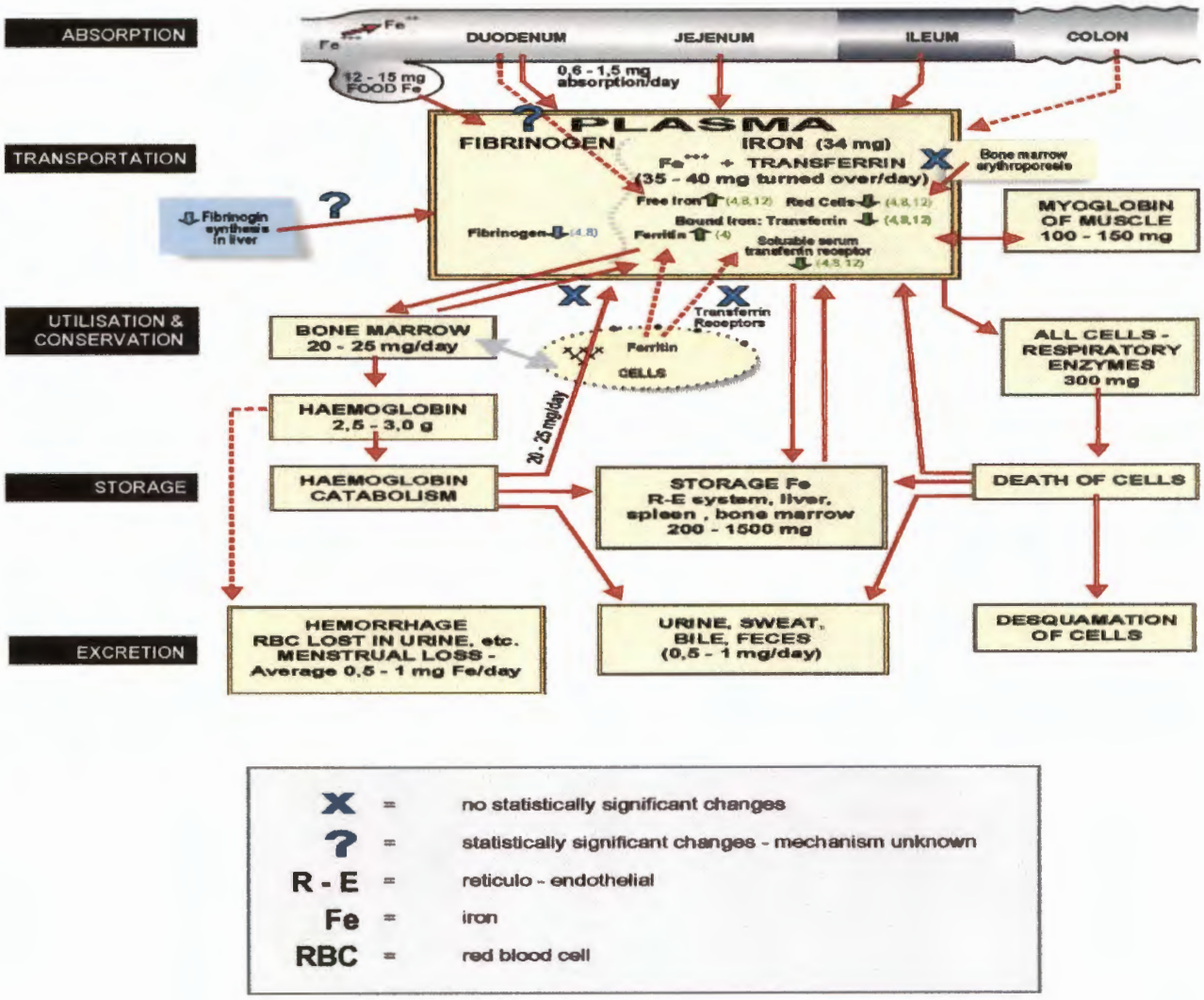

Figure 8.1 clearly indicates that the only possible interactions occurred between serum iron and vitamin A, as well as fibrinogen and vitamin A. No other interactions were observed in this study. Iron is distributed in the body in Hb iron, storage iron (tissues) and plasma (transport). Hb iron (present in RBC) constitutes ± 60-70 % of total body iron. At the end of the RBC lifespan, the aged RBC is phagolysed by cells of the R-E system. Nearly all the iron derived from breakdown of Hb is released into circulation (plasma) bound to iron-binding protein (transferrin). The ferritin concentration generally correlate well with tissue iron stores. Although the mechanism of the interaction was not studied, Figure 8.1 indicates that in this study no statistically significant interactions between vitamin A and ferritin, transferrin, Hb and Hct, but a significant change in total serum iron was observed.

8.2 Conclusions

From the results of this study, the following conclusions can be drawn:

- Compliance to the intake of the fortified sugar was good and this indicates that sugar is a suitable vehicle for vitamin A fortification.

- Serum retinol levels are maintained in healthy adults with normal serum retinol levels.

- Although dietary intake of vitamin A fortified sugar did not result in clinical improvements of iron status variables, statistically significant correlations between serum retinol and iron status variables in this homogeneous group of subjects, confirm a relationship between vitamin A and iron status.

- 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.

8.3 Recommendations

8.3.1 Practical implications of the results

This research project provided the opportunity to determine the effect of vitamin A fortified sugar consumption of iron status and fibrinogen levels in a community and individuals at risk of VAD and IDA. It was the first time that an intervention study with vitamin A fortified sugar was conducted in South Africa. One of the objectives of this study was to determine if sugar was a suitable vehicle for vitamin A fortification. This objective was achieved through analysis of data gathered by means of focus groups discussions and compliance and acceptability questionnaires.

The results of the clinical intervention trial provide information regarding the effect of fortified vitamin A on the iron status and fibrinogen levels of healthy young females with a high risk for vitamin A and iron deficiency. The results could therefore have an impact on public health policy regarding the treatment of micronutrient, specifically vitamin A and iron deficiency. Programmes aimed at the prevention of micronutrient deficiency, rather than treatment, may help to prevent the rising health costs in the country. The results clearly indicated that vitamin A fortification alone would not be sufficient, but should be accompanied by iron fortification/supplementation in this group.

Nutrition professionals should play leadership roles in addressing the alleviation of vitamin A and iron deficiencies. Many opportunities exist to improve the public's knowledge about micronutrient deficiencies including healthy eating habits and correct food storage and preparation. The disciplines of nutrition and food science can provide the means to enhance education, train professionals, educate the public, contribute to development of public policy, advise industry and to carry out research.

The following important contributions can be made by nutritional professionals regarding:

The field of education

- Efforts to improve compliance need to include education, counselling and the use of social support networks. Human interaction is the best way to enhance compliance because satisfaction with care is a contributing factor to compliance.
- Subjects should participate in nutrition education activities aimed to promote the use of fortified foods and dietary diversification.
- Nutrition professionals should develop innovative nutrition education programmes to increase the nutrition knowledge of the public.

The field of legislation

The South African Department of Health (DOH) has constituted a Food Fortification Task Group, representative of all stakeholders. Based on the results of the National Food Consumption Survey, maize meal and bread flour have been identified as vehicles for mandatory fortification and legislation is in preparation. The situation around sugar fortification is still in debate.

Although sugar is an ideal vehicle because it is eaten by the majority of vulnerable individuals, and because sugar fortification can be easily monitored (there are only seven sugar mills in the country and South Africa produces its own sugar), there are certain problems which have to be addressed. The first is concerns about dental caries of groups within DOH; the second is the multiple fortification needed and the lack of data on acceptability of sugar when other nutrients than vitamin A are also added. These aspects need clarification before a decision regarding mandatory sugar fortification will be taken.

Legislation must be drafted regarding fortification. It must clearly state that it is not appropriate and permitted to promote high consumption of sugar through ascribing healing properties to fortified sugar. The programme must have nutritional rather than medical or commercial objectives. The vitamin A fortification programme should thus be compatible with the ethos and principles of the Department of Health. The vitamin A programme design must focus on impact and quality, not just coverage and inputs.

8.3.2 Further research

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

- The mechanism by which vitamin A and iron interact needs to be studied and determined in order to plan future intervention programmes.
- Optimal fortification levels need to be determined through more research in order to identify safe vitamin A levels and especially the minimum iron fortification that will reduce iron deficiency without increasing risk of iron overload.
- Cost-effective analysis should be done to determine the most cost-effective interventions for the situation in SA in rural, urban and poorest rural areas. A mix of strategies is needed to ensure adequate vitamin A intakes for all people.
- There is a need for fortified food targeting the age group 13 to 25 years because the vitamin A capsule programmes do not reach this segment of the population.
- The role of vitamin A, specifically vitamin A fortified sugar, may form part of the arsenal needed to combat HIV. However, this was not investigated in this study. This element should be investigated in further studies.

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CONFERENCE PARTICIPATION & 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).
- Oldewage-Theron, W.H; Dicks, E. & Selepe, M. Consumption of tea amongst young Black males and females in the Vaal Triangle (poster).
- Oldewage-Theron, W.H; Dicks, E; Selepe, M; Hanekom, S.M. & Vorster, H.H. Food consumption patterns and nutritional intake 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).
- Dicks, E; Oldewage-Theron, W.H; Selepe, M; Hanekom, S.M. & Vorster, H.H. The use of vitamin A fortified sugar in a clinical intervention trial in the Vaal Triangle (poster).

2 **Bioavailability 2001 Conference from 29 May to 1 June 2001, Interlaken, Switzerland (poster presentation)**

Wilna H Oldewage-Theron, EG Dicks, M Selepe, C Grobler, SM Hanekom and HH Vorster. The effect of dietary vitamin a fortification on iron status of females aged 13-25 years old in the Vaal Triangle, South Africa (SA).

3 **17th International nutrition conference from 27-31 August 2001, Vienna, Austria (oral presentation)**

Wilna H Oldewage-Theron, EG Dicks, M Selepe, C Grobler, SM Hanekom and HH Vorster. The effect of dietary vitamin a fortification on iron status of females aged 13-25 years old in the Vaal Triangle, South Africa (SA).

4 **SASPEN 2001 congress from 25-27 September 2001, Stellenbosch SA**

Wilna H Oldewage-Theron, EG Dicks, M Selepe, C Grobler, SM Hanekom and HH Vorster. The effect of dietary vitamin a fortification on iron status of females aged 13-25 years old in the Vaal Triangle, South Africa (SA).

5 **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.
- Oldewage-Theron, W.H; Dicks, E. & Selepe, M. Consumption of tea amongst young Black males and females in the Vaal Triangle.
- Oldewage-Theron, W.H; Dicks, E; Selepe, M; Hanekom, S.M. & Vorster, H.H. Food consumption patterns and nutritional intake 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.
- Dicks, E; Oldewage-Theron, W.H; Selepe, M; Hanekom, S.M. & Vorster, H.H. The use of vitamin A fortified sugar in a clinical intervention trial in the Vaal Triangle.

Annexure 1 **TEA CONSUMPTION QUESTIONNAIRE**

SECTION A : DEMOGRAPHIC AREA

Please complete the following:

Date : .....

Name : .....

Sex : .....

Date of birth (dd/mm/yy) : .....

Age : .....

Institution : .....

Education level : .....

SECTION B : TEA CONSUMPTION RECORD

Please tick the applicable options

		YES	NO
1	Do you drink tea?		
2	Do you like tea?		

3	If yes, why do you like tea?

4 How many cups of tea do you drink per day?

0 cups	1 cup	2 cups	3 – 4 cups	5 – 6 cups	> 6 cups

5 What type of tea do you drink?

Ordinary tea leaves	Ordinary teabags	Rooibos tea	Flavoured tea

6 How do you drink your tea?

Black	With milk	With sugar	With honey	With lemon	Other

If other, please specify.....

7 How strong do you make your tea (per cup)?

1 teaspoon/teabag	2 teaspoons/teabags	>2 teaspoons/teabags

8 When do you drink tea?

Early morning	Breakfast	Mid-morning	Lunch	Mid-afternoon	Evening

9 Would you be prepared to buy more expensive tea that is fortified with vitamins or minerals?

YES	NO

Annexure 2

Vaal Triangle Technikon

SUGAR COMPLIANCE SURVEY 2000

For office use only
Site:
.....
.....

Dear Friend,

The purpose of this survey is to obtain information on sugar compliance patterns of the participants in this research. Please answer the following questions by ticking the appropriate block or printing your answer in the appropriate space. Thank you for your co-operation!

1. **Subject number** .....(must be completed)

	Yes	No
2. **Do you like the taste of the given sugar?**		
3. **Is there a difference in the colour of the given sugar and normal sugar?**		
4. **Does the given sugar smell different?**		
5. **Does the given sugar change the colour of the food in which it is used?**		
6. **Do you add sugar to the food while it is being cooked?**		
7. **Do you add more sugar to your food after it has been cooked?**		
8. **Do you keep the sugar in its original paper bag?**		
9. **If no, where do you normally store your sugar?**		
10. **Do you close the bag/container of sugar every time after use?**		
11. **Did you use more of the sugar that was given to you, than normally?**		
12. **Are you sure why fortified sugar should be taken?**		
13. **Does the fortified sugar make you feel ill?**		
14. **Did you tend to forget using the sugar?**		
15. **Do you like sweet foods?**		
16. **Do you think sugar is bad for your teeth?**		

Annexure 2

17. Will sugar make you fat?

		17.	

18. How many teaspoons of sugar do you use per day?

0 teaspoons	1-2 teaspoons	3-4 teaspoons	5-6 teaspoons	7-8 teaspoons	≥10 teaspoons

(Select one only)

18.1	
18.2	
18.3	
18.4	
18.5	
18.6	

19. How many teaspoons of sugar do you drink in your coffee or tea?

0 teaspoons	1-2 teaspoons	3-4 teaspoons	5-6 teaspoons	7-8 teaspoons	≥10 teaspoons

(Select one only)

19.1	
19.2	
19.3	
19.4	
19.5	
19.6	

20. How many spoons of sugar do you use over your "pap"?

0 teaspoons	1-2 teaspoons	3-4 teaspoons	5-6 teaspoons	7-8 teaspoons	≥10 teaspoons

(Select one only)

20.1	
20.2	
20.3	
20.4	
20.5	
20.6	

21. How many spoons of sugar do you use over your pumpkin?

0 teaspoons	1-2 teaspoons	3-4 teaspoons	5-6 teaspoons	7-8 teaspoons	≥10 teaspoons

(Select one only)

21.1	
21.2	
21.3	
21.4	
21.5	
21.6	

22. How many spoons of sugar do you use over your squash?

0 teaspoons	1-2 teaspoons	3-4 teaspoons	5-6 teaspoons	7-8 teaspoons	≥10 teaspoons

(Select one only)

22.1	
22.2	
22.3	

Annexure 2

22.4	
22.5	

23. How many spoons of sugar do you use over your sweet potatoes?

0 teaspoons	1-2 teaspoons	3-4 teaspoons	5-6 teaspoons	7-8 teaspoons	≥10 teaspoons

(Select one only)

23.1	
23.2	
23.3	
23.4	
23.5	
23.6	

24. How many spoons of sugar do you use over carrots (salad/cooked)?

0 teaspoons	1-2 teaspoons	3-4 teaspoons	5-6 teaspoons	7-8 teaspoons	≥10 teaspoons

(Select one only)

24.1	
24.2	
24.3	
24.4	
24.5	
24.6	

25. How many spoons of sugar do you use in tomato stew/tomato sauce?

0 teaspoons	1-2 teaspoons	3-4 teaspoons	5-6 teaspoons	7-8 teaspoons	≥10 teaspoons

(Select one only)

25.1	
25.2	
25.3	
25.4	
25.5	
25.6	

26. What type of sugar do you usually buy?

White sugar	**Brown sugar**	**Yellow sugar**	**Castor sugar**	**Icing sugar**

(Select one only)

26.1	
26.2	
26.3	
26.4	
26.5	

27. Would you be prepared to buy more expensive sugar if it is fortified with vitamins or minerals? **Yes** **No**

28. Do you think sugar is "healthy"? **Yes** **No**

27.	
28.	

Annexure 3

Declared levels of Vitamin A in the fortified sugar

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.

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

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\AlginI\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.....

Annexure 5

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 .....

.....

.....

Annexure 5

11.1 Is bogenoemde medisyne geregistreer? NVT .....

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 5

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 5

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.

Annexure 5

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:.....

Annexure 5

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.)

   .....

   .....

   .....

   .....

Annexure 5

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 5

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 5

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:

Annexure 5

Hiermee onderneem ek, ... (volle name), die eggenoot/-e van die proefpersoon in hierdie aansoek, om geen eis teen die Universiteit in te stel vir behandeling weens besering, skade of die 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: ……

199

Annexure 6

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 : .....

Annexure 7

Vaal Triangle Technikon

Vitamin A Fortification Project

Demographic questionnaire

1 Name: .....

2 Surname: .....

3 Subject number: | | | |

4 Birth date: 19 _ _ _ _ _ _

5 Age:

13	
14	
15	
16	
17	
18	
19	
20	
21	
22	
23	
24	
25	

6 Gender:

Female	
Male	

7 Language:

English	
South-Sotho	
Xhosa	
Zulu	
Twana	
Afrikaans	
North-Sotho	
Other	

8 Where do you live?

Rural village	
Township, Town, city	
Farm	
Hostel	
Informal settlement (squatter)	

9 How many persons stay in your home?

1	
2	
3	
4	
>5	

10 How many rooms are in your home?

1	
2	
3	
4	
>5	

11 Do you use sugar every day?

Yes	
No	

12 Do you smoke?

Yes	
No	

13 Do you drink alcohol?

Yes	
No	

14 Who prepares food and cooks your food?

Mother	
Granny	
Self	
Sister	
Aunt	
Father	
Other	

15 Do you suffer from any of the following illnesses?

High blood	
Diabetes	
Stroke	
Obesity	

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.....

Annexure 10

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

Annexure 10

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
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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 10

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 10

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

- 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.

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 10

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.

Annexure 11

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 12

EVALUATION OF THE FORTIFICATION OF SUGAR WITH VITAMIN A

QUANTIFIED FOOD FREQUENCY QUESTIONAIRE

Annexure 12

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?

Annexure 12

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)						4225 4250	
Maize-meal porridge	Soft (slappap)						4225 4250	
Maize-meal porridge	Crumbly (phutu)						4225 4250	
Ting								
Mabella Coarse Fine Rice	Stiff						4082	
Mabella	Soft						4082	
Oats							4032	

Annexure 12

FOOD	DESCRIPTION	Amount	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						4048 4134 4043	
Pastas	Macaroni Spaghetti Other:						4062	

Annexure 12

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						1531	
	Fried: in batter/crumbs Not coated						1634 1520	
	Roasted/grilled						1539	

Do you eat chicken skin **ALWAYS** 1 **SOMETIMES** 2 **NEVER** 3

Chicken bones stew							A003	
Chicken feet							A004 1609	
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	

Annexure 12

FOOD	DESCRIPTION	Amount	TIMES EATEN				CODE	AMOUNT/DAY
			Per day	Per week	Per month	Seldom/ Never		
Dried beans/peas/ lentils (10)	Soup Salad						3833 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:						2542	
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							

Annexure 12

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	

Annexure 12

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						8057	
	- 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 7052	
Apricots	Fresh Canned						7003 7004	
Mangoes	Fresh						7026	

Annexure 12

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						8004	
Wild fuit/berries	Specify type						7070	
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	

Annexure 12

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?							

Annexure 12

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						0009	
Fizzy drinks Coke, Fanta	Sweetened Diet						9001 9013	
Mageu/Motogo							9562	
Home brew							9516	
Tlokwe							9516	
Beer							9506	
Spirits							9518	
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	

Annexure 12

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								

Annexure 12

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	Sometimes	Never	Don't know
1	2	3	4

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

Always	Sometimes	Never
1	2	3

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

Very much	Like	Not al all
1	2	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 13

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 14

FOCUS GROUPS IN VITAMIN A FORTIFICATION STUDY

PURPOSE OF THE FOCUS GROUPS: SESSION 1 WEEK 0

The purpose of this focus group is to identify the participants knowledge, attitude and behaviour of the following aspects:

- How people store their food
- Problems experience with dental caries
- Problems experience with obesity
- The use of sugar and vitamin A rich foods in the diet

FOCUS GROUP QUESTIONS

BEGINNING:

Welcome the participants

Give an overview of the purpose.....

To find out you store your food, do you have problems with dental caries, obesity as well as the use of sugar in you diet and what you know about vitamin A.

GIVE THE GROUND RULES:

Please tell the truth, talk from your heart and be honest about any problems to do with food storage, obesity, dental caries and sugar consumption.

OPENING: FIRST DESCRIPTIVE QUESTION

Tell us your name and where you live.

INTRODUCTORY AND TRANSITORY QUESTION:

What is your favourite food?

KEY QUESTIONS:

If you hear the word sugar, what comes into your mind?

How do you store your sugar?

When do you add sugar to your food?

In what foods do you eat sugar?

What do you think are the main problems when eating sugar?

Tell us everything you know about vitamin A?

ENDING QUESTION:

Think back on what we have discussed and how do you think food must be stored?

What do you think is the biggest problem regarding sugar and regarding vitamin A?

FINAL QUESTION:

Have we missed any thing that you think is important?

Annexure 15

FOCUS GROUPS IN VITAMIN A FORTIFICATION STUDY

AIM OF THE FOCUS GROUP: SESSION 2 WEEK 1

The aim of this focus group is to identify what new knowledge and attitudes the participants learned during the nutrition education program regarding food storage, dental caries, obesity, and the use of sugar and vitamin A rich food in the diet.

FOCUS GROUP QUESTIONS

BEGINNING:

Welcome the participants

Give an overview of the purpose.....

To investigate what you learned about food storage, dental caries and obesity and the use of sugar in your diet.

GIVE THE GROUND RULES:

Please tell the truth, talk from your heart and be honest about any problems to do with food storage, obesity, dental caries and sugar consumption.

OPENING: FIRST DESCRIPTIVE QUESTION

What new information did you learn in the nutrition education program?

INTRODUCTORY AND TRANSITORY QUESTION:

Do you think the nutrition education program changed the choice of your favorite food?

KEY QUESTIONS:

If you hear the word sugar, what comes to your mind?

How will you store your sugar?

When do you add sugar to your food?

To which foods do you add sugar?

What do you think are the main problems when eating sugar?

What did you learn about vitamin A?

ENDING QUESTION:

Think back on what we have learned in the nutrition education program and how do you think food must be stored?

What do you think is the biggest problem regarding the use of sugar and of vitamin A?

FINAL QUESTION:

Have we missed any thing that you think is important?

Annexure 16

FOCUS GROUPS IN VITAMIN A FORTIFICATION STUDY

AIM OF THE FOCUS GROUP: SESSION 3 WEEK 4

The aim of the focus group is to identify problems the groups experienced with the use of the given sugar.

FOCUS GROUP QUESTIONS

BEGINNING:

Welcome the participants

Give an overview of the purpose.....

To find out what constraints you had regarding the sugar you received.

GIVE THE GROUND RULES:

Please tell the truth, talk from your heart and be honest about any problems to do with food storage, obesity, dental caries and sugar consumption and the sugar we gave you.

OPENING: FIRST DESCRIPTIVE QUESTION

What are the three things you think of when you hear the word sugar?

INTRODUCTORY AND TRANSITORY QUESTION:

To which foods do you add our sugar?

KEY QUESTIONS:

How do you store the sugar we gave you?

At what stage in the cooking process do you add the sugar we gave you?

How do you feel about our sugar?

In what beverages do you use our sugar?

Do your participation in this project make a difference in the amount of sugar you and your family use?

Do you think there is a difference in our sugar and the sugar you normally use?

Suppose that you were trying to encourage a friend to participate in this research. What would you say?

ENDING QUESTION:

Think back on what we have discussed and what do you think is the best qualities of the sugar given to you?

What do you think is the biggest problem with the given sugar?

FINAL QUESTION:

Is there anything that we should have talked about but didn't?

Annexure 17

FOCUS GROUPS IN VITAMIN A FORTIFICATION STUDY

AIM OF THE FOCUS GROUP: SESSION 4 WEEK 12

The aim of the focus group is to determine the group's compliance to and acceptance of the vitamin A fortified sugar.

FOCUS GROUP QUESTIONS

BEGINNING:

Welcome the participants

Give an overview of the purpose.....

To find out you if you would buy and use fortified sugar if it is available.

GIVE THE GROUND RULES:

Please tell the truth, talk from your heart and be honest about any problems to do with food storage, obesity, dental caries and sugar consumption and the sugar we gave you.

OPENING: FIRST DESCRIPTIVE QUESTION

Tell us your name and what you enjoyed the most by participating in this research project.

INTRODUCTORY AND TRANSITORY QUESTION:

What would you tell a best friend or a family member about this product?

KEY QUESTIONS:

If you could change one thing about this product, what would you change? What is the main reason for this change?

What would it take for this product to get a gold star? If this product is to receive an award, what would it be for?

What do you need to know about this product in order to accept or reject it?

Think back on the use of the sugar by your family. What was the greatest barrier to over come?

What is needed to overcome these barriers?

I'd like you to participate in a short exercise. Please complete the sugar compliance and acceptance questionnaire.

ENDING QUESTION:

Do you have any other advice for us when we introduce the new vitamin A fortified sugar?

FINAL QUESTION:

Our purpose today was to determine your compliance and acceptance to the vitamin A fortified sugar. Have we missed any thing?

Annexure 18

FOODS AND NUTRITION

Compiled by E G Dicks

Good eating habits
Healthy foods
Food preparation
Keeping fit

Annexure 18

FOODS AND NUTRITION

Section A

Healthy foods

What are healthy eating habits?

Healthy eating habits are when you eat enough of the right foods everyday.

If you eat the right foods then

- Your body will be more healthy
- You will look good
- You will have enough energy to do your work well, think and learn better
- You will enjoy life more for example social events and celebrations
- You will get sick and will feel tired all day.

What are the right foods?

From a nutritional point of view there are no good or bad foods. Your body needs a variety of nutrients like proteins, carbohydrates, fat, vitamins and minerals that come from the foods you eat.

1. Give you energy to do your work
2. Build your body and make it strong
3. Protect you against disease

1. **Foods that give energy**

One eats certain energy foods to give heat and energy so that you can

- Work
- Learn
- Play
- Grow normally

These energy foods are carbohydrates and fats:
Porridge (pap), brown bread, sorghum, wheat, potatoes, samp, maize rice, rice and cereals
Sugar, jams, fat, oils, butter, margarine

Annexure 18

Sugar

Because sugar does not contain other nutrients (vitamins or minerals) it can be called empty –calorie food, except in cases where it is fortified with some nutrients. Sugar cannot be single out as the sole cause of obesity since all excess calories are stored in the body as fat. As sugar consumption increases obesity rises, but obesity can also occurs where the sugar intake is low. As long as the diet is balanced and otherwise adequate, sugars emptiness can be accepted. The rule is to eat only a moderate amount of sugars and food containing added sugar. Nobody can eat sugar in very large portions or large portions of sugar-containing foods without effecting their blood glucose levels or their weight.

2. **Foods that build strong bodies**

Building foods build strong, healthy bodies
Building foods are important for

- Building bones, teeth, skin and muscles
- Help the body to grow

These building foods are called proteins
Milk, yoghurt, cheese and eggs
Meat, liver, kidneys, heart, offal, lungs and poultry such as chicken
Fresh and tinned fish
Dried beans, peas and lentils, soya beans and soya products
Nuts such as peanuts and peanut butter

3. **Foods that protect your body**

Your body needs protecting foods to protect it against diseases
These protecting foods are important to protect your body against

- Infections
- Other diseases

These protecting foods are called vitamins and minerals
Vegetables, dark green and yellow vegetables such as spinach, marogo, cabbage, pumpkin and carrots, dark yellow sweet potato, mango, papaya
Fruits such as oranges,

Annexure 18

Vitamin A

It is needed for building and maintaining healthy tissues throughout the body, particularly eyes, skin, bones and tissues of the respiratory and digestive track. It is also important for the immune system. Vitamin A deficiency can lead to poor night vision (night blindness) and in sever cases permanent blindness. This occurs mainly in undernourished children, especially those with measles and other infections. It can lead to increased illness and death from infection.

Section B

Healthy balanced meals

What are healthy meals?

To prevent your body from getting sick you must just eat food every day from the three food groups which

- Give energy
- Build strong bodies
- Protect against disease

Every time you eat, your plate must have foods from all three groups. Then you will have a balanced meal. It means that you must eat a wide variety of foods available to you. You may need to take extra care at different times of your life to make sure you get the right amount of certain nutrients. For example:

During pregnancy
If you are breastfeeding
Teenagers. They grow rapidly and so have very high energy needs. It is important that adolescent girls in particular are well-nourished both to meet their immediate needs and the future stresses of childbearing.
Active people
And when you want to gain or lose weight.

How should you plan healthy meals?

The meal in the morning can be like this

- Energy food porridge/ bread
- Building food milk (not cremora)
- Protecting food orange/ fruit

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The meal at lunch time can be like this

- Energy food — porridge or bread or samp
- Building food — meat or fish or eggs or dried beans
- Protecting food — vegetables: cabbage, marogo or pumpkin

The evening meal can be like this

- Energy food — porridge or bread or samp
- Building food — meat or fish or dried beans
- Protecting food — vegetables: cabbage, marogo or pumpkin

Remember:

- Eat and enjoy a variety of foods
- Eat just enough to have the right body weight
- Be careful of too much fat in the diet
- Eat enough fiber foods
- Drink plenty of water
- Use alcohol in moderation
- Children must eat building foods everyday
- Do not allow your family to eat too many sweets, cold drinks, chips and cookies and it do not help to build strong healthy bodies

Section C

Preparation and handling of food

Germs in the environment and on food can easily multiply and contaminate your food. Eating contaminated food can lead to serious illness. To reduce the risk of food poisoning it's important to follow the basic rules of good hygiene whenever and where ever you prepare food. Even though clean things can look clean, people, insects and other items can make it dirty again. Even in clean surroundings food will go bad over time.

Handling of food

- Wash your hands with soap and water before you touch or eat food
- Do not touch your hair, nose, mouth or any sores on your body while working with food
- Make sure that all utensils, plates, bowls, pots and cutlery are clean

Annexure 18

- Make sure that dish cloths are clean

Preparation of food

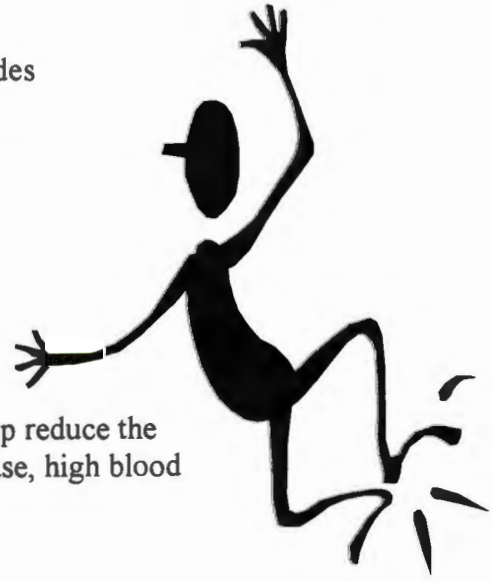
- Make sure that the kitchen or place where you prepare the food and all utensils are very clean, and wash with hot water and soap after each meal and rinse them in clean water.
- Do not cough, sneeze or smoke near food
- Wash all vegetables and fruit well in clean water before use.
- When cooking vegetables and fruit
  - Use as little water as possible
  - cook for a short time
  - Eat the food as soon as possible after cooking
- Only use fresh food, discard spoiled, smelly or moldy foods. Always use fresh water form a safe supply.
- When using tinned food, make sure the tin
  - Is not swollen
  - Is not rusted and corroded
  - Does not leak
- When cooking, heat food thoroughly
- Keep hot foods hot and cold foods cold until they are eaten.
- Do not keep cooked or raw food to long. Throw it away if it look or smells bad or spoiled.

How to store food

- Store food in the coolest part of the house
- Keep food in a clean dry place or cupboard
- Do not store food in containers that are used for other purposes for example chemicals
- Cover food to protect it from insects, dust and smoke
- Make sure that rats, mice and insects cannot reach food
- Do not store food on the ground or floor
- Do not store food near poisons such as paraffin or insecticides

Section D

<u>Keep active and stay fit</u>



Physical activity will help regulate your appetite so that you eat the right amounts of food for your energy needs. It will also help you sleep better and work more efficiently. It also helps to build strong bones. Keep fit and avoid being fat to help reduce the risk of developing some chronic diseases, including heart disease, high blood pressure and diabetes.

Annexure 18

Dental care

With care your teeth can last a live time. Bacteria in the mouth live on carbohydrate foods for example sugar. As the bacteria grow and multiply they produce acids which attacks tooth surfaces and this can cause tooth decay. Adults and children should therefore brush their teeth regularly, preferably twice a day using toothpaste with fluoride. This helps to protect the teeth from decay and avoids gum disease later in life. Another way to reduce the risk of decay is to avoid nibbling carbohydrate foods throughout the day.

A family who eats the right food, is a healthy, strong family.

Question time:
Why are healthy eating habits important?
What are the right foods food good health?
What should be included in a healthy, balanced meal?
What should you remember when you buy food?
How will you store a bag of sugar?
In how mush water must you cook vegetables?
What must you do if you scratched your head while preparing food?

Annexure 19

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 19

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

Annexure 20

POTCHEFSTROOM UNIVERSITY FOR CHE

SUGAR ACCEPTANCE AND CONSUMPTION SURVEY 2000

For office use only
Site:
.....
.....

Dear Friend,

The purpose of this survey is to obtain information on sugar consumption patterns of the participants in this research. Please answer the following questions by ticking the appropriate block or printing your answer in the appropriate space. We thank you for your co-operation!

SECTION A

1.	**Today's date**	1	
2.	**Your birth date (dd mm yy)**	2	
3.	**Male** ☐ **Female** ☐	3	
4.	**In what type of area do you live?**		
4.1	**Rural village** ☐	4.1	
4.2	**Farm** ☐	4.2	
4.3	**Township, town or city** ☐	4.3	
4.4	**Informal settlement(squatter)** ☐	4.4	

SECTION B

		Yes	No		
5.	**Do you like the taste of the given sugar?**			5.	
6.	**Is there a difference in the colour of the given sugar and the sugar you normally use?**			6.	
7.	**Does the given sugar smell different?**			7.	
8.	**Does the given sugar change the colour of the food in which you used it?**			8.	
9.	**Do you add sugar to the food while it is being cooked?**			9.	
10.	**Do you like sweet foods?**			10.	
11.	**Do you add sugar your food after it has been cooked?**			11.	

Annexure 20

12. How many spoons of sugar do you use per day?

0 teaspoon	1 teaspoon	5 teaspoons	4 teaspoons	6 teaspoons	>6 teaspoons

12.1	
12.2	
12.3	
12.4	
12.5	
12.6	

13. How much sugar do you use per month?

1 kg	2,5 kg	5 kg	10 kg	>12,5 kg

13.1	
13.2	
13.3	
13.4	
13.5	

14. Would you be prepared to buy more expensive sugar that is fortified with vitamins or minerals? **Yes** **No**

14.	

15. Do you think sugar is "healthy"? **Yes** **No**

15.	