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Associations between indices of iron status, anthropometric and biological markers of cardiovascular disease risk

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ABSTRACT

***Background*:** In South Africa, as in many other developing countries, iron deficiency (the most common micronutrient deficiency) still remains unresolved; while obesity has emerged as a public health challenge causing increases in the incidence and prevalence of cardiovascular diseases (CVDs). Research has shown that certain iron indices are associated with both anthropometric and biological markers of CVDs. Adiposity is thought to modulate the pathway linking iron status to CVDs.

Objective: To examine the associations between iron indices, anthropometric and biological markers of CVDs in an African population undergoing transition.

Methods: This thesis was based on secondary analysis of data generated during the Transition and Health during Urbanisation of South Africans (THUSA) study; and primary and secondary analysis of the baseline Prospective Urban and Rural Epidemiological (PURE) study. Both studies were cross-sectional in design and were conducted between 1996-1998 and in 2005 respectively in the North West Province of South Africa. The 1854 men and women participants in the THUSA study (≥15years) and 1262 women participants in the PURE study (≥35years) were included in the analysis. The relationship between iron and anthropometric indicators of CVD risk was examined in the THUSA study while that of iron status, anthropometric and biological markers of CVD risk was examined in the PURE study.

Results: In the THUSA study, ferritin was positively associated with body mass index (BMI), waist circumference (WC), waist to hip ratio (WHR), body fat and subscapular skinfold ($r=0.141$, 0.359, 0.396, 0.308, 0.141 respectively for men and 0.126, 0.232, 0.319, 0.126, 0.105 respectively for women; $p<0.01$). Only the women showed decreased serum iron concentration with increasing BMI ($p<0.05$). WC and WHR increased with increasing serum ferritin concentration for both genders ($p<0.05$). As for the PURE study, associations between iron status parameters and CVD risk factors were generally weak ($r<0.3$, $p<0.01$) and were not retained after adjusting for valid confounders. WC and WHR increased with increasing ferritin concentration ($p<0.05$).

Conclusion*:* Although these results do not indicate any significant association between iron indices and biological markers of CVD, its association with anthropometric indices gives an indication of the possible contribution of iron in the aetiology of CVDs. Thus, it

may be necessary to exercise caution on the emphasis placed on iron as a nutrient and iron intervention programmes because of the suggestive role of iron in CVD development.

KEYWORDS: Iron indices, ferritin, obesity, anthropometry, adiposity, cardiovascular diseases, developing countries, African, THUSA, PURE.

UITTREKSEL

Agtergrond: In Suid-Afrika, soos in baie ander ontwikkelende lande, is ‘n ystertekort (wêrelwyd die algemeenste mikronutriënt-tekort) nog steeds onopgelos. Terselfdertyd het obesiteit ’n publieke gesondheidsorg uitdaging geword, met ‘n gevolglike verhoging van die ontwikkeling en voorkoms van kardiovaskulêre siektes (KVS). Daar is bewyse dat sekere merkers van ysterstatus geassosieer word met beide antropometriese en biologiese risiko-faktore van KVS. Dit is dus moontlik dat adipositeit ysterstatus se verband met KVS mag moduleer.

Doel: Om vas te stel of daar ‘n assosiasie is tussen merkers van ysterstatus, antropometriese verandelikes en biologiese risiko-faktore van KVS in ’n swart Suid-Afrkaanse populasie wat ’n voedingsoorgang ondergaan.

Metode: Die proefskrif is gebaseer op ’n sekondêre analise van data wat gegenereer was in die THUSA-studie (“Transition and Health during Urbanisation of South Africans”) en primêre sowel as sekonêre analises in die PURE-studie (“Prospective Urban and Rural Epidemiology”). Beide THUSA en basislyn PURE was dwarsdeursnee studies en was uitgevoer in die Noordwes provinsie van Suid-Afrika tussen 1996-1998 en in 2005, onderskeidelik. Die 1854 manlike en vroulike deelnemers in die THUSA-studie ($\geq$15 jaar) en slegs die 1262 vroulike proefpersone van die PURE-studie ($\geq$35 jaar) was ingesluit in die analises. Die verband tussen yster en antropometriese metings is in die THUSA-studie ondersoek terwyl die ysterstatus en KVS risiko-faktore in die PURE-studie ondersoek is.

Resultate: In die THUSA-studie was ferritien positief geassosieer met liggaamsmassa indeks (LMI), middel omtrek (MO), middel tot heup verhouding (MHV), liggaamsvet en die subskapulêre velvou ($r=0.141, 0.359, 0.396, 0.308, 0.141$ onderskeidelik vir mans en $0.126, 0.232, 0.319, 0.126, 0.105$ onderskeidelik vir vroue; $p<0.01$). Slegs in die vroue was verlaagde serum yster geassosieer met ’n verhoogde LMI ($p<0.05$). Die MO en MHV het verhoog met ’n toename in serum ferritien konsentrasie vir beide geslagte ($p<0.05$). In die PURE-studie was assosiasies tussen die merkers van ysterstatus en KVS risiko-faktore oor die algemeen swak ($r<0.3$, $p<0.01$). Die assosiasies het verwdyn nadat daar vir geldige veranderlikes, wat moontlik ’n invloed op die resultate kon gehad het, aangepas is. MO en MHV het ook toegeneem met ’n toename in ferritien konsentrasies.

Gevolgtrekking: Alhoewel hierdie resultate nie ’n betekenisvolle assosiasie tussen merkers van ysterstatus en biologiese risiko-faktore van KVS aangedui het nie, gee die assosiasie met antropometriese metings ’n aanduiding van ‘n moontlike bydrae van yster tot die ontwikkeling van KVS. Dit mag dus nodig wees om versigtig te wees met intervensies waarin ysterstatus aangespreek word, omdat yster moontlik ’n rol in die ontwikkeling van KVD mag speel.

KERNWOORDE Yster indikatore, ferritien, obesiteit, antropometrie, adipositeit, kardiovaskulêre siektes, ontwikkelende lande, Afrika, THUSA, PURE

SUMMARY

BACKGROUND

Many developing countries are experiencing a demographic, health and nutrition transition (Lee, 2003). The challenge of undernutrition still remains, while obesity that was thought to be characteristic of developed countries has become a major public health problem in developing countries (Prentice, 2006). Both conditions can co-exist in the same household or individual (Doak *et al*., 2002).

Iron deficiency has been identified as the most prevalent single nutrient deficiency affecting almost two billion people worldwide (WHO, 2008). Iron deficiency and iron deficiency anaemia are mainly prevalent in developing countries (Heath *et al.,* 2002). Iron deficiency can occur at all stages of the life cycle, but the most vulnerable groups are women (reproductive age, pregnant and breastfeeding women) and children. In South Africa, several *ad hoc* studies have reported anaemia in 7-29% of pregnant women (Mamabolo *et al*., 2004; Dannhauser *et al*., 1999; Kruger *et al.,* 1994), 57% of pregnant teenage girls (Bopape *et al.*, 2008), 21% of infants and young children (Faber *et al.*, 2007), 26% of non-pregnant teenage girls (WHO, 2008) and 13% elderly persons (Oldewage-Theron *et al.,* 2008; Charlton *et al.,* 1997). Iron deficiency and iron deficiency anaemia have important public health implications. Diminished iron status affects energy metabolism, immune function, bone health, and work capacity (Harris *et al.,* 2003; Dallman*,* 1986).

Conversely, excess iron could be harmful because it may catalyse the formation of highly reactive oxygen and hydrogen radicals when present in the unbound state (Cook *et al.*, 1992). These radicals can cause permanent damage to intracellular proteins and deoxyribonucleic acid (Sullivan, 1981). The consequences of excess iron are of major public concern (Basset *et al.,* 1986). Excess iron may lead to fibrosis, cirrhosis, excess skin pigmentation, diabetes, arthritis, cardiac failure, and fatal arrhythmias (Basset *et al.,* 1986).

Iron deficiency has been associated with obesity in several populations (Eftekhari *et al.,* 2009; Iwasaki *et al.,* 2005; Gillum, 2001; Micozzi *et al.,* 1989). While obese people have been shown to be deficient of functional iron, their iron stores have been reported to be in the range of those classified as overload (Zafon *et al.,* 2009; Gillum, 2001). Additionally, epidemiological studies (Ramakrishnan *et al.,* 2002; Milman *et al*., 1999; Iribarren *et al*., 1998) have indicated that ferritin concentration correlates positively with cardiovascular diseases (CVDs) risk factors. Adiposity has been hypothesised to be a mediator for this association (Robinson *et al.,* 2004; Williams *et al*., 2002).

The associations between iron indices, obesity and CVD risk factors are of public health significance as the prevalence of obesity and obesity–related morbidity continues to increase along with existing micronutrient deficiency, especially iron (Beard, 2001).

AIMS AND OBJECTIVES

The main aim of this thesis was to examine the relationship between iron indices, anthropometric and biological markers of CVD risk in African volunteers in the North-West Province of South Africa. Specific objectives were to:

1. Review the literature on iron as a nutrient and its metabolism, obesity and CVDs. Additionally, this thesis reviewed (narratively) the relationship between iron status and adiposity in women in developing countries with special focus on the factors that may influence this association.

2. Examine relationships between iron indices and selected anthropometric indicators of CVD risk in an African population using the data from the Transition and Health during Urbanisation of South Africans (THUSA) study.

3. Examine the relationship between iron status and CVD risk markers in black South African women using data from the Prospective Urban and Rural Epidemiological (PURE) study.

STUDY DESIGN

This thesis was based on both primary analysis of biological samples (PURE study) and secondary analysis of pre-existing data (THUSA study). Figure 1 illustrates the design of this thesis; it indicates the contributions and activities of the candidate.

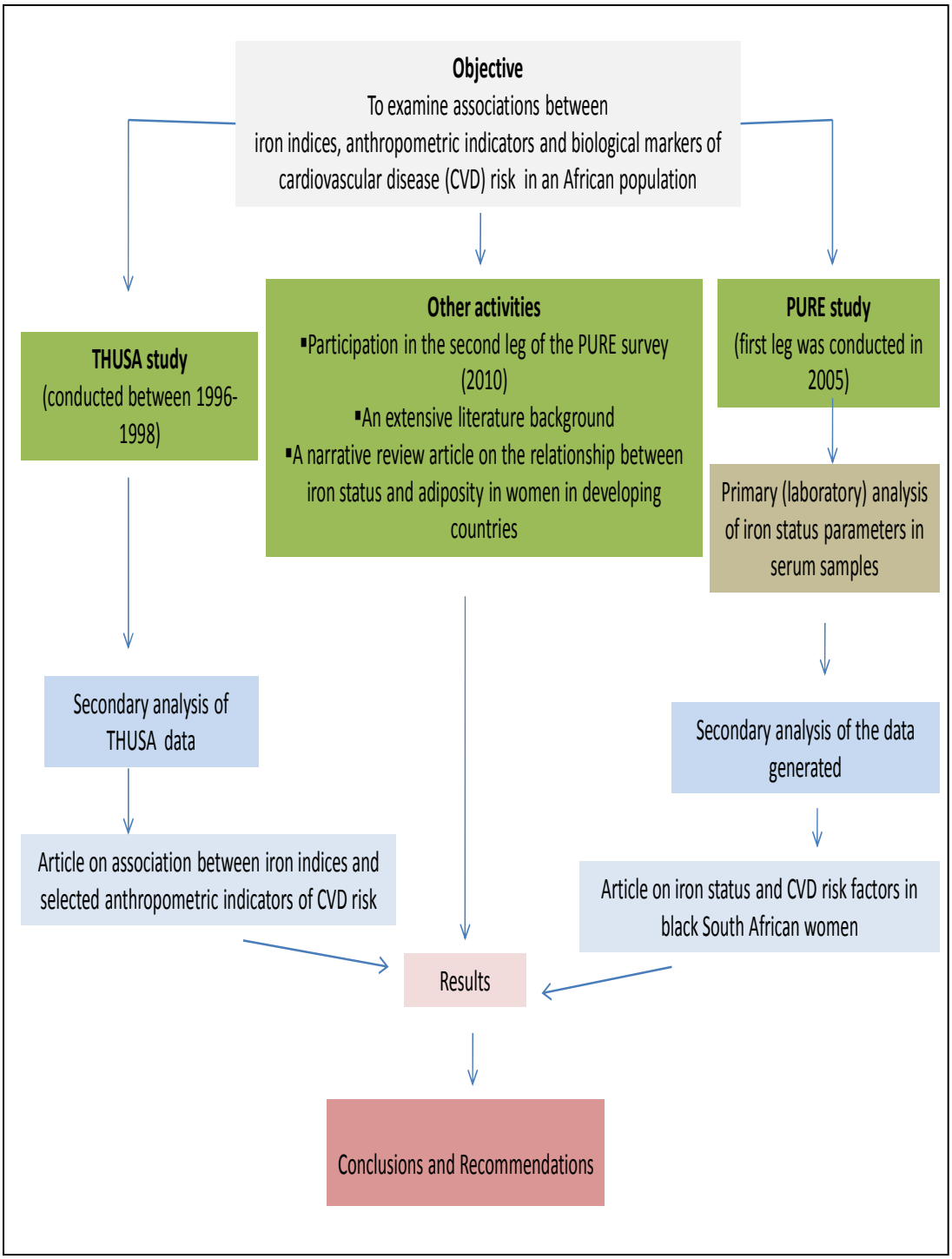

Figure 1: The design of this thesis

The THUSA study

In this cross-sectional, comparative, population-based study, 1854 men and women aged 15 years and older and from five levels of urbanisation (deep rural tribal areas, farms, informal housing area or squatter camps, established urban townships and 'upper' urban areas) voluntarily participated. The THUSA study was conducted between 1996 and 1998. Thirty-seven randomly selected sites were investigated in rural and urban areas covering all districts of the North West Province of South Africa. Pregnant and lactating women as well as subjects taking any form of chronic medication, with body temperatures above 37^0C or who were inebriated were excluded.

The PURE study

This cross-sectional epidemiological survey was part of the North West Province South African (NWPSA) leg of the 12-year PURE study which investigates the health transition in urban and rural subjects. The main selection criterion was that there should be migration stability within chosen rural and urban communities. The rural community (A) was identified 450km west of Potchefstroom on the highway to Botswana. A deep rural community (B), 35km east from A and only accessible by gravel road was also included. Both communities are still under tribal law. The urban communities (C and D) were chosen near the North-West University (Potchefstroom Campus). Community C was selected from the established part of the township next to Potchefstroom and D from informal settlements surrounding community C. The baseline data for NWPSA were collected from October-December 2005. A total of 2010 apparently healthy African volunteers (35 years and older), with no reported chronic diseases of lifestyle, tuberculosis (TB) or known human immunodeficiency virus (HIV) were recruited from a sample of 6000 randomly selected households. The present study examined a total of 1262 women volunteers in the PURE study.

METHODS

A variety of quantitative and qualitative research techniques were used by a multi-disciplinary team to collect, analyse and interpret data generated from biological samples and questionnaires. Data were analysed using the Statistical Package for Social Sciences (SPSS), version 17. In the THUSA study, data were marginally not normally distributed and were presented as means and standard errors. Participants were grouped into different categories using standard cut-off points for waist to hip ratio (WHR), body mass index (BMI) and ferritin. A Spearman correlation test was used to examine associations between variables while a multivariate analysis of variance (MANOVA) was used to test significant difference between groups. As for the PURE study, data were also not normally distributed and were logarithmically transformed. Data were presented as means, medians and 95% confidence interval. Participants were grouped into quartiles of ferritin, transferrin receptor (TfR) and TfR/ferritin ratio. A Pearson correlation test was used to examine associations between variables. A MANOVA test was used to examine significance difference between quartiles. In both studies, a partial correlation test was used to examine the associations between variables after adjusting for valid confounding factors.

RESULTS

After an extensive literature review on iron, obesity and CVDs, a review paper was prepared discussing the factors influencing the relationship between iron status and adiposity in women from developing countries. The results indicate an inconsistent relationship between iron indices and adiposity. Furthermore, other factors such as infection, alcohol consumption, type of diet and genes were shown to affect the relationship between iron status and adiposity in women from developing countries.

The THUSA study

In this study, serum ferritin was positively associated with BMI, waist circumference (WC), WHR, body fat and subscapular skinfold (r=0.141, 0.359, 0.396, 0.308, 0.141

respectively for men and 0.126, 0.232, 0.319, 0.126, 0.105 respectively for women; $p<0.01$). Serum ferritin concentration was higher in the high WHR category than the normal WHR category for both genders ($p<0.05$). Additionally, WC and WHR increased with increasing ferritin concentration in both genders ($p<0.05$). Serum iron was lower in the obese than the normal weight and pre-obese women only ($p<0.05$).

The PURE study

Associations between iron status parameters and CVD risk factors were generally weak ($r<0.3$, $p<0.01$). These associations were not retained after adjusting for age, BMI, smoking, alcohol consumption and C-reactive protein (CRP). WC and WHR were significantly higher in the fourth quartile of serum ferritin than the third, also, in the third quartile compared to the second quartile ($p<0.05$).

DISCUSSION AND CONCLUSION

Both the THUSA and PURE studies were conducted in the North West Province of South Africa from 1996-1998 and in 2005 respectively. The results indicate that abdominal obesity as defined by increased WC and WHR associated positively with serum ferritin concentration in this population. However, there is no evidence of a significant association between any iron index and biological markers of CVD in the women that were examined.

Several mechanisms have been speculated to be responsible for the elevation of serum ferritin in obese people. One is the low grade inflammation that occurs during fat deposition. This could contribute to the elevation of serum ferritin concentration since ferritin is an acute phase protein.

It is concluded that both WC and WHR are associated positively with serum ferritin concentration for both genders and no significant association exists between iron indices and CVD risk factors in the women. It may be necessary to scale up obesity interventions as it has been shown to play a significant role in the iron status of this population. Although no significant association was observed between iron indices and

CVD risk factors, the role iron plays in the development of CVD cannot be overlooked as the number of iron replete individuals continues to rise as a result of various intervention programmes (such as micronutrient fortification of staple foods, and provision of supplement to pregnant women) that are taking place.

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LIST OF ABBREVIATIONS

µg/l	Micrograms per litre
AIDS	Acquired immune deficiency syndrome
ANOVA	Analysis of variance
BP	Blood pressure
BMI	Body mass index
BMP6	Bone morphogenetic protein 6
CI	Confidence interval
cm	Centimeter
CRP	C-reactive protein
CV	Coefficient of variance
CVDs	Cardiovascular diseases
DXA	Dual energy X-ray absorptiometry
DCYTb	Duodenal cytochrome b
DMT1	Divalent metal transporter 1
DNA	Deoxyribonucleic acid
DOH	Department of Health
EDTA	Ethylenediamine tetra acetic acid
FAO	Food and Agricultural Organisation
FFQ	Food frequency questionnaire
GH	Growth hormone
g/d	Grams per day
Hb	Haemoglobin
HCP1	Haeme carrier protein 1

HDL-C	High density lipoprotein cholesterol
HFE	Symbol for haemochromatosis gene
HIV	Human immunodeficiency virus
HJV	Haemojuvelin
IASO	International Association for the Study of Obesity
IL-6	Interlukin 6
IREG1	Iron regulated gene 1
JAK	Janus activated kinase
KDa	KiloDalton
kg/m^2	Kilograms per meter squared
Km	Kilometers
LDL-C	Low density lipoprotein cholesterol
MCV	Mean corpuscular volume
mg	Milligrams
mmol/l	Millimole per litre
mmHG	Millimeter of mercury
mRNA	Messenger ribonucleic acid
NTB1	Non-transferrin bound iron
NWPSA	North West Province South Africa
PGF_2	Prostaglandin F2
PURE study	Prospective Urban and Rural Epidemiological study
oxLDL	Oxidised low density lipoprotein cholesterol
RBCs	Red blood cells
RDA	Recommended daily allowance
ROS	Reactive oxygen species

SD	Standard deviation
SE	Standard error
SMAC	Sequential multiple analyser computer
SPSS	Statistical package for social sciences
SSF	Subscapular skinfold
STAT3	Signal transducer and activator of transcription 3
STEAP3	Six-transmembrane epithelial antigen of prostrate protein 3
TB	Tuberculosis
TC	Total cholesterol
TfR	Transferrin receptor
TG	Triglyceride
TGF	Transforming growth factor
THUSA study	Transition and Health during Urbanisation of South Africans study
TIBC	Total iron binding capacity
TSF	Triceps skinfold
UN	United Nations
UNICEF	United Nations Children's Fund
VLDL	Very low density lipoprotein
WC	Waist circumference
WHO	World Health Organisation
WHR	Waist to hip ratio

LIST OF SYMBOLS

^{o}C	Degree Celsius
%	Percentage
μ	Micro
β	Beta
α	Alpha
r	Correlation coefficient
r_s	Spearman correlation coefficient
R	Partial correlation coefficient
>	Greater than
≥	Greater than or equal to
<	Less than
≤	Less than or equal to
+/-	Plus or minus
N	Number
Fe^{2+}	Ferrous ions
Fe^{3+}	Ferric ions

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CHAPTER 1

INTRODUCTION

CHAPTER 1: INTRODUCTION

1.1 BACKGROUND AND MOTIVATION

Iron is crucial for life; every cell needs iron for metabolism and growth. Iron deficiency has been identified as the most prevalent single nutrient deficiency affecting over 2 billion people worldwide (WHO, 2008). The developing countries have the highest prevalence of iron deficiency (Heath & Fairweather-Tait, 2002) with Asian countries topping the list followed by countries in Sub-Sahara Africa (Dreyfuss *et al*., 2000). Women of reproductive age and children are mostly affected by iron deficiency (Stoltzfus *et al.,* 2004). In South Africa, anaemia has been reported in 7-29% of pregnant women (Mamabolo *et al*., 2004; Dannhauser *et al*., 1999; Kruger *et al.,* 1994), 57% of pregnant teenage girls (Bopape *et al.*, 2008), 21% of infants and young children (Faber & Wenhold, 2007), 26% of non-pregnant teenage girls (WHO, 2008) and 13% elderly persons (Oldewage-Theron *et al.,* 2008; Charlton *et al.,* 1997). Additionally, iron deficiency may exist without anaemia (WHO, 2001). The main causes of anaemia are: intake of a diet poor in iron and infectious diseases such as malaria, hookworm infestations and schistosomiasis. Deficiencies of other key micronutrients including folate, vitamin B_{12} and vitamin A, and inherited conditions such as thalassaemia may also cause iron deficiency anaemia (WHO, 2004).

Poor iron status has adverse effects on health (Cook *et al.*, 1992). Iron deficiency has been associated with obesity among men, women and children (Eftekhari *et al.,* 2009; Iwasaki *et al*., 2005; Gillum, 2001; Micozzi *et al.,* 1989). Larairai *et al.* (2007) observed that body mass index (BMI) increased as the proportion of women who did not meet their estimated average requirement for iron increased. Additionally, a cross-sectional study observed that more than 50% of overweight children and adolescents who exhibited iron deficiency anaemia had BMI greater than the 97^{th} percentile (Pinhas-Hamiel *et al*., 2003).

Conversely, excess iron could be detrimental to health. Iron in the unbound state may catalyse free radical formation which may damage intracellular proteins and

deoxyribonucleic acid (DNA) (Cook *et al.*, 1992). Because of the ease with which additional iron can be provided to people through iron fortified foods and iron supplements and the limited ability to excrete the mineral, the consequences of iron overload are of public health significance (Basset *et al.,* 1986). Excess iron may lead to fibrosis, cirrhosis, excess skin pigmentation, diabetes, arthritis, cardiac failure, and fatal arrhythmias (Basset *et al.,* 1986).

South Africa is experiencing a health transition that is characterised by a triple burden of disease comprising of under nutrition-related infectious diseases, non-communicable diseases and the human immunodeficiency virus/acquired immune deficiency syndrome (HIV/AIDS) (Vorster, 2002). The prevalence of obesity and obesity–related morbidity continues to increase (Beard, 1994). In South Africa, 30% of men and 55% of women have been classified as overweight or obese (DOH, 2007). Evaluation of disease risk factors resulting from under and over nutrition is, therefore, important.

It is pertinent to know the extent of the threat posed by different risk factors before better health can be achieved. Risk factors for cardiovascular disease (CVD) are many, and there is vast interaction between them. With both iron deficiency and obesity existing in South Africa, it is important to establish whether they are related. Establishing this relationship and relating it to biological health outcomes may provide additional clues to the aetiology of CVD. This is particularly important in a population undergoing a health transition like South Africa.

1.2 Associations between iron indices, anthropometry and cardiovascular disease risk factors

Measures of iron stores have been reported to correlate positively with CVD risk factors (Ramakrishnan *et al.,* 2002; Milman & Kirchhoff, 1999; Iribarren *et al*., 1998; Halle *et al*., 1997; Magnusson *et al*., 1994). Research evidence has indicated that adiposity may play a modulating role in the pathway linking iron stores to CVD risk (Robinson & Graham, 2004; Williams *et al*., 2002). Serum ferritin concentration has been found to associate positively with higher plasma triglycerides (TG) (Salonen *et al*., 1992), glucose

(Halle *et al.,* 1997), total cholesterol (TC) (Magnusson *et al*., 1994), fibrinogen concentrations (Oshuag *et al*., 1995), and systolic (Salonen *et al*., 1992) or diastolic blood pressure (Milman & Kirchhoff*,* 1999). In addition, a negative association between high density lipoprotein cholesterol (HDL-C) and ferritin concentration has been reported (Oshuag *et al*., 1995; Salonen *et al.,* 1992). Furthermore, adiposity has been shown to be a strong determinant of ferritin concentration (Gillum, 2001).

However, other studies do not corroborate this result (Sempos *et al.,* 2000; Sempos *et al*., 1994; Stampfer *et al*., 1993). It was proposed that increased serum ferritin levels in obesity results from inflammation and not necessarily increased iron stores (Oshuag *et al.*, 1995; Herbert *et al.,* 1995; Alexander, 1994; Lipschitz *et al*., 1974). However, the ratio of serum transferrin receptors to serum ferritin was used by Tuomainen *et al.* (1998), who found that the risk of acute myocardial infarction among Finnish men doubled across tertiles, even after adjusting for inflammation and alcohol intake. Interaction of iron stores with established CVD risk factors like low density lipoprotein cholesterol (LDL-C) and TC could also explain this observation (De Valk & Mark*,* 1999).

There is an indication that iron functions in the maintenance of body weight and composition. In addition, recent data have shown that adipocytes are not just storage for energy, they are endocrine organs, with multiple metabolic roles in regulating whole-body physiology (Andrew *et al.,* 2006) including iron metabolism. Hepcidin, which is expressed in obese individuals, is thought to play a role in anaemia associated with obesity (Nemeth *et al.*, 2003; Fleming & Sly*,* 2001). There is growing evidence that the inflammatory state that characterises obesity may play a causal role in the development of insulin resistance, type 2 diabetes, and the metabolic syndrome (Weisberg *et al.,* 2003; Xu *et al*., 2003; Grimble, 2002).

In summary, therefore, these intriguing, but often controversial findings mentioned above motivated this study to examine the relationships between iron status and obesity, and a possible modulating role of iron in the relationships between obesity and CVD risk, in a population where iron deficiency, a positive iron balance and obesity are prevalent.

1.3 Measures of iron status to be explored in this study

Iron deficiency is usually considered to develop in three sequential stages; depletion of iron stores, iron deficient erythropoiesis and overt anaemia with low haemoglobin (Hb) levels. The direct indicators for assessing iron deficiency utilize blood parameters that are reflective of one or more of the above stages.

1.3.1 Haemoglobin

Hb assessment is relatively easy and inexpensive, and this measurement is frequently used as a proxy measure of iron deficiency. However, it is not a sensitive indicator of iron status as the levels only drop in the third stage of iron deficiency. It is also not a specific indicator of iron deficiency as other micronutrient deficiencies, parasitic infections and certain diseases also affect Hb concentrations (Gibson, 2005; WHO, 2001).

1.3.2 Serum iron

Serum iron concentration declines after iron stores are fully depleted. Its utilisation is limited by the wide diurnal variations in iron concentration. Concentrations in healthy subjects may vary by as much as 100% during a 24-hour interval. This variation is not diminished significantly by sampling at a uniform time each day because roughly one-third of the subjects cycle in the reverse direction (Bothwell *et al.,* 1979).

1.3.3 Total Iron Binding Capacity (TIBC)

TIBC is the amount of added iron that can be specifically bound by plasma. It increases in iron deficiency but falls with inflammation. It provides some additional discriminating evidence although it is usually within the normal range when iron deficiency and chronic inflammation coexist. Because the serum iron and TIBC move in a reciprocal fashion in iron deficiency and iron overload, an

informative expression of iron transport is the serum iron expressed as a percentage of the TIBC (Bothwell *et al.,* 1979).

1.3.4 Transferrin saturation

Transferrin is a protein with a molecular mass of 79.6 kDa which consists of a single amino acid chain. Each transferrin molecule can bind two ferric (Fe^{3+}) ions. Around 90% of transferrin is formed in the liver. Under normal conditions, about a third of the transferrin is loaded with iron. The transferrin saturation can be easily calculated using the equation transferrin saturation (%) = (iron X 100/ transferrin). In cases of iron deficiency, the transferrin saturation is lowered; with iron overload, it is increased. Determining the transferrin saturation significantly increases the diagnostic sensitivity compared to the measurement of ferritin alone when screening for iron overload and monitoring therapy. The main limitation of the transferrin saturation measurement relates to the wide diurnal variations in serum iron concentration (Hinzmann, 1999).

1.3.5 Ferritin

Ferritin is a ubiquitous intracellular protein that stores iron and releases it in a controlled fashion (Casiday & Frey, 2000). It is an acute phase reactant and, therefore, elevated in response to any infectious or inflammatory process (WHO, 2001). Serum ferritin levels below 12µg/l are indicative of depleted iron stores (Beard, 1994).

1.3.6 Transferrin receptors (TfR)

TfR increases in iron deficiency and unlike serum ferritin it appears to be a more promising indicator of iron status. The TfR rises only when iron stores are exhausted and serum ferritin has fallen below 12µg/L and, therefore, it is considered a good indicator of functional iron deficiency (Skikne *et al.,* 1990).

1.4 Aims and objectives

The main aim of this thesis is to examine the relationship between measures of iron status, anthropometric indices and biological markers of CVD risk in an African population. Within this umbrella aim, specific projects, each with clearly defined objectives were done.

- Firstly, an extensive literature survey on iron and its metabolism, obesity and CVDs was conducted. Additionally, a review paper discussing factors influencing the relationship between iron status and adiposity in women from developing countries was prepared and submitted for publication in Critical Reviews in Food Science and Nutrition.

  Specific objectives

  - ✓ To examine relevant literature concerning iron and its metabolism, obesity and CVDs in order to have a good understanding of the link between them.

  - ✓ To examine the factors influencing the relationship between iron status and adiposity in women from developing countries.

- Secondly, the relationship between iron indices and selected anthropometric CVD risk factors was examined using the data generated from the Transition and Health during Urbanisation of South African (THUSA) study. An original article was prepared; this has been accepted for publication in the Cardiovascular Journal of Africa.

  Specific objective

  - ✓ To examine the associations between the following iron and anthropometric indices:

- Serum ferritin
- Serum iron
- Hb
- TIBC
- Transferrin saturation
- BMI
- Waist circumference (WC)
- Waist to hip ratio (WHR)
- Percentage body fat
- Skinfolds (triceps and subscapular)

Data from the THUSA study were used to examine the relationship between iron status variables and anthropometric indices (paper in press). These relationships were then examined in more depth using data from women in the PURE study to examine relationships between iron status variables and other CVD risk factors.

- Thirdly, the relationship between iron status and CVD risk factors was examined in women participants in the Prospective Urban and Rural Epidemiological (PURE) study. An original article was written and submitted to the Public Health Nutrition Journal.

Specific objectives

- ✓ To conduct laboratory analysis of iron indices (TfR and ferritin) on serum samples of women participants collected during the PURE baseline survey in 2005.

- ✓ To examine the relationship between the following iron and CVD risk factors:

- Serum ferritin
- TfR
- TfR/Ferritin ratio
- BMI
- WC
- WHR
- TC
- LDL-C
- HDL-C
- TG

- Blood pressure
- Blood glucose
- C-reactive protein

1.5 Structure of the thesis

This thesis is presented in article format and consists of six chapters which contain one review paper and two original papers submitted for publication, one of which was accepted during the course of writing up this thesis.

Following this introductory chapter:

Chapter 2 comprises of an extensive literature background on iron as a nutrient and its metabolism, obesity and CVDs.

Chapter 3 is a narrative review paper that elucidates the relationship between iron status and adiposity in women in developing countries. This article has been submitted to the Critical Reviews in Food Science and Nutrition.

Chapter 4 comprises of an original article which examines the relationship between indices of iron status and selected anthropometric indicators of CVD in an African population. This article is in press in the Cardiovascular Journal of Africa.

Chapter 5 is an original article on iron status and CVD risk factors in black South African women living in the North West Province. This article is being reviewed by Public Health Nutrition.

Chapter 6 comprises of a general discussion, recommendation and conclusions.

The references are provided at the end of each chapter according to author's instruction as specified by each journal to which papers were submitted. The relevant references used in Chapters 1, 2 and 6 are provided according to the requirement stipulated by the North-West University (Potchefstroom Campus). The technical style used in the unpublished chapters is uniform but differs in other chapters. Addenda for both the THUSA and PURE studies close this thesis.

Note: Both the THUSA and PURE studies recruited "apparently" healthy subjects. However, some of the participants tested human immunodeficiency virus (HIV) infected. Secondary analysis of the PURE data showed that HIV infection was not a confounder in the relationships tested. Therefore, HIV-infected persons were included in these analyses.

A conceptual framework that illustrates the possible interrelationships between iron, adiposity and CVDs is given in Figure 1.1

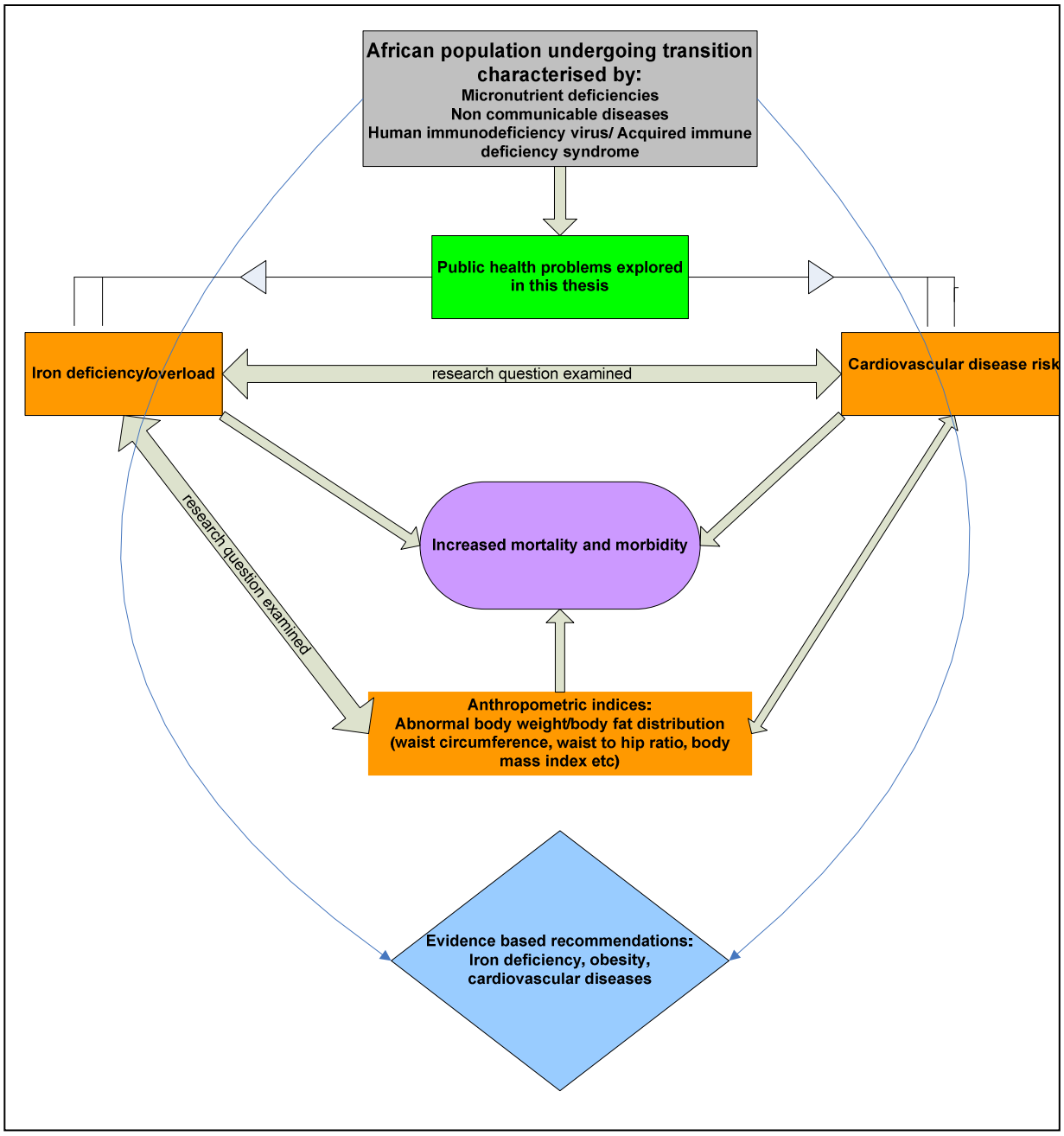

Figure 1.1 Conceptual framework for this thesis

1.6 Ethical consideration

The study forms part of the broader THUSA and PURE studies and the collection of information and relevant biological samples from informed volunteers has the necessary ethical clearance from the Ethics Committee of the previous Potchefstroom University Christian Higher Education (THUSA) and the Ethics Committee of the North-West University and North West Department of Health (PURE). The reference numbers for ethical approval are **4M5-95** (THUSA) and **04M10** (PURE).

1.7 Author's contributions to the separate papers in this thesis

The studies reported in this thesis were planned and executed by a team of researchers and contribution of each researcher is illustrated in Table 1.

Table 1 Research team members and their roles

Name	Role in the study
O.R. Aderibigbe (PhD candidate)	Writing and compilation of this thesis, serum sample analysis, all the statistical analyses in this thesis, interpretation of results and writing of publications, first author of 3 articles (Chapters 3,4 & 5) in this thesis
Dr P.T. Pisa (Supervisor)	Supervised this thesis, statistical analyses, interpretation of results, co-authored 3 articles (Chapters 3,4 & 5) in this thesis
Prof H.H. Vorster (Co-supervisor)	Co-supervised this thesis, planning and coordinating the THUSA study, interpretation of results, co-authored 3 articles (Chapters 3,4 & 5) in this thesis
Dr R.L. Mamabolo	Co-authored 2 articles (Chapters 4 & 5) in this thesis
Prof H.S. Kruger	Co-authored 3 articles (Chapters 3,4 & 5) in this thesis
Prof A. Kruger	Planning and coordinating the PURE study, co-authored 1 article (Chapter 5) in this thesis

I declare that as a co-author I have approved the above mentioned article, that my role in the study, as indicated above, is a representation of my actual contribution and that I hereby give consent that the manuscript may be used for the PhD thesis of Mrs. O.R. Aderibigbe.

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Dr P.T. Pisa Prof H.H. Vorster

_____ _____

Dr R.L. Mamabolo Prof H.S Kruger

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Prof A. Kruger

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CHAPTER 2

EXTENSIVE LITERATURE BACKGROUND

CHAPTER 2: EXTENSIVE LITERATURE BACKGROUND

OVERVIEW

This segment of the thesis describes the nutrition and health transition experienced in many developing countries; iron as an important nutrient and its role in health; obesity; cardiovascular diseases (CVDs) and the link between iron, obesity and CVDs.

2.1 INTRODUCTION AND BACKGROUND

The world's population is increasingly becoming urbanised as a result of natural demographic growth of urban populations, migration from rural to urban areas and the development of rural towns into urban centres (UN Population Division, 2002). The urbanisation occurring in developing countries is being accompanied by an epidemiological transition (Lee, 2003). This has been described as a change in the health, disease and mortality pattern of populations (Omran, 1971). While hunger, infectious and parasitic disease still remain unresolved in many developing countries, non-communicable diseases that were thought to be confined to the developed countries are becoming increasingly prevalent in developing countries (Prentice, 2006).

As poor countries become more prosperous, they acquire some of the benefits of rich countries as well as some of the problems. One of the acquired problems is obesity (Lee, 2003). This is as a result of changes in diet, physical activities, health and nutrition patterns (Vorster, 2002). It has been estimated that more than one billion adults are overweight, one-third of whom are already obese globally (IASO, 2004; WHO, 2002). Countries undergoing economic development such as China, Brazil and South Africa are experiencing a rapid increase in the prevalence rates of obesity across all age groups and economic levels (Yip & Ramakrishnan, 2002).

South Africa is an emerging economy that is experiencing a triple burden of disease like many other developing countries. This is characterised by a high prevalence of undernutrition-related infectious diseases, the emergence of non-communicable diseases,

and the human immunodeficiency virus/acquired immune deficiency syndrome (HIV/AIDS) pandemic (Vorster, 2002). In South Africa, 17% of children aged 1-9 years, 55% of adult females and 30% of adult males have been classified as overweight or obese (DOH, 2007; Steyn *et al.*, 2005). It has been shown that women tend to become obese at a faster rate than men (WHO, 2002; Steyn *et al.*, 2000). Obesity is an established risk factor for many non-communicable diseases such as insulin resistance, diabetes, hypertension, dyslipidaemia, osteoarthritis, ishaemic heart disease and certain cancers (WHO, 2000).

Despite the increasing prevalence of obesity in South Africa, micronutrient deficiency still remains a public health challenge. The diet supplying excess energy leading to overweight/obesity could be deficient in micronutrients (Quinion, 2010). Iron deficiency is the most common micronutrient deficiency in developing countries (WHO, 2008). It affects approximately two billion people globally (WHO, 2008). Iron deficiency is more prevalent in developing countries than developed countries (UN, 2000). Almost half of infants and pregnant women in developing countries are anaemic and 20-50% of non-pregnant women of reproductive age are iron deficient (Yip & Ramakrishnan, 2002). Prevalence of iron deficiency in South Africa varies from 26% in non pregnant women to 29% in pregnant women and 57% in pregnant teenage girls (Bopape *et al.,* 2008; WHO, 2008; Mamabolo *et al.,* 2004).

Iron deficiency has a detrimental effect on cognition, reproduction, respiration, immunity and work capacity (WHO, 2001). However, excess iron has additionally been shown to have adverse effects on health (Sullivan, 1981). Excess iron is able to catalyse oxidative reactions that initiate the development of CVDs (Salonen *et al.,* 1992).

Scientific evidence suggests that obesity may be an important determinant of iron status (Seltzer & Mayer, 1963). Obesity has been shown to associate negatively with functional iron while associating positively with iron stores (Chambers *et al.,* 2006). Furthermore, increased iron stores have been associated with increased risk for developing CVDs (Kelly, 2002).

2.2 IRON

Iron is an essential element. It is the fourth most abundant element and the second most abundant metal in the earth's crust. However, because it exists in an oxidised state its accessibility is limited (Hallberg & Asp, 1996). Iron takes part in many processes in the human body, including oxygen transport and storage, energy release and propagation of genetic information (Cook *et al.*, 1992). Given the critical dependence of life on iron, human beings have a unique capacity to store iron, but a very limited capacity to absorb it from the diet (Gibson, 2005).

2.2.1 Laboratory assessment of iron status

Body iron is found in three major compartments, namely: (i) storage compartment (ii) transport compartment (iii) erythroid iron compartments. During iron deficiency, these three compartments are depleted sequentially and assessment is always targeted at examining the stage and severity of iron depletion (Beard, 1994).

2.2.1.1 Storage iron

Iron stores serve as buffer during periods of increased iron need as the case may be during pregnancy or blood loss (Bothwell *et al.,* 1979). The storage iron compartment is located primarily in the reticulo-endothelial cells of the liver, spleen and bone marrow (Bothwell *et al.,* 1979). Iron is stored in two forms (ferritin and haemosiderin) which are related structurally and functionally (Herbert *et al.,* 1997). Ferritin is the diffuse soluble fraction while haemosiderin is the aggregated insoluble fraction. Serum ferritin concentration seems to be the most accurate measure of iron stores (Iancu, 1992). However, serum ferritin concentration is elevated during infection or other inflammatory conditions, thereby limiting it as a measure of iron stores (Cook *et al*., 1992).

2.2.1.2 Transport iron

Approximately 0.1% of body iron is found in transport compartments (Baker and Morgan, 1994). Iron is transported in the serum bound to a protein called transferrin and then delivered to receptors present on cell surfaces. Transferrin maintains extracellular iron in a soluble form, prevents iron-mediated free radical toxicity and facilitates transport into cells (Baker & Morgan, 1994). Serum transferrin is an 80 KDa glycoprotein with homologous N-terminal and C-terminal iron-binding domains (Huebers & Finch, 1987). Serum transferrin is often determined in the laboratory as the total iron binding capacity (TIBC). TIBC is the amount of serum iron that can be bound by plasma transferrin, thus, serum iron and TIBC are inversely related (Ponka & Lok, 1997). Transferrin saturation is usually calculated as a percentage of the TIBC (% transferrin saturation = serum iron X 100/TIBC) (Ponka & Lok, 1997). Serum iron, another measure of transport iron, is affected by diurnal variations, and this also affects percentage transferrin calculated from it (Baynes, 1996). Soluble serum transferrin receptor (TfR) concentration has been suggested as a better measure of functional iron. It is elevated in iron deficiency and differentiates between iron deficiency anaemia and anaemia of chronic diseases (Kohgo *et al.,* 1986).

2.2.1.3 Erythroid iron

Red blood cells (RBCs) are referred to as erythroid cells. They are haeme containing proteins that function in oxygen transport. The largest percentage of body iron is contained in the erythroid cells (Schlieper *et al.,* 2004). Haemoglobin (Hb), the essential component of RBCs, transports oxygen through the bloodstream from the lungs to all the tissues of the body. Hb also carries carbon dioxide back to the lungs to complete the process of respiration (Bothwell *et al.,* 1979).

Myoglobin, a single chain globular protein, is located in muscle cells. It is the primary oxygen-carrying pigment of muscle. In addition, myoglobin serves as a

reserve supply of oxygen (Schlieper *et al.,* 2004). During periods of oxygen deprivation oxy-myoglobin releases its bound oxygen which is then used for metabolic purposes (King, 2010).

Hb or haematocrit (Hct) are used to determine the advanced stage of iron depletion and it is the only laboratory assay that provides a quantitative measure of the severity of iron deficiency once anaemia has developed (Cook *et al*., 1992). Hb and Hct determinations are basically interchangeable with respect to assessment of iron status, the choice depending on the available instrumentation (Cook *et al.,* 1992). However, sensitivity and specificity of Hb measurement to iron deficiency is limited (Gibson, 2005). Mean corpuscular volume (MCV) and the level of free protoporphyrin are reliable indices of reduced Hb synthesis, but they do not become elevated until several weeks after the onset of iron deficiency (Jensen *et al.*, 1990; Bothwell *et al*., 1979). Moreover, none of these measures differentiate between iron deficiency anaemia and anaemia of chronic diseases (Piomelli *et al.,* 1973). Figure 2.1 shows the distribution of iron in the different compartments.

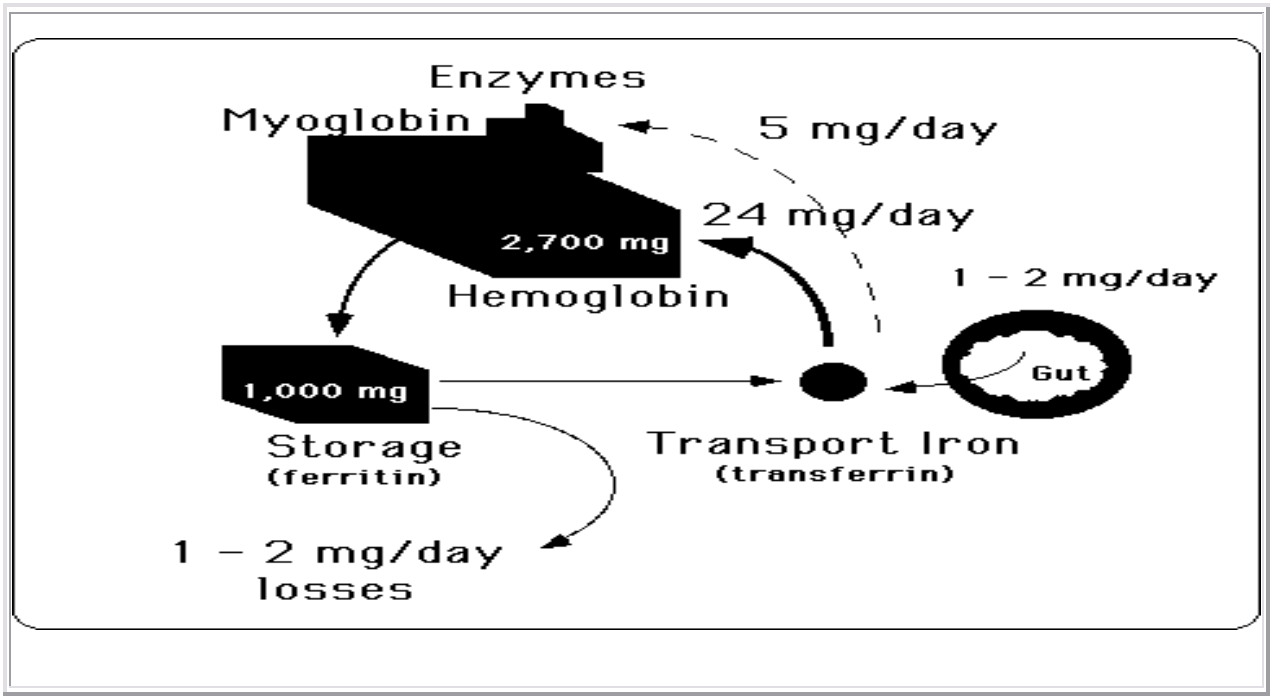

Figure 2.1 Distribution of iron in body compartments (adapted from Okam, 2007)

2.2.2 Dietary assessment of iron status

Diets are rated in quality according to the amount of nutrients (in this case, iron) they provide. It often involves an interviewer who tries to get the dietary data from the respondent. Dietary assessment is challenging because it is largely dependent on the ability of the respondent to recall what he/she has eaten (Thompson & Byers, 1994). Dietary assessment methods include:

2.2.2.1 24 hour recall

This is a quick and easy method. Information about the foods the respondent has eaten in the past 24 hours is collected. It does not truly represent the person's usual intake (Gibson & Fergusson, 2008).

2.2.2.2 Food frequency questionnaire (FFQ)

In this method, a respondent is given a list of foods to indicate his/her intake (frequency and quantity) per day, week or month. It is more representative of a person's usual diet. It is inexpensive and easy to use. There is the difficulty of estimating serving size. It puts more burden on the respondent (Schatzkin *et al.,* 2003). Kunneke (2008) developed a short FFQ specifically to assess iron intake. The validity of the short FFQ was tested on a sample of 140 pre-menopausal women aged 18-45 years. It was concluded that the short FFQ could be used to assess dietary iron intake in groups, but not in individuals (Kunneke, 2008).

2.2.2.3 Dietary history

This method aims to cover usual food intake pattern of individuals over a relatively long period of time. It is subject to the respondent's memory and interviewer's experience (Wrieden *et al*., 2003).

2.2.2.4 Food dairy technique

The type and quantity of food eaten is recorded by the respondent at the time of consumption. The data collection period varies between 1-7 days. It depends on the respondent's reliability (Wrieden *et al*., 2003).

2.2.3 Definition of iron status

Iron balance is defined as the net difference between the amount of iron that is excreted and the amount of iron that is absorbed from nutritional sources (McClung & Karl, 2008). The maintenance of iron balance is highly regulated and is controlled mainly at the site of absorption (McClung & Karl, 2008). The following terms describe the different stages of iron load in the body:

2.2.3.1 Normal iron status

This is a state where the functional needs of the erythroid mass and other tissues are met and the iron store is not being threatened (Hallberg & Asp, 1996).

2.2.3.2 Iron depletion

This is the stage where storage iron is being used up. Though this stage is not associated with any adverse effects, it does indicate a risk for iron deficiency (Bothwell *et al*., 1979).

2.2.3.3 Iron deficient erythropoiesis

At this stage, the needs of the erythroid mass are no longer met, and the level of erythrocyte protoporphyrin and serum TfR concentration goes up while Hb concentration may still be normal (Skikne *et al.*, 1990).

2.2.3.4 Iron deficiency anaemia

This is the last and most severe stage of iron deficiency. Hb concentration is reduced and production of RBCs is affected (Mahan & Escott-Stump, 2008).

2.2.3.5 Iron overload

Accumulation of excess storage iron to an extent that it causes damage to tissues (Kelly, 2002).

Table 2.1 describes the concentration of iron status variables at the different levels of iron load in adults. Note that the terms *positive and negative iron balance* are used to describe overload and deficiency states in some literature.

Table 2.1 Sequential stages of iron depletion in adults

	POSITIVE IRON BALANCE		NORMAL	NEGATIVE IRON BALANCE			
	STAGE II	***STAGE I***		***STAGE I***	***STAGE II***	***STAGE III***	***STAGE IV***
TIBC(µg/l)	<30	<30	33±3	30-36	36	39	41
Serum ferritin (µg/l)	>300	>150	100±60	<25	20	10	<10
Serum iron (µg/l)	>17	>15	11±5	<12	11	<6	<6
Transferrin saturation (%)	>60	>45	35±15	30	30	<15	<15
RBC Protoporphyrin	30	30	30	30	30	100	200
Haemoglobin (g/l)	--	--	>13(male) >12(female)	--	--	--	--
Serum transferrin receptor (mg/l)	--	--	<8.5	>8.5	--	--	--

Adapted from Herbert *et al.* (1997) and Cook *et al.* (1992)

TIBC: total iron binding capacity, RBC: red blood cell

2.2.4 Causes of iron deficiency and/or iron deficiency anaemia

2.2.4.1 Poor/inadequate diet

Inadequate dietary intake is the major cause of iron deficiency (Umbreit, 2005). A diet that is composed mainly of foods containing non-bioavailable iron and substances inhibiting iron absorption (e.g. phytates) will eventually lead to iron deficiency (Hurrell, 1997). Iron deficiency may also be aggravated by poor nutritional status, especially when it is associated with deficiencies in folic acid, vitamin A or B_{12}, as is often the case in populations living in developing countries (Herbert, 2001; Hurrell, 1997). The best sources of iron are meat, poultry, fish, eggs and iron-fortified foods (foods that have iron added). Plant sources of iron (spinach and other dark green leafy vegetables, certain types of beans, dried fruits, and cereals) are not readily absorbed and sometimes contain inhibitors of iron absorption (Mahan & Escott-Stump, 2008).

Food fortification has demonstrated to be an efficient strategy to prevent iron deficiency (Bothwell *et al*., 1992). However, any iron source used for food fortification must meet certain requirements (Boccio *et al*., 1992; Cook & Reusser, 1983). These are: 1) high iron bioavailability, 2) inertness of the iron in relation to the sensory properties of the fortified food, 3) absence of toxicity, 4) stability during storage or elaboration process and 5) absorption mechanism of the iron in the fortified food should follow the same pattern as dietary iron (Fairwearher-Tait & Teucher, 2002)

2.2.4.2 Blood loss

In women, low iron levels may be due to blood loss from long or heavy menstrual periods or bleeding fibroids in the uterus (WHO, 2008). Blood loss that occurs during childbirth could additionally cause low iron levels in women (Legett *et al*., 1990). Internal bleeding may result from ulcers, regular aspirin use and urinary tract bleeding. This may lead to iron-deficiency anaemia (Valeri *et al*., 1997).

Furthermore, blood loss from severe injuries, surgery, or frequent blood drawings can cause iron-deficiency anemia (Valeri *et al*., 1997). Some parasitic infestations cause bleeding in the gastrointestinal tract (see *2.4.6*).

2.2.4.3 Increased physiological need

The adolescent growth spurt, pregnancy and breastfeeding are situations when the body requires more iron. If this increased need is not met, a deficiency can quickly occur (Whitfield *et al*., 2003).

2.2.4.4 Exercise

Athletes are prone to iron deficiency because regular exercise increases the body's need for iron in a number of ways; for example, rigorous training promotes RBC production, while iron is lost through sweating (Lyle *et al.,* 1992).

2.2.4.5 Inability to absorb iron

Healthy adults absorb about 10 to 15 percent of dietary iron but in certain conditions the body is unable to absorb or use iron from food. This may occur in persons suffering from coeliac disease, a condition that damages the lining of the intestines or after a gastrectomy, a medical procedure to surgically remove the stomach (Bushara, 2005).

2.2.4.6 Infestations

Malaria and other parasitic infestations are major causes of anaemia, affecting 300-500 million people globally. In endemic areas, it may be the primary cause of half of all severe anaemia cases (WHO, 2000). Hookworm infestation and in some places schistosomiasis also contribute to anaemia (Doherty, 2007). Approximately 44 million pregnant women have hookworm infestations and 20 million people are severely infested with schistosomiasis (WHO, 2002). Parasites

need iron for survival and multiplication and thereby compete with the host for the available iron (Doherty, 2007).

2.2.5 Metabolism of iron

2.2.5.1 Absorption and transportation of iron

Dietary iron exists in two forms: haeme and non-haeme iron (Lammi-keef *et al.,* 2008). After digestion, haeme iron in the ferrous state is absorbed into the intestinal enterocytes by interacting with the haeme carrier protein 1 (HCP1) (Baker & Morgan, 1994). Non-haeme iron primarily is in the ferric state and is reduced to the ferrous state through the action of ferrireductases (Cook & Reddy, 2001). In the duodenum this reduction is carried out primarily by duodenal cytochrome *b* (DCYTB) (Frazer & Anderson, 2005). The acidic nature of the gastric secretion also enhances its reduction and solubility. The reduced form of iron (ferrous) is preferred for intestinal absorption (Frazer & Anderson, 2005).

After solubilisation, the divalent metal transporter 1 (DMT1) is responsible for importing dietary non-haeme iron through the apical site of absorptive enterocytes in the duodenum (Gunshin *et al*., 1997). The iron can be stored within intestinal enterocytes bound to ferritin or transported across the basolateral membrane of intestinal enterocytes into circulation, through the action of the transport protein ferroportin (also called IREG1=iron-regulated gene 1) (Mckie *et al*., 2000). Associated with ferroportin is the enzyme hephaestin (a copper-containing ferroxidase with homology to ceruloplasmin) which oxidises the ferrous form back to the ferric form (Mahan & Escott-Stump, 2008). Once in circulation, the ferric form of iron is bound to transferrin and passes through the portal circulation of the liver. The liver is the major storage site for iron. The major site of iron utilisation is the bone marrow where it is used for haeme synthesis (Finch, 1994).

Transferrin can bind two moles of ferric iron. Iron-laden transferrin is taken up into the cell by forming complexes with transferrin receptor-1, TfR1 (Andrews, 1999; Ponka & Lok, 1997). Recently, another homologous receptor, TfR2 has been identified (Kawabata *et al.,* 1999). Unlike TfR1, which is ubiquitously expressed, TfR2 is mainly expressed in the liver (Kawabata *et al.,* 1999). Internalisation of the iron-TfR complexes is initiated following receptor phosphorylation by protein kinase C. Following internalisation, the iron is released due to the acidic nature of the endosomes. The TfR is then recycled back to the cell surface (Baker & Morgan, 1994). Figure 2.2 illustrates the transportation of iron from the intestinal lumen to the blood.

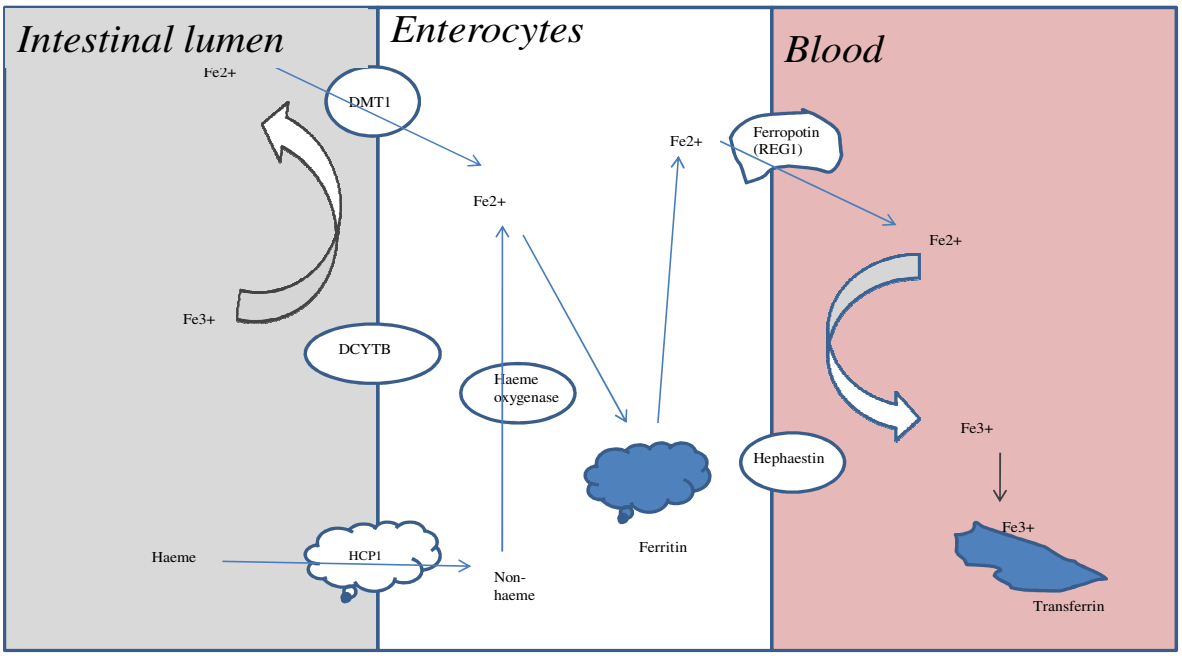

Figure 2.2 Transportation of iron (adapted from King, 2010)

2.2.5.2 Storage of iron and release of iron from stores

The main cellular site of iron storage is the liver (Finch, 1994). Iron bound to transferrin is taken up from the blood by hepatocytes through binding of transferrin to the TfR (Mahan & Escott-Stump, 2008). Free iron, called non-transferrin bound iron (NTBI) in the plasma can also be absorbed by hepatocytes via the DMT1 (Bothwell *et al.,* 1979). However, the ferric form predominates in the blood and must first be reduced by ferrireductases prior to DMT1 transport (Valberg *et al.,* 1975). Upon binding to transferrin, the TfR is internalised via receptor-mediated endocytosis. The acidic environment of the endosome results in the release of ferric iron from transferrin (Valberg *et al.,* 1975). The ferric iron is reduced in the endosome to the ferrous form via the action of an endosomal ferrireductase, usually the six-transmembrane epithelial antigen of prostate protein 3 (STEAP3). The ferrous iron is transported out of the endosome via DMT1 and can then be stored in the hepatocyte bound to ferritin as in intestinal enterocytes (Bothwell *et al.,* 1979). The transferrin-TfR complexes are recycled back to the surface of the hepatocyte and the transferrin is released to the blood where it can bind more ferric iron in circulation. Ferrous iron is released from hepatocytes to the circulation through the action of ferroportin. When in circulation, ferrous iron is oxidised to the ferric form by plasma ferroxidase known as ceruloplasmin. The ferric iron can then be bound by transferrin and delivered to other tissues of the body (Eisenstein & Blemings, 1998).

Ferritin is the major protein used for intracellular storage of iron (Finch, 1994). Ferritin without bound iron is referred to as apo-ferritin. Apo-ferritin is a large polymer made up of 24 polypeptide subunits. This multimeric structure of apo-ferritin is able to bind up to 2000 iron atoms in the form of ferric-phosphate (Herbert *et al*., 1997). The majority of intracellularly stored iron is found in the liver, skeletal muscle and reticuloendothelial cells. If the storage capacity of the ferritin is exceeded, iron will be deposited adjacent to the ferritin-iron complexes in the cell. These amorphous iron deposits are referred to as haemosiderin (Iancu,

1992). The iron present in haemosiderin is not readily available to the cell and thus cannot supply iron to the cell when it is needed. Haemosiderin is found most frequently in macrophages and is most abundant following haemorrhagic events (Iancu, 1992).

2.2.5.3 Iron excretion

The body's complex system of iron regulation and recycling ensures that only little iron is excreted. On average, an adult male loses only 0.9mg a day through the intestines, skin cell exfoliation, sweat and urine (Brune, 1986). Bleeding can also deplete iron reserves (Heeney & Andrews, 2004).

Women lose more iron than men because of menstrual bleeding and the high iron demands of pregnancy and lactation. Iron lost due to menstrual bleeding adds 0.4 mg to a woman's daily iron loss, so women lose 1.3mg for every 0.9mg of iron lost by men. Iron loss due to pregnancy adds yet another 4mg to this total. These are the approximate daily losses of iron in healthy individuals: intestinal excretion: 0.6mg, sweat and skin cell exfoliation: 0.2mg, urine: 0.1mg, menstruation: 0.4mg, pregnancy or lactation: 4mg (Green, 1968).

2.2.6 Regulation of body iron

The major process responsible for iron homeostasis is intestinal absorption. The different factors that influence this process are briefly discussed here.

2.2.6.1 Iron status

Iron absorption depends on the current iron status of an individual (Magnusson *et al.*, 1981). Iron absorption is up-regulated by a factor of two to three in iron deficiency states when compared to normal iron states. Iron deficiency probably

acts at the level of crypt-cell programming in response to the saturation of plasma transferrin with iron (Frazer & Anderson, 2005).

2.2.6.2 Dietary factors

Haeme iron is readily absorbed across the intestinal mucosa and is less regulated than non-haeme iron (approximately 25% compared to 5-10%). The presence of other dietary components can either enhance (e.g. ascorbic acid and citric acid) or inhibit (e.g. phytate and calcium) non-heam iron absorption ((Lammi-keef *et al.,* 2008). Furthermore, iron absorption may be inhibited after ingesting a large amount of dietary iron as a result of blockage of the absorptive enterocytes; this may occur even in the presence of iron deficiency (Amine & Hegsted, 1971).

2.2.6.3 Hepcidin

Hepcidin is a circulatory peptide hormone that regulates iron metabolism. By modulating hepcidin production, an organism controls intestinal iron absorption and mobilisation from stores to meet body iron need (Fleming & Sly, 2001). Hepcidin is primarily, but not exclusively, secreted by hepatocytes and is highly conserved among different species. There is evidence that hepcidin is also expressed in the heart, kidney, adipose tissue, pancreas and haematopoietic cells, although the biological relevance of extra-hepatic hepcidin is not well defined (Nemeth & Ganz, 2006). The mature bioactive form of hepcidin is a peptide composed of 25 amino acids. It is derived from a precursor (pre-prohepcidin) of 84 amino acids after undergoing two enzymatic cleavages. Other isoforms of hepcidin which are composed of 20 and 22 amino acids are detectable in human serum and urine. The biological significance of these isoforms is uncertain (De Domenico *et al*., 2008; Kemna *et al*., 2008). Hepcidin acts by binding to ferroportin, an iron transporter present on cells of the intestinal duodenum, macrophages, and cells of the placenta. This binding induces ferroportin internalisation and degradation (Nemeth *et al*., 2004). The loss of ferroportin from

the cell surface prevents iron entry into plasma. Decreased iron entry into plasma results in low transferrin saturation, and less iron is delivered to the developing erythroblast. Decreased expression of hepcidin leads to increased cell surface ferroportin and increased iron absorption. Hepcidin is modulated by different stimuli, which act as positive or negative regulators:

1. Positive regulators: Increased iron store and inflammation activate hepcidin transcription in the hepatocytes.

2. Negative regulators: Hypoxia, anaemia, increased erythropoiesis and reduced iron stores all negatively regulate hepcidin expression (Nemeth & Ganz, 2006). Figure 2.3 illustrates the role of hepcidin in iron regulation and Table 2.2 shows the effect of other proteins in hepcidin regulation of iron.

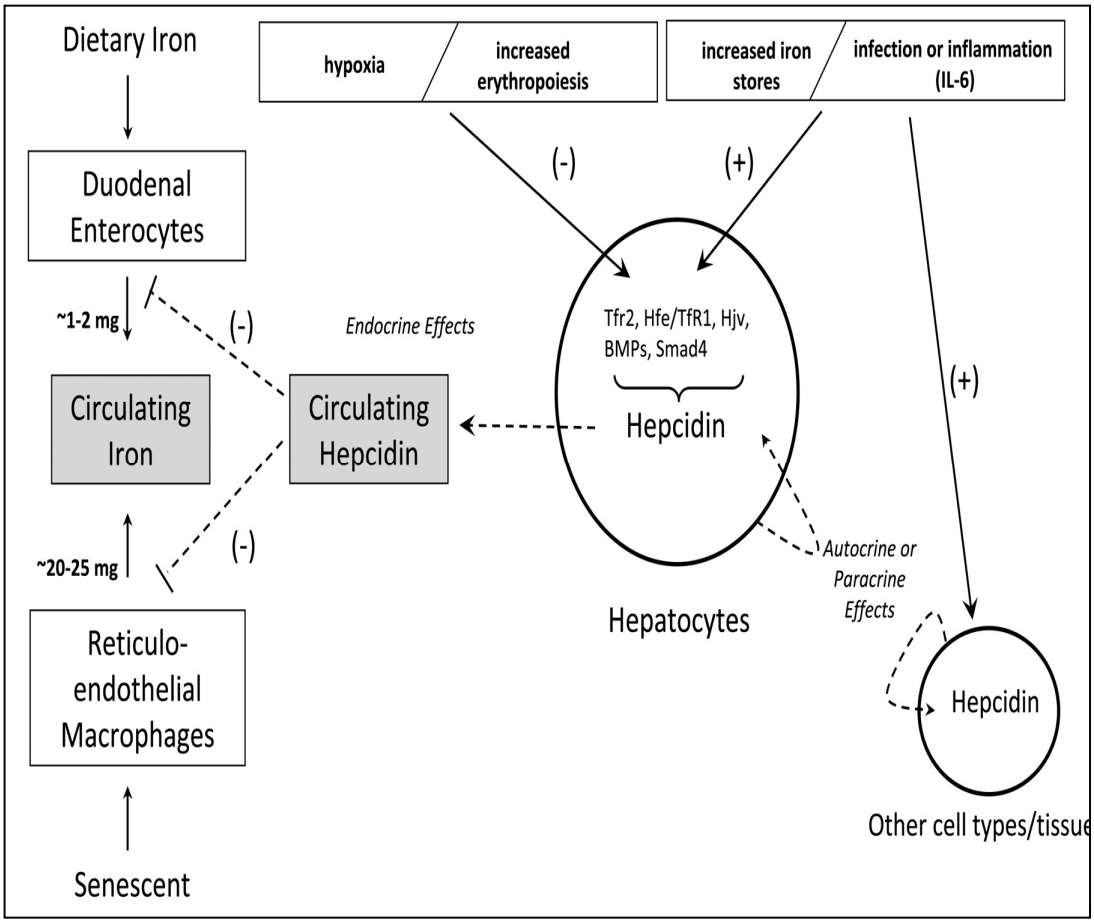

Figure 2.3 Hepcidin regulation of iron (Nemeth & Ganz, 2006)

Table 2.2 Proteins involved with hepcidin regulation of iron transport

Protein	Properties	Role in hepcidin regulation of iron transport
Ferroportin	Transmembrane protein	Hepcidin receptor and iron transporter
HFE	Single point mutation responsible for most iron overload	Mutations in HFE are associated with low hepcidin production; precise function unknown
TfR2	Homologous to TfR1; abundantly expressed in liver	May sense plasma iron level through HFE
BMP6	Member of the TGFβ superfamily of cytokines; expression is induced by iron	Binds to BMP receptors I and II, inducing phosphorylation of SMAD proteins
HJV	Membrane-bound and soluble protein	Serves as a BMP coreceptor to activate SMAD
SMADs	Cellular mediators of transcriptional activation	Activated SMAD induce transcription of hepcidin
JAK	Involved in a signaling pathway that regulates ferroportin trafficking; transcriptional reaponse	May phosphorylate ferroportin; activates STAT3[1] in response to IL-6
STAT3	Cellular mediator of transcriptional activation	Activated STAT3 induces transcription of hepcidin

Adapted from Donovan *et al*. (2005) and Nemeth & Ganz (2006)

HFE: symbol for the haemochromatosis gene, TfR2: transferrin receptor 2, BMP6: bone morphogenetic protein 6, TGF-β: transforming growth factor-β, HJV: haemojuvelin, JAK: Janus activated kinase, STAT3: signal transducer activator of transcription 3, IL-6: interleukin 6.

2.2.7 Other factors influencing iron status

2.2.7.1 Age

Ageing is associated with changes in metabolism and status of various nutrients (Ahluwalia *et al*., 2000). There is some evidence that haematologic variables such as Hb and Hct may decline with ageing in humans (Lipschitz, 1991) while serum ferritin, which is usually indicative of body iron stores increases with ageing (Milman *et al.,* 1986). The different iron requirements placed on the body during growth and reproduction play a major role in how age affects iron status (Whitfield *et al*., 2003).

2.2.7.2 Gender

Men have been shown to usually have better iron status than women in adolescence and adulthood (Looker *et al.,* 1997; Legett *et al*., 1990). These differences are considered to be primarily due to menstrual loss of iron in fertile women. Recent studies in adolescents and adults also suggest that there may be other sex-related differences, such as hormonal changes and differences in growth needs (Ilich-ernst *et al*., 1998; Bergstrom *et al*., 1995).

2.2.7.3 Alcohol

Although, the exact mechanism is not well elucidated, alcohol has been reported to have direct or indirect effects on iron absorption (Moirand *et al.,* 1990). Charlton *et al.* (1964) showed that a single dose of alcohol increased the absorption of ferric iron in normal subjects (alcohol abstainers). It was first postulated that increased body iron in alcoholics was due to heavy ingestion of iron contained in wines and home-brewed alcoholic beverages. However, some alcoholic beverages only contain trace amounts of iron (e.g. whisky, gin). It was therefore, proposed that alcohol increases iron absorption at the luminal level (Powell, 1966). Another proposed mechanism is that alcohol may disturb the

distribution of iron between the storage and cellular compartments (Moirand *et al*., 1990). Additionally, it has been suggested that alcohol may influence iron absorption in man by its effects on folic acid metabolism. Alcohol induces folic acid deficiency, which in turn increases iron absorption by increasing plasma iron turnover or by a direct effect on intestinal mucosal membrane (Celada *et al*., 1979).

2.2.7.4 Smoking

Past or present smoking status has been associated with higher serum ferritin concentrations among various populations (Larue *et al*., 1993; Wu *et al.,* 1994). Furthermore, it has been shown that smoking increases the fetal red mass in pregnant women. This has been attributed to increased erythropoietin induced by hypoxia (Rollins *et al*., 1993). Iron stores are mobilised for erythrocyte production, thereby explaining the reduction in ferritin concentration in pregnant women (Jazayeri *et al.,* 1998).

2.2.7.5 Infestation

Parasitic infestations have been associated with poor iron status (Di, 2009). Infestations causing loss of blood (e.g. hookworm and Schistosome specie) may further exacerbate a low iron status (Stephenson & Holland, 1987). Malaria causes anaemia through haemolysis and suppression of haematopoiesis. This acute phase response causes sequestration of iron leading to increased ferritin concentration (Friis *et al*., 2001).

2.2.7.6 Exercise

Exercise places a greater demand on the iron requirement of the body due to increased blood volume and muscle mass (Lyle *et al*., 1992). Both aerobic and anaerobic exercises have been shown to lead to lower iron status. Exercise can influence iron balance through increased gastrointestinal blood loss after running and haematuria as a result of erythrocyte rupture within the foot during running

(Lyle *et al*., 1992). In addition, blood Hb concentration has been shown to reduce after several weeks of weight lifting in both men and women trainers (Deruisseau *et al.,* 2004).

2.2.8 Other forms of anaemia

2.2.8.1 Megaloblastic anaemia

There are two forms of megaloblastic anaemia, namely: (i) folic acid deficiency anaemia and (ii) pernicious anaemia (Savage *et al.,* 1994). Both reflect a disturbed synthesis of deoxyribonucleic acid (DNA), which results in morphologic and functional changes in erythrocytes, leucocytes, platelets and their precursors in the blood and bone marrow (Baynes, 1994). While the first type results from folic acid deficiency, the later is a consequence of vitamin B_{12} deficiency. The deficiency of intrinsic co-factors that absorb Vitamin B_{12} from food in the stomach can result in pernicious anaemia as well. Alcoholism has been shown to be a contributing factor in these forms of anaemia (Baynes, 1994). Symptoms of the disorder include weakness, fatigue, memory lapses and irritability (Savage *et al.,* 1994). This condition can be avoided by including foods with folic acid in the diet e.g. leafy vegetables, beef, liver, asparagus, and red beans (Mahan & Escott-Stump, 2008).

2.2.8.2 Copper deficiency anaemia

Copper is needed for optimum Hb formation. Ceruloplasmin, a copper containing protein, is required for transporting stored iron to the plasma (Jain & Willians, 1988). If copper is insufficient in the body, iron transportation is impaired leading to low Hb levels even in the presence of optimum iron stores (Jain & Williams, 1988). The amount of copper required is minimal and can often be met by commonly consumed diet. However, copper deficiency has been found in infants

fed cow's milk or copper deficient formulas or in adults with malabsorption syndrome (Masugi *et al.,* 2005; Hein, 2003).

2.2.8.3 Anaemia of protein-energy malnutrition

Many of the iron transporters are protein molecules. During protein deficiency, Hb production declines. Thus, fewer tissues are oxygenated (Macdougal *et al.,* 1982). The condition mimics iron deficiency because blood volume remains the same with reduced RBCs (Macdougal *et al.,* 1982).

2.2.8.4 Aplastic anaemia

Aplastic anaemia is a disorder in which the bone marrow does not make enough new RBCs. This can happen through injury where the blood forming tissue in the bone marrow is destroyed. Because of this, the sufferer is unable to fight infection and is likely to be a heavy bleeder. There is no definite known cause for aplastic anaemia but it is thought to be caused by exposure to certain toxins and also to the hepatitis virus (Locasciulli *et al.,* 2007).

2.2.8.5 Sports anaemia

During the early stages of a vigorous training programme there could be increased RBC destruction coupled with decreased Hb, serum iron and ferritin concentration. Symptoms include lethargy, paleness, purpura, bleeding, rapid heartbeat, infections, and congestive heart failure (Wolski, 2010).

2.2.8.6 Sickle cell anaemia

Sickle cell anaemia is an autosomal recessive genetic blood disorder in which RBCs are abnormal, rigid and sickle shaped (Desai & Dhanani, 2004). This form of anaemia is hereditary. Sickle cell anaemia is a life threatening disease and there

is no prevention. Symptoms of this condition include painful attacks in arms, legs and stomach, jaundice in whites of the eyes, fever, chronic fatigue, rapid heartbeat, paleness. Complications include leg ulcers, shock, cerebral haemorrhage, and orthopedic disorders (WHO, 2005).

2.2.8.7 Polycythemia vera

Polycythemia vera is a blood disorder in which the bone marrow makes too many RBCs. It may also result in overproduction of white blood cells and platelets. There is fast and intense reproduction of these cells and the bone marrow cells mature more rapidly than usual. Most of the health concerns associated with *polycythemia vera* are caused by a blood-thickening effect that results from an overproduction of RBCs (Passamonti *et al.,* 2003). The cause of this condition is unknown. Symptoms include purplish coloured skin, bloodshot eyes, headache, dizziness and enlarged spleen (Tefferi, 2007).

2.2.8.8 Anaemia of chronic diseases

This occurs during inflammation, infection or malignant disease. There is decreased RBC production. Increased concentrations of inflammatory cytokines such as interleukin-1 and tumor necrosis factor have been observed in persons suffering from this condition (Spivak, 2002). Ferritin levels are normal or increased while serum iron and TIBC are reduced (Spivak, 2002).

2.2.9 Disorders of iron metabolism associated with iron overload

2.2.9.1 Haemochromatosis

Haemochromatosis is the most common form of iron overload disease. Primary haemochromatosis, also called hereditary haemochromatosis, is genetic. It is mainly caused by a defect in a gene called *HFE* (symbol for haemochromatosis

gene), which helps regulate the amount of iron absorbed from food. The two known mutations of *HFE* are *C282Y* and *H63D* (Adams *et al*., 2005). Individuals who are homozygous for the gene may die of iron overload unless they donate blood often. Those who are heterozygous do not usually develop the disease, but may have higher than average iron absorption (Gertig *et al.,* 2003). Secondary haemochromatosis is caused by alcoholism and other disorders (Adams *et al.*, 2005). In developing countries, secondary iron overload may develop as a result of eating foods cooked in iron-pots (Kew & Asara, 2007). African iron overload has been attributed to the consumption of traditional beers brewed in iron containers (Mcnamara *et al.,* 1998). Juvenile haemochromatosis leads to severe iron overload in the liver and heart; it is found among adolescents and young adults between the ages of 15 and 30 (Kelly *et al.,* 1998). The neonatal form causes rapid iron buildup in the baby's liver that can lead to death (Andrews, 1999). Symptoms of the disease include joint pain, fatigue, lack of energy, abdominal pain, loss of sex drive, and heart problems (Mcnamara *et al.,* 1998).

2.2.9.2 Sideroblastic anaemia

This is a condition in which iron that has neither been used nor stored is kept in immature RBCs. These cells are called sideroblasts (Caudil *et al.,* 2008). These iron-containing cells do not function normally and the development and production of RBCs is diminished. The symptoms include that of iron deficiency and iron overload. This form of anaemia is responsive to pyridoxine (vitamin B_6) treatment except if it is as a result of drug therapy or alcoholism (Camaschella, 2008).

2.2.9.3 Haemolytic anaemia

This occurs when defects in RBCs membranes lead to oxidative damage and eventually to cell lysis. This condition is usually associated with vitamin E deficiency, an antioxidant that prevents oxidative reactions (Wright, 1999).

2.2.9.4 Thalassemia

Thalassemia is a genetic disorder in which there is decreased and defective production of Hb. RBCs are microcytic, hypochromic and short-lived leading to an increase plasma volume due to increased iron absorption and deposition in tissues (Funchs *et al*., 1996). Additionally, bone marrow is expanded and facial deformities develop. Patients may require blood transfusion to live (Funchs *et al*., 1996). There are two types; namely: alpha-thalassemia and beta-thalassemia. Their names describe which part of the Hb molecule that is affected, the alpha or the beta chain.

2.2.10 Standard recommendations for iron intake

Adequate iron has been defined as iron intake that is sufficient enough to prevent iron deficiency anaemia (Umbreit, 2005). Standard recommendations advise an intake that prevents iron deficiency in the majority of individuals in a specific population (Marx & Van Asbeck, 1996). Total absolute requirements include requirement for growth, basal losses and, in females, menstrual losses.

Iron requirements during pregnancy are not equally distributed over the period of pregnancy. The total daily iron requirements, including the basal iron losses (0.8mg), increase during pregnancy from 0.8mg/day to about 10mg/day during the last six weeks of pregnancy. In lactating women, the daily iron loss in milk is about 0.3mg and basal loss is 0.8mg. The total iron requirement during lactation period amounts to 1.1mg/day (FAO/WHO, 1998). Table 2.3 shows the standard iron requirement for children, adult males and females, and postmenopausal women as recommended by the Food and Agriculture Organisation (FAO) and World Health Organisation (WHO).

Table 2.3 Iron requirements and recommended iron intakes by age and gender

Groups	Age (*years*)	Mean body Weight (*kg*)	Required iron intake for growth (*mg/day*)	Median iron losses (*mg/day*)		Total absolute iron requirements *(mg/day)*
				Basal	***Menstrual***	
Children	0.5-1	9.0	0.55	0.17		0.72
	1-3	13.3	0.27	0.19		0.46
	4-6	19.2	0.23	0.27		0.50
	7-10	28.1	0.32	0.39		0.71
Males	11-14	45.0	0.55	0.62		1.17
	15-17	64.4	0.60	0.90		1.50
	18+	75.0		1.05		1.05
Females	11-14	46.1	0.55	0.65	0.48	1.68
	15-17	56.4	0.35	0.79	0.48	1.62
	18+	62.0		0.87	0.48	1.46
Post-menopause		62.0		0.87		0.87

Adapted from vitamin and mineral requirements in human nutrition FAO/WHO (1998).

2.3 OBESITY

Obesity has been defined as an excess of body fat (WHO, 2004). The escalating number of obese individuals in developing countries is a major factor in the shift in the health status of the people. About one hundred million people living in developing countries have been classified as obese (WHO, 2000). Research results have correlated obesity with an increased risk of developing a number of health conditions including metabolic syndrome, type 2 diabetes, hypertension, coronary artery disease, colon cancer, postmenopausal breast cancer, endometrial cancer, gallbladder disease, osteoarthritis, and obstructive sleep apnea (Powers *et al*., 2007).

2.3.1 Causes of obesity

Obesity could result from factors that are either genetic or environmental.

2.3.1.1 Genetic factors

Genes affect a number of weight-related processes in the body, such as metabolic rate, blood glucose metabolism, fat storage and hormonal functions. Molecular and genetic research has identified substances that modulate feeding behaviours. Leptin, a product of the ob gene has been implicated in this pathway. It is a hormone secreted by the adipose tissue. Leptin is able to send signals that stimulate the production of neurotransmitters that are capable of either inhibiting or enhancing feeding and energy metabolism (Wang *et al*., 1999).

2.3.1.2 Environmental factors

Obesity results from the consumption of excess of dietary energy required to support normal body needs. Additionally, a sedentary lifestyle may lead to accumulation of fat. These factors are modifiable unlike the genetic factors (Mokdad *et al*., 2004; WHO, 2000).

2.3.2 Lipogenesis

Lipogenesis is the formation fat. It is a metabolic process in animals that converts simple sugars to fatty acids and synthesizes triacylglycerols through the reaction of fatty acids with glycerol (Kalant *et al*., 2003). Fatty acid synthesis starts with acetyl-CoA and builds up by the addition of two carbon units. After lipogenesis, the triacylglycerols are packaged into very low-density lipoprotein (VLDL) and secreted by the liver (Schutz, 2004). VLDL helps transport lipids and cholesterol throughout the body. Lipogenesis occurs in the cytosol. The main sites of triglyceride synthesis are the liver, adipose tissue, and intestinal mucosa (Trayhurn, 2007).

Carbohydrates from the human diet are converted into energy, stored as glycogen, or converted into fat. Fatty acids, in the form of triglycerides can also be consumed through diet. In addition, amino acids from proteins are used for new protein synthesis or they can be converted to carbohydrate and fat (Powers *et al.*, 2007). If the diet supplies energy in excess of what the body requires, lipogenesis converts the excess energy into fat. If the diet consumed does not produce enough energy to support a person's activity level, the energy stored in body fat reserves is used instead (Kersten, 2001).

Excess energy is stored in adipose tissue which are located in three sites: under the skin (subcutaneous), around internal organs (visceral) and in the bone marrow (Albright & Stern, 1998). Subcutaneous adipose tissue has the largest capacity for storing fat (80%) and when it reaches its threshold, fat is stored in other organs in the body (Albright & Stern, 1998).

Lipogenesis is responsive to dietary and hormonal changes. According to Kersten *et al.* (1999), polyunsaturated fatty acids decrease lipogenesis while diets rich in carbohydrates stimulate lipogenesis. Fasting decreases lipogenesis in adipose tissues but increases it in the liver because of the large amount of fatty acids released from adipose tissues (Kersten *et al*., 1999). Insulin, a hormone secreted by the Beta cells of the pancreas, plays an important role in the lipogenic process. The net effect of insulin is to increase storage and block mobilisation and oxidation of fatty acids (Nakae & Accili, 1999). Other hormones

that play a significant role in lipogenesis are leptin and growth hormone (GH). Leptin has been reported to inhibit the gene expression of lipogenic enzymes (Swierczynski, 2006). GH decreases insulin sensitivity resulting in down regulation of fatty acid synthesis (Yin *et al.*, 1998).

2.3.3 Biological role of adipocytes

Adipocytes, also known as lipocytes and fat cells, are the cells that primarily compose adipose tissue, specialised in storing energy as fat (Lubeck, 2007). However, recent discoveries have shown that adipocytes are not just storage depot for energy. Adipocytes are also endocrine organs, with multiple metabolic roles in regulating whole-body physiology (Andrew *et al.,* 2006). Small adipocytes in lean individuals promote metabolic homeostasis while enlarged adipocytes in obese individuals recruit macrophages and promote inflammation and the release of a range of factors including hepcidin (McClung & Karl, 2008). Obesity is associated with chronic and low grade inflammation which may cause hypoferremia as seen in patients with chronic inflammatory disease (Powers *et al.,* 2007).

Furthermore, scientific evidence has shown that the distribution of fat in the body is more important than the total body fat when considering the interplay between body fat and health. Excess fat located around the central region of the body has been associated with CVDs (Wajchenberg, 2000).

2.3.4 Measurement of obesity: Anthropometry

Anthropometry is the study of body sizes and proportions. Anthropometric indices provide the most portable, universally applicable, inexpensive and non-invasive technique for assessing the size, proportions and composition of the human body. It reflects both health and nutritional status and predicts performance, health and survival

(WHO, 1995). Anthropometric measurements are compared to reference data (standards) in order to evaluate the status of an individual. However, anthropometric measurements are not sensitive to short-term changes in nutritional status (WHO, 1995). They include:

2.3.4.1 Body mass index (BMI)

BMI is the ratio of body weight to the square of height (Gray & Fujioka, 1991). It is an estimate of whole body fat. BMI has long been established as the preferred method of measuring adiposity in epidemiological studies (WHO, 2000), and for most populations a good correlation exists between BMI and percentage body fat for all ages and both genders. Evidence shows that high BMI is associated with type 2 diabetes and increased risk of CVD morbidity and mortality. The World Health Organisation (1998) gave the ranges for classifying persons according to BMI as follows:

BMI<18.5kg/m^2: underweight

BMI 18.5-24.9kg/m^2: normal weight

BMI 25.0-29.9 kg/m^2: overweight

BMI$\geq$30.0 kg/m^2: obese.

2.3.4.2 Body circumferences

These include waist, hip, thigh, mid upper arm and muscle arm circumferences. They are estimates of regional body fat (WHO, 2002).

2.3.4.3 Skinfold thicknesses

These are measured at several body sites (subscapular, triceps, biceps, suprailliac, mid-thigh, mid-calf) and have been shown to detect changes in subcutaneous fat distribution (Sarria *et al*., 1998).

2.3.4.4 Others

At the molecular level, the body can be divided into fat mass and lean mass. The fat mass refers to the essential and non-essential lipids found in tissues and organs while the lean mass refers to the water, protein, minerals, glycogen and other residual compounds in the body (Wang *et al*., 1992). These can be determined using several methods such as near-infrared interactance, dual energy X-ray absorptiometry (DXA), body density measurement, bioelectrical impedance analysis or calculated from other anthropometric indices (Cogill, 2001). Ratios such as head to chest, waist to hip and waist to thigh have also been shown to be good indicators of adiposity (Gibney *et al*., 2004).

2.4 CARDIOVASCULAR DISEASES

CVD is a leading cause of global morbidity and mortality and is responsible for one-in-three deaths (WHO, 2002). CVD has reached near epidemic proportions in Africa. According to the World Health Report (2002), CVD accounted for 9.2% of total deaths in the African region in 2001, and hypertension, stroke, cardiomyopathies and rheumatic heart disease were the most prevalent causes (Peden *et al.,* 2004). In South Africa, ischaemic heart disease and stroke have been reported to account for 35% of cardiovascular deaths (Norman *et al.,* 2006). CVDs include diseases that involve the heart or blood vessels (arteries and veins) such as aneurysm, angina, atherosclerosis, cerebrovascular accident (stroke), cerebrovascular disease, congestive heart failure, coronary artery disease, myocardial infarction (heart attack) and peripheral vascular disease .

CVDs have both direct and indirect effects. Direct effects involve heart attack (angina pectoris, unstable angina, and myocardial infarction). Angina pectoris results from plaques that narrow the diameter of coronary arteries to the extent that the heart becomes significantly deprived of blood during times of exertion. This typically causes chest pressure or pain. Other symptoms include a sensation of indigestion, dizziness, breathing

difficulty, sweatiness, nausea and numbness in the neck, jaw or arms. Indirect effects include irregular heart rhythms, cardiac arrest and congestive heart failure (Gibney *et al.,* 2004).

Growing evidence indicates that chronic and acute overproduction of reactive oxygen species (ROS) under patho-physiologic conditions are integral in the development of CVD. ROS mediate various signaling pathways that underlie vascular inflammation in atherogenesis. These include the initiation of fatty streak development through lesion progress and the ultimate plaque rupture (Madamanchi *et al*., 2005). A variety of cells and lipids are involved in the pathogenesis of CVDs. These include lipoproteins, cholesterol, triglycerides, platelets, monocytes, endothelial cells, fibroblasts and smooth muscle cells (Madamanchi *et al*., 2005).

Nutrition can influence the development of CVDs by modifying one or more of the cells involved in the pathogenesis of CVDs. Diets rich in saturated fat, the use of cocaine, and high levels of other chemicals like homocysteine in the blood have been reported to be positively associated with increased risk for CVDs while consumption of polyunsaturated fatty acids and vegetables are associated with lower risk (Nicolosi *et al*., 2001).

2.4.1 Risk factors for cardiovascular diseases

2.4.1.1 Physical activity

Exercise burns energy, helps to control cholesterol levels and diabetes, and may lower blood pressure. Exercise also strengthens the heart muscle and makes the arteries more flexible (Ornish *et al*., 1990). Physical inactivity has been reported to be associated with almost twice the risk of developing CVD and results in a poorer prognosis in survivors of myocardial infarction compared with their active counterparts (Briffa *et al*., 2006).

2.4.1.2 High blood pressure

High blood pressure increases the risk of heart disease, heart attack, and stroke. Though other risk factors can lead to high blood pressure, a person can have high blood pressure without having other risk factors (Anderson *et al.,* 2010). Hypertension has been reported to be the commonest CVD risk factor among South Africans with 59% of black African people, 55% of Indian and coloured people and 50% of white people diagnosed with the disease (Connor *et al*., 2005).

2.4.1.3 Abnormal lipid profile

When blood cholesterol levels are high, the excess cholesterol is deposited in the arteries, including those of the heart, which can lead to narrowing of the arteries and heart disease (Rainwater *et al.,* 1999).

2.4.1.4 Smoking

Research has shown that smoking increases heart rate, tightens major arteries, and can create irregularities in the timing of heartbeats, all of which make the heart work harder. Smoking also raises blood pressure (Chen & Boreham, 2002). Although nicotine is the main active agent in cigarette smoke, other chemicals and compounds like tar and carbon monoxide are also harmful to the heart (Winstainley *et al.,* 1995). These chemicals lead to the buildup of fatty plaque in the arteries, possibly causing injury to the vessel walls. They also affect the levels of cholesterol and fibrinogen. This increases the risk of a blood clot that can lead to a heart attack (Burke & Fitzgerald*,* 2003).

2.4.1.5 Obesity

Extra weight is thought to lead to increased total cholesterol levels, high blood pressure, and an increased risk of coronary artery disease (Poirier *et al*., 2006).

2.4.1.6 Diabetes

The health consequences of diabetes include increased risk for heart disease and stroke. Heart disease and stroke contribute to approximately 65 percent of deaths among diabetics, with heart disease being the leading cause of diabetes-related death (Grundy *et al*., 1999). Diabetic adults compared to non-diabetic adults have heart disease death rates about 2 to 4 times higher. In addition, stroke risk is 2 to 4 times higher among people with diabetes (Grundy *et al.,* 1999).

2.4.1.7 Gender

Overall, men have a higher risk of heart attack than women. The difference narrows after women reach menopause. After the age of 65, the risk of heart disease is about the same between the genders when other risk factors are similar (Fodor & Tzerovska*,* 2004).

2.4.1.8 Age

Older age is a risk factor for heart disease. Reports have shown that about 4 out of every 5 deaths due to heart disease occur in people older than 65 (Gibney *et al.,* 2004).

2.4.1.9 Genes

There is evidence that CVD is more common among certain racial and ethnic groups. For example, studies have shown that African Americans have more severe high blood pressure and a greater risk of heart disease than Caucasian Americans. In addition, a person with a family history of CVD is at a greater risk of having the disease (Sing *et al*., 2003).

2.5 IRON, OBESITY AND CARDIOVASCULAR DISEASES: MAKING THE LINK

The potential role of iron in CVD derives from its ability to serve as a catalyst in oxidation-reduction reactions. Its toxicity is enhanced by the limited capacity of the human body to excrete iron (Bothwell *et al*., 1979). Furthermore, the co-existence of abnormal iron status and obesity has been shown to increase the amount of free iron available to initiate oxidative stress (Garcia *et al.,* 2009). Obese persons have been reported to suffer from iron deficiency even in the presence of optimal or overloaded iron stores. Ferritin, the most important measure of iron store, has been reported to be elevated in obese persons and increased ferritin has been associated with increased risk for developing CVDs in many populations (Zafon *et al*., 2010).

The specific mechanism responsible for this observation is not fully understood but there are several speculations. Since obesity is an inflammatory condition, it has been suggested that the low grade inflammation induced by obesity leads to increased ferritin (Zafon *et al*., 2010). Hepcidin, the hormone that down regulates iron absorption has been implicated in this pathway. Hepcidin is thought to reduce iron absorption while inhibiting the release of iron from the stores (Nemeth *et al*., 2006). This could lead to a functional iron deficiency. The increased blood volume in obese individuals may also contribute to iron deficiency (McClung & Karl, 2008).

In summary, it is pertinent to know the extent of the threat posed by different risk factors before better health can be achieved. Risk factors for CVD are many, and there is interaction between them. An example is that of abnormal iron status and obesity. This is intriguing because it is not clear which one leads to the other (Wilson *et al.,* 1998). Evaluating one risk factor separately will overlook the effect of changes of the other risk factor. Assessing the relationships between the levels of the different risk factors may, therefore, help in predicting the rate of the disease (Wilson *et al*., 1998; Robins, 1986). The main objective of the experimental work in this thesis was to examine the relationship between abnormal iron status and obesity, and a possible modulating role of

iron in the relationship between obesity and CVD risk, in a population known to suffer from these nutrition related conditions.

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CHAPTER 3

THE RELATIONSHIP BETWEEN IRON STATUS AND ADIPOSITY IN WOMEN FROM DEVELOPING COUNTRIES: A REVIEW

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CHAPTER 3: THE RELATIONSHIP BETWEEN IRON STATUS AND ADIPOSITY IN WOMEN FROM DEVELOPING COUNTRIES: A REVIEW

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Abstract

Scientific reports have shown that iron deficiency is positively associated with adiposity. With the high prevalence of iron deficiency and obesity in developing countries and women being particularly affected, this review was carried out with aim of elucidating the link between iron status and adiposity in women from developing countries and to examine factors influencing this relationship. An extensive literature search was conducted using several search engines. A systematic approach with pre-specified inclusion criteria was used in selecting relevant literature. Eight studies that met the inclusion criteria were selected for review. The relationship between iron status indices and adiposity in women in developing countries varied widely. While some studies observed negative relationships, some reported positive relationships and others no significant relationships. Furthermore, other factors such as infection, alcohol consumption, type of diet and genes were shown to affect the relationship between iron status and adiposity in women in developing countries. In conclusion, the possibility of iron status playing a role in adiposity in women from developing countries is likely, and it may be influenced by several other factors as described in the results. Thus, it is recommended that a special research effort should be directed towards this area.

Keywords: Iron status, adiposity, women, developing countries, review.

Introduction

Iron deficiency and its associated anaemia is among the top ten contributors to the global burden of disease, affecting billions of people worldwide (WHO, 2001). The majority of iron deficient or anaemic people are in developing countries (UN, 2000). Forty percent of the total attributable global burden of iron deficiency occurs in the South-East Asia Region and almost another quarter in the African Region (Dreyfuss et al., 2000; WHO 2008). The greater demand for iron as a result of growth, menstruation and lactation makes adolescent girls and women of reproductive age a vulnerable group for iron deficiency (Whitfield et al., 2003). The World Health Organization/World Bank ranks iron deficiency anaemia as the third leading cause of disability-adjusted life years lost for females aged 15–44 years (Tolentino and Friedman, 2007).

Iron deficiency usually develops in a sequential manner, starting with iron depletion, progressing into iron-deficient erythropoiesis and finally overt anaemia (Cook et al., 1992). Iron deficiency can be detected by measurements of serum iron, total iron binding capacity (TIBC), serum ferritin, blood haemoglobin (Hb), blood haematocrit (Hct) and soluble transferrin receptor (TfR) concentration (Killip et al., 2007). Consequences of iron deficiency include reduced work capacity, impaired cognitive functioning, poor reproductive performance and reduced immune functioning (Killip et al., 2007).

While dietary iron remains the major determinant of iron status, scientific evidence suggests that adiposity may be an additional determinant of iron status (Seltzer and Mayer, 1963). An inverse association between adiposity and iron status has been described (Chambers et al., 2006). While studies have shown that adiposity might increase the risk of iron deficiency, adiposity has additionally been shown to induce elevation of serum ferritin concentrations (Zafon et al., 2010). This has been attributed to the fact that ferritin is an acute phase protein which may be elevated by the low grade inflammation that occurs when adipose tissues are enlarged (Zafon et al., 201o).

Contrary to conventional knowledge that the obesity epidemic is limited to industrialized nations, the rising impact of obesity is becoming apparent in developing countries. Approximately 115 million people in developing countries have become obese leading to

a rise in associated diseases such as heart diseases, diabetes and cancer (WHO, 2000). The prevalence of obesity in adolescents in countries like Egypt, Brazil and Mexico is now comparable to that in developed nations (Wang and Lobstein, 2006). African women are generally more obese than men (WHO, 2000). For example, 33% of Gambian women aged 35 years and above are obese compared to 2% of men in the same age group (Prentice, 2006). Additionally, reports have shown that obesity prevalence is rapidly increasing among women in Asian countries (WHO, 2000).

Given the increasing burden of diseases in developing countries as well as the existence of factors such as poor diets (Hurrell, 1997), traditional beer consumption (Kew and Asare, 2007), infection (Di, 2009) and gene mutation (Mcnamara et al., 1998), all known to influence iron status and adiposity, it is important to have a good understanding of the relationship between iron status and adiposity in this setting. Therefore, this review was carried out with the aim of elucidating the link between iron status and adiposity in women from developing countries, and to examine the effect of factors in developing countries that may influence this relationship.

Methods

Search Strategy

A comprehensive literature search of English-language studies was performed in Ebsco host, Google Scholar, Medline, Web of Science, Science Direct, the Cochrane Library, Scisearch and PubMed. Search words and phrases used included: iron, ferritin, anaemia, iron deficiency, TfR, Hb, hypoferremia, hyperferritinaemia, anthropometry, body fat, adiposity, overweight, obesity, body composition, fat deposition, body mass index (BMI), chronic diseases, developing countries, women, girls, females and adolescents. These words and phrases were also used in different combinations and looked for in title, abstract and full text. A secondary search was conducted through the reference lists of identified articles. Although, this paper is not a generic systematic review, it employed a systematic approach to select relevant literature that may help in understanding the link between iron status and adiposity in women from developing countries and the effect of factors in developing countries that may play a role in this association.

Selection criteria

Studies that were included in this review had to meet certain criteria as detailed as follows:

1. The study must report on the relationship between at least one of the iron indices (Hb, Hct, serum iron, serum ferritin, TIBC, percentage transferrin saturation and TfR) and any anthropometric variable (weight, BMI, waist circumference (WC), waist to hip ratio (WHR), skinfolds, percentage body fat, lean body mass).
2. The study population was restricted to women from developing countries that were at least 12 years of age.
3. The study must have a cross-sectional design or report the cross-sectional baseline data from other types of studies.

Definition of terms

1. Adiposity: refers to a state of being fat. This includes total body fat as indicated by BMI$\geq$25kg/m^2, central fat as indicated by WHR>0.8 or WC>88cm and fatness in other regions of the body such as arm, chest, hip, thigh etc (Methot et al., 2010; WHO, 1998).
2. Obesity: BMI$\geq$30kg/m (UN, 2000; Kasdan, 2000).
3. Iron deficiency: serum ferritin concentration<12µg/l (Kasdan, 2000, Yip and Ramakrishnan, 2002).
4. Iron deficiency anaemia: serum ferritin concentration<12µg/l and Hb<12g/dl (Kasdan, 2000, Yip and Ramakrishnan, 2002).

Results

Eight studies met the inclusion criteria (Aderibigbe et al., 2010a; Aderibigbe et al., 2010b; Eckhardt et al., 2008; Eftekhari et al., 2009; Ettyang et al., 2003; Famodu and Awodu, 2009; Nemati et al., 2007; Paknahad et al., 2008). The publication year ranged from 2003 to 2010. The number of female participants examined in the selected studies ranged from 76 to 6841. Two of the studies were conducted in adolescent girls (Eftekhari

et al., 2009; Nemati et al., 2007), five in adult women that were neither pregnant nor lactating (Aderibigbe et al., 2010a; Aderibigbe et al., 2010b; Eckhardt et al., 2008; Famodu and Awodu, 2009; Paknahad et al., 2008) and one in lactating women (Ettyang et al., 2003). Five of the studies included ferritin as a measure of iron status (Aderibigbe et al., 2010a; Aderibigbe et al., 2010b; Eftekhari et al., 2009; Ettyang et al., 2003; Nemati et al., 2007). Paknahad et al. (2008) and Nemati et al. (2007) only used Hb and Hct, while Famodu and Awodu (2009) used Hct only. One of the studies did not include BMI as an anthropometric index (Nemati et al., 2007). Only three studies examined WHR as an indicator of adiposity (Aderibigbe et al., 2010a; Aderibigbe et al., 2010b; Famodu and Awodu, 2009) The detailed description of the articles is illustrated in Table 1.

Eftekhari et al. (2009) found a negative association between ferritin and BMI ($r= -0.38$, $p<0.01$) in iron deficient Iranian girls aged 13-20 years while Nemati et al. (2007) observed a positive association between ferritin and body weight ($r=0.158$, $p<0.05$) in adolescent Iranian girls aged 12 years. In addition, there was no significant difference ($p<0.05$) between the weight and height of anaemic and non anaemic Iranian girls aged 12 years (Nemati et al., 2007). Hb and Hct increased ($p<0.05$) across BMI quartiles in adult Iranian women between the ages 15 and 49 years (Paknahad et al., 2008).

Ettyang et al. (2003) reported no significant difference ($p<0.05$) in BMI among lactating Kenyan women at different levels of iron depletion (as defined by ferritin), but Hb was shown to increase with increasing BMI. In the report by Famodu and Awodu (2009) Hct increased with increasing BMI and WHR in Nigerian women with an average age of 59 years. Aderibibge et al. (2010a and 2010b) showed that WC and WHR increased with increasing ferritin concentration in South African women, while serum iron decreased with increasing BMI in women. A lower odds ratio of anaemia was additionally reported in over-weight and obese women than normal weight women from Egypt (Eckhardt et al., 2008).

No significant difference in odds of anaemia was observed in Mexican women in different categories of BMI (Eckhardt et al., 2008). A lower odds ratio of anaemia was reported in over-weight and obese women than normal weight women from Peru (Eckhardt et al., 2008).

Table I: Description of studies examining the link between iron status and adiposity in women from developing countries

Reference	Objective of study	Study population	No of subjects	Results	Observed trends	Statistical significance	Factors implicated in iron & adiposity link	Factors corrected for in the study
Eftekari et al., 2009 Iran	To investigate the association between Fe deficiency and weight status	Iron deficient adolescent Iranian girls aged 13-20	431	Ferritin & BMI associated negatively	↑Ferritin ↓BMI	r = -0.38 p <0.001	Urbanization Diet Low physical activity	Systemic diseases
Nemati et al., 2007 Iran	To determine the prevalence of Fe deficiency anaemia and its relation to height, weight and school success	School girls aged 12 in Ardebil, Iran	170	Ferritin & weight associated positively (r=0.158, p<0.05)	↑Ferritin ↑ weight	r = 0.158 P<0.05	------	------
Paknahad et al., 2008 Iran	To assess the BMI status in premenopausal women and its relationship with iron biochemical indices.	Non-pregnant, non-lactating 15-49 years old Iranian women	1049	Hb increased across BMI quartile Hct in increased across BMI quartiles	↑Hb/Hct ↑BMI	P<0.05	Industrialization Low physical activity Parity	Infection
Ettyang et al., 2003 Kenya	To establish the prevalence and the relationship of vitamin A and iron to maternal body composition	Lactating Kenyan women aged 15-45 years	88	As ferritin concentration decreases, BMI, %body fat and fat free mass did not vary significantly among lactating mothers	↓Ferritin ---- BMI, %body fat & fat free mass	p>0.05	------	Age Parity Child's age Child's birth weight Lactation period
Eckhardt et al., 2008 Mexico Egypt Peru	To compare the odds of anaemia in overweight and obese versus non-overweight women in three countries at different stages of the nutrition transition	Non pregnant women aged 18-49 years from Mexico, Peru and Egypt	Mexico 11,965 Egypt 6841 Peru 5078	Odds of anaemia did not differ by BMI in Mexico Odds of anaemia was lower for over-weight and obese group than normal weight women in Peru	↑ Odds of anaemia --- BMI in Mexico ↓Odds of anaemia ↑BMI in Peru	p>0.05 p<0.05 OR=0.83	Other micronutrient deficiencies (Zn, folate & Vit C) Malaria and other chronic parasitic infections Smoking	Socio-demographic characteristic (rural/urban) Education level Parity Age Altitude Socio-

				Odds of anaemia was lower for over-weight and obese group than normal weight women in Egypt	↓ Odds of anaemia ↑BMI in Egypt	P<0.05 OR=0.78		economic status
Famodu et al., 2009 Nigeria	To study the relationship of fibrinogen, plasma viscosity & Hct with measures of obesity	Nigerians women (average age 59 years)	76	Hct increased across BMI percentile up until the 75th percentile	↑ Hct ↑WHR	P=0.0074	------	Diseases
Aderibigbe et al., 2010a South Africa	To examine relationship between iron indices and selected anthropometric CVD risk factors	South African women aged between 15 years and older	952	WC and WHR increased with increasing ferritin concentration Serum iron decreased with increasing BMI. Ferritin associated positively with BMI, WC, WHR, body fat and SSF	↑ Ferritin ↑WC & WHR ↓ Serum Fe ↑ BMI	P<0.05	------	Age BMI smoking
Aderibigbe et al. 2010b South Africa	This study examined the associations between iron status parameters and CVD risk factors in black South African women.	South African women aged between 35 years and older	1262	WC and WHR increased across ferritin quartiles.	↑ Ferritin ↑WC & WHR	P<0.05	------	Age BMI Smoking Alcohol

↑ increasing; ↓ decreasing; ---- no significant variation.
Hct, haematocrit; BMI, body mass index; WHR, waist to hip ratio, WC, waist circumference; CVD, cardiovascular disease.

Discussion

With the high prevalence of obesity and iron deficiency in developing countries, this review elucidates the link and factors influencing the associations between iron status parameters and indicators of adiposity in women from developing countries. The literature search resulted in eight studies that examined the relationship between iron status and adiposity in women from developing countries. Assessment indicators used for iron status varied widely; and there was no uniformity in the physiological state of women participants included in the studies that met the inclusion criteria. The results of the studies reviewed varied widely; while some observed positive associations, some reported negative associations and some did not observe any significant associations.

The negative association between circulating iron and adiposity was first demonstrated in adolescents (Wenzel et al., 1962) and thereafter several studies have confirmed this finding in different populations (Eftekhari et al., 2009; Nead et al., 2004; Pinhas-Hamiel et al., 2003; Seltzer and Mayer, 1963; Tussing-Humphreys et al., 2009). In addition, it has been reported that obese adolescents have increased ferritin concentration despite the deficient serum iron (Nemati et al., 2007; Zafon et al., 2010). However, the study conducted in Iranian adolescent girls contradicts this observation (Eftekhari et al., 2009). A negative association was reported between ferritin and BMI in this population (Eftekhari et al., 2009). The adolescent girls in this study were already iron deficient (Eftekhari et al., 2009). Furthermore, adolescence is the transition period between childhood and adulthood. The overall iron requirements increase from a preadolescent level. The available data on iron intakes in adolescents suggest that adolescent girls are unlikely to acquire substantial iron stores during this time period. Iron stores are further depleted by the growth spurt that occurs during adolescence (Beard, 2000). This could explain the negative association between ferritin and adiposity in adolescent Iranian girls. Moreover, Asian countries have a greater prevalence of iron deficiency than other developing regions; this has been attributed to less care given to girls and women in this region (Osman and Alok, 1998).

Studies conducted in adult premenopausal women have confirmed the presence of iron deficiency in obese adult women. Zimmerman et al. (2008) showed that independent of

iron stores, a higher BMI Z-score was associated with decreased iron absorption in Thailand women aged 18-50 years. Aderibigbe et al. (2010a) reported a decreasing serum iron concentration with increasing BMI in South African women aged 15 years and above. An increased risk of anaemia with increasing pre-pregnancy BMI has been reported for postpartum women (Bodnar et al., 2004). There is evidence of an increased risk of iron deficiency in menstruating women, pregnant women and adolescents after bariatric surgery (Flancbaum et al., 2006; Love and Bilett, 2008). On the contrary, reports from Peru and Egypt showed that the odds of anaemia decreased with increasing BMI in adult premenopausal women while in women from Mexico no significant variation in odds of anaemia with change in BMI was found (Eckhardt et al., 2008). Additionally, Hct increased with increasing BMI in Nigerian women (Famodu and Awodu, 2009) and BMI of lactating women from Kenya was not significantly associated with ferritin concentration (Ettyang et al., 2003).

It has been suggested that overweight or obese women from rural areas (for instance in Egypt, Nigeria and some part of Mexico) may have higher energy intakes that convey enough additional iron to lower the risk of anaemia compared to their non-overweight counterparts (Lonnerdal, 2000; Lopez and Martos, 2004). The lifestyle changes of the urban poor may have pressurized them to choose only the high carbohydrate foods while their rural counterparts have a wider choice from grown and hunted foods from their surroundings. This could bring the iron status of obese rural women at par to that of the normal weight women in the urban areas (Lonnerdal, 2000; Lopez and Martos, 2004).

Studies that examined the relationship between iron indices and adiposity in postmenopausal women were conducted in developed countries (Crist et al., 2008; Lecube et al., 2006; Liu et al., 2003). A moderate degree of iron deficiency in obese postmenopausal women has been reported (Lecube et al., 2006). A positive association between ferritin and central adiposity has been reported in postmenopausal women (Lecube et al., 2006). Increased secretion of serum ferritin occurs at menopause but reports have shown that the ferritin level in postmenopausal women is still low compared to that found in men of the same age group (Berge et al., 1994; Kato et al., 2000; Zacharski et al., 2000).

Mechanisms

The aetiology of the hypoferremia of obesity is not clear. Among proposed causes are inadequate intake of dietary iron and greater iron requirement in obese individuals because of larger blood volume (Failla et al., 1988; Pinhas-Hamiel et al., 2003; Newman et al., 2003). Kennedy et al. (1986) reported that chronic obesity was negatively associated with iron in the plasma, liver, bone and muscle of genetically obese mice compared to lean mice. This observation was irrespective of gender and age of the mice (Kennedy et al., 1986).

However, the relationship between adiposity and serum ferritin has been observed to go in an opposite direction compared to that between serum iron and adiposity. A positive association has been demonstrated between ferritin and adiposity (Gillum, 2001). Ferritin, an acute phase protein, is elevated in inflammatory conditions (Tomkins, 2003). Obesity has been identified as an inflammatory condition (Yanoff et al., 2007). Moreover, recent findings have shown that adipocytes are not just storage organs for fat, they play a regulatory role in body homeostasis (Andrew et al., 2006). Hepcidin, a hormone secreted mainly in the liver but also found in adipocytes is highly expressed in obese individuals (McClung and Karl, 2008). Hepcidin is able to prevent release of iron from the stores, and absorption of intracellular iron (McClung and Karl, 2008). This has been speculated as one of the mechanisms that could explain iron deficiency in obese individuals despite optimum or overloaded stores. Liver hepcidin messenger ribonucleic acid (mRNA) expression has been shown to correlate with transferrin saturation whereas adipose hepcidin mRNA expression did not, but rather correlated positively with markers of inflammation, which indicates that hepcidin may have tissue-specific regulation (Berki et al., 2006). Lipid peroxidation increases during fat deposition as a result of the reactivity of intracellular iron with lipids, sequestration of intracellular iron into the stores could occur in order to reduce lipid peroxidation thereby leading to reduced functional iron and increased iron stores (Festa et al., 2000).

Increased ferritin concentration observed in obese individuals has been argued to be the result of chronic inflammation and not necessarily increased iron stores (Zafon et al., 2010). In the study conducted by Lecube et al. (2006), the increased ferritin concentration

found in obese women with metabolic syndrome was not accompanied by any significant changes in other iron status parameters. Additionally, the observation that serum ferritin concentration increased while transferrin saturation decreased in those with excess body fat gave support to the inflammatory cause for hyperferritinaemia of obesity (Dreyfuss et al., 2000; Ford and Cogswell, 1999; Gillum, 2001). Some studies have reported elevated amounts of inflammatory cytokines such as tumor necrosis factor and interlukin-6 in patients with obesity and other metabolic syndromes. (Hotamisligil, 1999; Pickup et al., 2000). BMI has been positively associated with serum C-reactive protein, another marker of inflammation (Hak, 2002).

Though inflammation is considered to be one of the consequences of obesity, some studies conducted in adults have suggested that inflammation may occur before obesity (Barzilay et al., 2006; Engstrom et al., 2003). It was reported that inflammatory markers were prospectively predictive of weight gain. Though the mechanism of this effect is not fully understood; it has been proposed that inflammation may stimulate hunger and inhibits satiety through interaction with the feeding and satiety centre of the brain (Barzilay et al., 2006; Engstrom et al., 2003). There is a growing view that the inflammatory state that characterizes obesity may play a causal role in the development of insulin resistance, type 2 diabetes and the metabolic syndrome (Grimble, 2002; Weisberg et al., 2003; Xu et al., 2003).

Other factors that could affect the link between iron status and adiposity in developing countries

Iron status is influenced by the amount of iron present in the diet and other dietary components that may influence iron absorption. The diets in many developing countries are monotonous, composed mainly of cereals and legumes, with minimal amounts of bioavailable iron, and high in inhibitors of non haeme-iron absorption like phytates (Hurrell, 1997). In a study, a cohort of Moroccan children who have been made iron replete by fortification were left to follow their habitual diet (non-fortified cereal and legume), it was reported that the prevalence of iron deficiency increased from 0-43%

within 15 months among the children (Zimmermann et al., 2005). This finding demonstrated that low iron bioavailability from a legume and cereal– based diet can be a cause of iron deficiency in developing countries. Furthermore, this diet that is deficient in micronutrient could be energy dense such that it leads to increased adiposity (Quinion, 2010).

Unfortunately, data on the dietary intake of women in developing countries are few. Dietary data from South Africa and Kenya showed that adult women were consuming a diet high in fat and saturated fats but deficient in micronutrients, including iron (Steyn and Nel, 2006). In both countries, significant differences between the urban and rural women were observed (Steyn and Nel, 2006). Urban women consumed more foods rich in bio-available iron than rural women (Steyn and Nel, 2006). However, distinct difference in nutritional status has been reported between the rich and poor urban women (Hattingh et al., 2008). Urban black South African women were shown to meet $\geq$67% of the recommended daily allowance (RDA) for iron (Hattingh et al., 2008). However, only 54% of these women showed total iron intakes $\geq$67% of the RDA (Hattingh et al., 2008). This report supports others that have showed large discrepancies between the poor and rich in urban areas of developing countries (Becquey and Martin-Prevel, 2010; Sukchan et al., 2010).

Deficiency of other micronutrients such as folic acid and cobalamin (vitamin B_{12}) which are prevalent in developing countries can influence the relationship between iron status and adiposity (Ramakrishnan et al., 1999; Seshadri, 2001). Folic acid deficiency causes disturbance in synthesis of deoxyribonucleic acid (DNA), which leads to damage of red cells (Baynes, 1994). Casanueva et al. (2000) reported that folic acid deficiency was associated with anaemia in non-pregnant, non-lactating Mexican women aged 23-40 years. Additionally, folic acid deficiency was associated with obesity in the same population. Menzie et al. (2008) compared the amount of haeme iron, non-haeme iron and other dietary factors that influence iron absorption in a sample of obese and non-obese adults. The authors reported that obesity-related hypoferremia cannot be explained by differences in reported dietary iron or vitamin intakes.

Alcohol consumption has been shown to influence both the iron status (Aderibigbe et al., 2010b; Kew, 2007) and level of adiposity (Gopane et al., 2010; Rohrer et al., 2005) of an individual. Gopane et al. (2010) reported that the amount of alcohol consumed was a strong predictor of ferritin in a South African study. Alcohol consumption rate is high in Sub-Saharan Africa where the consumption of traditional beers that are brewed in iron pots is prevalent (Kew, 2007). This has been reported to contribute greatly to dietary iron overload in Sub-Saharan Africa (Kew, 2007). However, the effects of alcohol use on the risk of obesity have not been thoroughly explored ((Rohrer et al., 2005). Pisa et al. (2010) reported a significant drop in BMI of South African women ($\geq$35years) with increasing self-reported alcohol consumption. In the same population, increased WC and WHR has been reported to contribute significantly to increased ferritin concentration in a black South Africans even after adjusting for self-reported alcohol consumption (Aderibigbe et al., 2010b).

Malnutrition in developing countries is aggravated by the burden of infestations with malaria parasite, intestinal worms and the human immunodeficiency virus (HIV) (Di, 2009). Micro-organisms in need of iron for survival and multiplication compete with the host for the available iron (Di, 2009). It is being speculated that the anaemia associated with chronic asymptomatic malaria may be due to an inflammatory mediated effect on iron redistribution to storage compartments and a resultant deficit in erythropoietin production with or without bone marrow responsiveness (Abdalla et al., 1980). In chronic malaria, sequestration of iron into the bone marrow coexists with iron-deficient erythropoiesis (Verhoef et al., 2002). HIV infected persons suffer from iron metabolism disorders. At the advanced stage, anaemia can coexist with elevated ferritin and increased bone marrow iron content (De Monge et al., 1999). Obesity as an inflammatory condition co-existing with infection may further deplete the available functional iron while increasing iron stores.

A genetic pre-disposition to iron overload has been suggested in Africans but the putative gene has not been identified. It is speculated that the gene may be a result of mutation as described in hereditary haemochromatosis gene (HFE) (Mcnamara et al., 1998). The condition was first attributed to excess intake of traditional home made beers. However,

not all beer drinkers develop excess iron overload and not everyone who develops iron overload is a traditional beer drinker. Investigators have concluded that heterozygosity for an unidentified iron overloading gene confers susceptibility while homozygous persons may be severely affected (Moyo et al., 1998).

Conclusion

The results of the studies reviewed in this paper showed an inconsistent relationship between iron status parameters and adiposity in women living in developing countries. The likelihood of iron playing a role in adiposity is possible. Besides, factors like infection, consumption of traditional beer and poor diet have the potential to affect the iron and adiposity relationship. Further research is, therefore, required in the developing regions in order to understand the link between iron status and adiposity fully. This new research should take into consideration the effect of the factors that have been discussed in this paper.

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CHAPTER 4

THE RELATIONSHIP BETWEEN INDICES OF IRON STATUS AND SELECTED ANTHROPOMETRIC CARDIOVASCULAR DISEASE RISK MARKERS IN AN AFRICAN POPULATION: THE THUSA STUDY

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CHAPTER 4: THE RELATIONSHIP BETWEEN INDICES OF IRON STATUS AND SELECTED ANTHROPOMETRIC CARDIOVASCULAR DISEASE RISK MARKERS IN AN AFRICAN POPULATION: THE THUSA STUDY

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Abstract

There is evidence that certain indices of iron status are associated with anthropometric measures which are used independently as markers of cardiovascular disease risk. This study examined whether this association exists in an African population. The study is a cross-sectional comparative study that examined a total of 1854 African participants. Ferritin was positively associated with BMI, WC, WHR, body fat and subscapular skinfold. Serum ferritin concentration was higher in the high WHR category than the normal WHR category for both genders. Additionally, WC and WHR increased with increasing ferritin concentration in both genders. Serum iron was lower in the obese than the normal weight and pre-obese women only. In this population-based study, increased serum ferritin concentration associated positively with increased WHR and WC, indicating that individuals or populations at risk of iron overload as defined by high serum ferritin concentration may be at a greater risk of developing CVDs.

Keywords: Iron indices, anthropometry, cardiovascular diseases, African, THUSA study.

Introduction

South Africa is experiencing a health transition, associated with a triple burden of disease characterised by a high prevalence of undernutrition-related infectious diseases, the emergence of non-communicable diseases, and the human immunodeficiency virus/acquired immune deficiency syndrome (HIV/AIDS) pandemic.[1] Micronutrient deficiencies still remain a major public health challenge in most developing countries**.** Iron deficiency anaemia is the most common nutritional deficiency in the world and brings negative consequences on growth and health.[2, 3] The prevalence of iron deficiency is highest in the developing countries and its causes are multi-factorial.[4] In South Africa, anaemia has been reported in 7-29% of pregnant women[5-7] and 57% of pregnant teenage girls.[8] The World Health Organisation[2] estimated that 26.4% of non-pregnant women of reproductive age in South Africa had haemoglobin concentration below 12µg/dl. Charlton[9] also reported a 13% prevalence of anaemia among an elderly South African population.

Iron is a key element in many biochemical processes and shortage of iron causes damage to cells and organs. On the other hand, excess iron could be harmful because it is able to catalyse the formation of highly reactive oxygen and hydrogen radicals when present in the unbound state.[10] Because of the ease with which additional iron can be provided to iron replete individuals through iron fortified foods or iron supplements and the limited ability to excrete the mineral, the consequences of iron excess are as relevant nutritionally as the liabilities of iron deficiency. A high prevalence of iron overload (15%) was reported among male blacks across Sub-Saharan Africans that have the custom of drinking a traditional fermented beverage with high iron content.[10] The genetic predisposition to iron overload has also been identified in Africans [10]

Due to rapid urbanisation, lifestyle changes and adoption of western diets, obesity has become a growing problem in developing countries. Countries undergoing transition such as China, Brazil, and South Africa, are particularly affected and have an increasing prevalence of obesity across all economic levels and age groups.[11] In South Africa, 30% of men and 55% of women have been classified as overweight or obese.[12]

Cross-sectional studies have indicated that measures of iron status are positively associated with cardiovascular disease (CVD) risk factors and this association is hypothesised to be mediated by adiposity.[14, 15] Additionally, iron deficiency has been reported to be positively associated with anthropometric indicators like waist circumference (WC), body mass index (BMI) and waist to hip ratio (WHR)[13,14] which are now used independently as markers for CVD risk.[15] Establishing the relationship between measures of iron status and these anthropometric CVD risk markers may give an indication whether the iron status of a population can predict their CVD risk. In view of this, the present study is aimed at examining the relationship between measures of iron status (ferritin, serum iron, haemoglobin, total iron-binding capacity, and percentage transferrin saturation) and selected anthropometric CVD risk markers in an African population.

Methods

Study design, subject selection and organisational procedures

The THUSA (Transition and Health during Urbanisation of South Africans) study was conducted from 1996-1998 in the North West Province of South Africa.[16] It was a cross-sectional comparative study in which a community based sample of 1854 apparently healthy black volunteers (15 years and older) were recruited from 37 randomly selected sites, using a statistical model that ensured a representative sample from 5 levels of urbanisation: deep rural, commercial farms, informal settlements, "middle class" urban and "upper class" urban. Pregnant and lactating women, individuals taking chronic medication, those with oral temperatures above 37^0C and inebriated volunteers were excluded.

Permission to conduct the study in specific areas with advice on recruitment procedures were obtained from the North West Department of Health, tribal chiefs, community leaders, headmasters of high schools and mayors. The study was approved by the Ethics Committee of the North-West University (**Ethics number: 4M5-95)** and all participants signed an informed consent form. Participants were fasted (10-12 hours) for the baseline

blood sampling and other measurements. They received lunch after completion of the tests. All participants received feedback regarding their blood pressure, fasting glucose concentrations and haemoglobin values. Where necessary, participants were referred to their nearest health facility for further diagnosis and treatment. Travelling expenses of participants were covered.

Questionnaires

Questionnaires were designed for this study population and were validated using appropriate methods.[16] The questionnaires were administered during individual interviews conducted by the researchers and specially trained African field workers in the language of the participant's choice.

The *demographic questionnaire* included questions on type of housing, access to electricity, water source, sanitation, personal and household income, health history (also of close family members), number and ages of people living in the same house, ownership of property, education level as well as smoking and drinking habits.

Anthropometric measurements

Anthropometric measurements were done in triplicate by postgraduate Biokinetics students and standardised by a level III anthropometrist. Height was measured to the nearest 0.5cm with a stadiometer (Invicta, IP 1465, UK) and weight was determined on a portable electronic scale to the nearest 0.1kg (Precision Health Scale, A & D Company, Japan) with the participants in light clothing. Skinfold thickness and body circumferences of participants in their underwear were measured with calibrated instruments (Holtain® unstretchable metal tape; John Bull® calipers). All the measurements were done according to International Society for the Advancement of Kinanthropometry (ISAK) standards.[17] BMI was calculated by dividing weight in kilograms by height in meter squared. Waist to hip ratio was calculated by dividing WC by hip circumference. Percentage body fat was calculated using Siri's equation (4.95/density – 4.50) X 100.[18] Total body density was derived from Durnin and Womersley's equation[19] using the sum of biceps, triceps, subscaplar and suprailiac skinfold thicknesses.

Clinical examinations

Two nurses examined the participants for clinical signs of malnutrition. Oral temperatures were taken and blood pressure recorded, also in triplicate using a sphygmomanometer (Tycos®) with adjustable cuffs of different sizes.

HIV test

HIV status was determined anonymously with an enzyme-immunological method (enzymum-Test®, anti-HIV 1 and 2 and subtype Ø, Boehringer Mannheim, Germany, cat no 1557319).

Blood, serum and plasma samples

Blood was drawn from the *vena cephalica* using a sterile butterfly infusion set (Johnson & Johnson, 21G, 19mm) and syringes. For preparation of serum, 5ml blood was allowed to clot in glass tubes, centrifuged at 3000rpm for 15 minutes (Universal 16R™, Hettich, with cooling facilities), and transferred into storage tubes. Citrated blood was prepared by drawing 4.5ml blood into a syringe containing 0.5ml 1mol/l citrate (pH 4.5-4.8). These samples were centrifuged for 10 minutes at 3000rpm in plastic siliconised tubes and the plasma stored in tubes. All serum and plasma samples were immediately stored at -18^{0}C to -20^{0}C in the field for 2-4 days and afterwards at -84^{0}C in the laboratory.

Biochemical analyses

All laboratory analyses were done within a year of blood collection. Haemoglobin concentration was measured in the field on EDTA (ethylenediaminetetraacetic acid) blood. Serum ferritin was measured using an immunoradioactive assay (Ferritin MAb Solid Phase Component System; Benton Dickson & Co, Orangeburg, NY) with an Auto Gamma 500C counting system (United Technologies, Packard, III). Serum iron and total iron-binding capacity (TIBC) were determined spectrophotometrically with an RA-1000 automated system (Technicons, Tarrytown, NY) using a colorimetric method (Fe SYS 1 and Test-Combination Iron-binding Capacity; Boehringer Mannheim), and percentage transferrin saturation was calculated by finding the molar ratio of serum iron and twice

the serum transferrin using the formula percentage transferrin saturation = (serum iron (µg/dl)*100/transferrin (mg/dl)*2).

Statistical Analysis

Data were analysed using SPSS (Statistical Package for Social Sciences), version 17 and presented as geometric means and standard error of means (SE). The minimum and maximum values were also stated. Spearman correlation was used to assess the relationship between iron indices and anthropometric indicators. Partial correlations after adjusting for age, BMI and smoking were further assessed. A stepwise regression method was used to identify valid confounders in this particular population. Age, BMI and smoking were treated as confounders. HIV status did not modify or confound the iron status parameters, so it was not adjusted for in the analysis. To assess further the relationship between iron status and anthropometric indicators, men and women were grouped and analysed in different WHR and BMI categories.[20] Additionally, men and women were grouped and analysed in three ferritin groups: 1. low ferritin group (serum ferritin concentration below 12µg/L), 2. normal ferritin group (serum ferritin concentration between 12 and 150µg/L) and 3. high ferritin group (serum ferritin concentration above 150µg/L). These cut-off points are the clinical cut-off points recommended by standard dietetic practice.[21] Multivariate analysis was used to assess significant differences between different groups before and after adjusting for age, BMI and smoking. Statistical significance was set at $P<0.05$.

Results

Table 1 outlines the anthropometric and iron indices of participants. All iron indices were better in men than women ($p<0.0001$) before and after adjusting for age, BMI and smoking. Women had a higher mean BMI ($p<0.0001$) and body fat ($p<0.0001$) than men. Waist circumference ($p=0.006$) and WHR ($p<0.0001$) were significantly higher in men than women before adjusting for age, BMI and smoking. Age did not differ significantly ($p=0.076$) between men and women.

Correlations between iron indices and anthropometric indicators for both men and women are displayed in Table 2. In men, the strongest correlations were found between ferritin and WC (r_s=0.359, p<0.01) and ferritin and WHR (r_s=0.396, p<0.01). Likewise in women, the strongest correlations were found between ferritin and WC (r_s=0.232, p<0.01) and ferritin and WHR (r_s=0.319, p<0.01).

Iron indices are compared according to WHR and gender categories in Table 3. In men, mean serum ferritin concentration was significantly higher in the high WHR group than the normal WHR group (p<0.0001), though this disappeared after adjusting for age, BMI and smoking. In addition, mean serum iron was also significantly higher in the high WHR group than the normal WHR group (p=0.020) after adjusting for age, BMI and smoking. In women, mean serum ferritin and haemoglobin concentration were significantly higher in the high WHR group than the normal WHR group before (p<0.0001, p=0.003 respectively) and after (0.004, p=0.018 respectively) adjusting for age, BMI and smoking. Women in the normal WHR group had higher mean serum TIBC than those in the high WHR group before (p<0.0001) and after (p=0.019) adjusting for age, BMI and smoking. No significant differences were observed for serum iron and transferrin saturation between the two WHR categories before and after adjusting for age, BMI and smoking.

The comparison of anthropometric indices according to three different ferritin concentrations (low, normal and high ferritin) is presented in Table 4. In men, mean WC (p<0.0001) and WHR (p<0.0001) were significantly higher in the high ferritin group than the low ferritin group before adjusting for age, BMI and smoking but WC only remained significant (p=0.014) after adjusting for age, BMI and smoking. Moreover, mean WC (p<0.0001) and WHR (p<0.0001) of the men were significantly higher in the high ferritin group than the normal ferritin group before adjusting for age, BMI and smoking. This was not retained after adjusting for age, BMI and smoking. A significantly higher mean BMI (p=0.001) was found in the high ferritin group as compared to normal ferritin group before adjusting for age and smoking. The high ferritin group had a significantly higher mean body fat as compared to the low ferritin group (p=0.015) and the normal ferritin group (p<0.0001) before adjusting for age, BMI and smoking. As for women, mean WC

and WHR were significantly higher in the high ferritin group than the low ferritin group before ($p<0.0001$, $p<0.0001$ respectively) and after ($p=0.002$, $p=0.018$ respectively). WC and WHR were also higher in the high ferritin group than the normal ferritin group before ($p=0.033$, $p=0.005$ respectively) and after ($p<0.0001$, $p=0.014$ respectively) adjusting for age, BMI and smoking. Women in the normal ferritin group had higher mean BMI ($p=0.032$) than those in the low ferritin group before adjusting for age and smoking.

Table 5 compares the mean iron indices among four BMI categories. In men, mean serum TIBC was significantly higher in the normal weight group as compared to the underweight group before ($p=0.016$) and after ($p=0.018$) adjusting for age and smoking. Additionally, mean serum TIBC was significantly higher in the overweight than the underweight ($p=0.047$) after controlling for age and smoking. No significant differences were observed in ferritin, serum iron, percentage transferrin saturation and haemoglobin among men in the different BMI categories before and after adjusting for age and smoking. As for women, mean serum ferritin concentration was significantly higher in the underweight group than the normal weight group, overweight group and obese groups before ($p=0.001$, $p=0.020$, $p=0.014$ respectively) and after ($p=0.017$, $p=0.037$, $p=0.007$ respectively) controlling for age and smoking. A significantly higher mean haemoglobin concentration was observed in the underweight as compared to the overweight ($p=0.036$) and obese ($p=0.013$), though only the difference between the underweight and obese was retained ($p=0.022$) after adjusting for age and smoking. Percentage transferrin saturation was higher in the overweight than obese women before ($p=0.014$) and after ($p=0.016$) adjusting for age and smoking. Obese women had a lower mean serum iron than the normal weight ($p=0.047$) and overweight ($p=0.006$) women but only the difference between the overweight and obese remained significant ($p=0.013$) after adjusting for age and smoking. No significant differences in TIBC were observed for the different BMI categories before and after adjusting for age and smoking.

Table 1: Anthropometric and haematological characteristics of participants

	Men (n=711, 42.80%)		Optimum cut-off point	Women (n=952, 57.20%)		Optimum cut-off point
Variable	*Mean (SE)*	*min, max*		*Mean (SE)*	*min, max*	
Age (years)	34.32 (0.58)	15.00, 82.00	---	35.04 (0.46)	15.00, 90.00	---
Serum Fe (µmol/l)	16.52 (0.33)	0.74, 73.42	≥11[22]	13.13*# (0.24)	0.26, 59.85	≥11[22]
Serum TIBC (µmol/l)	63.86 (0.49)	28.77, 197.22	≤ 73[22]	68.21*# (0.44)	29.13, 171.12	≤ 73[22]
Transferrin Saturation (%)	25.86 (0.53)	1.34, 102.22	20-50[21]	19.18*# (0.39)	0.35, 97.03	20-50[21]
Serum Ferritin (µg/l)	104.12 (12.04)	1.00, 2877.17	12-150[21]	39.40*# (5.10)	0.28, 2678.53	12-150[21]
Hb (g/dl)	13.30 (0.79)	4.70, 22.90	13-18[21]	12.00*# (0.67)	4.70, 31.10	12-16[21]
BMI (kg/m^2)	20.80 (0.15)	13.76, 65.39	18.5-24.9[20]	26.13* (0.21)	14.60, 53.64	18.5-24.9[20]
WC (cm)	73.95 (0.35)	53.60, 126.20	≤ 80[23]	77.25*# (0.42)	46.50, 130.20	≤ 80[23]
WHR	0.84 (0.00)	0.59, 1.52	≤ 0.95[20]	0.76*# (0.00)	0.47, 1.00	≤ 0.80[20]
Body fat (%)	20.63 (0.26)	9.17, 39.30		47.06*# (0.45)	13.30, 83.79	
TSF (mm)	7.36 (0.19)	1.80, 37.30		18.89*# (0.33)	3.50, 52.10	
SSF (mm)	9.63 (0.22)	3.00, 40.00		18.69*# (0.42)	4.40, 60.00	

*Significant difference between men and women before adjusting for age, BMI and smoking ($p<0.05$).
#Significant difference between men and women after adjusting for age, BMI and smoking ($p<0.05$).
BMI: body mass index, Hb: Haemoglobin, SD: standard deviation, TIBC: total iron binding capacity, TSF: triceps skinfold, SSF:Subscapular skinfold, WC: waist circumference, WHR: waist to hip ratio

Table 2: Correlations of iron and anthropometric indices of participants

Anthropometric indices	***Iron Indices***									
	Serum Fe (µmol/L)		**TIBC (µmol/L)**		**Transferrin Saturation (%)**		**Ferritin (µg/L)**		**Hb (g/dl)**	
	(r_s)	***(r_p)***	***(r_s)***	***(r_p)***	***(r_s)***	***(r_p)***	***(r_s)***	***(r_p)***	***(r_s)***	***(r_p)***
				MEN						
BMI (kg/m2)	.015	__	119**	__	-.032	__	.141**	__.	-.056	__
WC (cm)	.010	-.043	.000	. 004	.006	-.049	.359**	.068	-.047	-.064
WHR	.027	.067	-.157**	-.004	.079*	.062	396**	.014	.003	.016
Body fat (%)	-.048	-.009	.-029	.008	-.037	.012	.308**	.102	-.111*	-.096
TSF (mm)	.012	-.044	.097*	.051	071	-.052	.076*	-.028	-.022	-.056
SSF (mm)	.019	-.031	.035	.001	-.005	-.027	.141**	.010	-.018	-.059
				WOMEN						
BMI (kg/m2)	-.039	_	-.041	__	-.021	__	.126**	__	-.065*	__
WC (cm)	-.036	-.007	-.087**	-.045	-.008	.014	.232**	.024	-.008	.089
WHR	-.044	-.016	-.166**	-.026	.004	.008	.319**	.031	.083*	.126
Body fat (%)	-.013	-.021	-.023	.082	--.002	-.054	.126**	.012	-.063	-.011
TSF (mm)	--.062	-.053.	-.025	-.012	-.048	-.047	.040	-.059	-.037	-.015
SSF (mm)	-.029	-.035	-.040	-.008	-.014	-.027	.105**	-.038	.072*	-.025

r_s: Spearman correlation, ** Spearman correlation coefficient is significant at the 0.01 level (2-tailed).
* Spearman correlation coefficient is significant at the 0.05 level (2-tailed).
r_p: Partial correlation after adjusting for age, BMI and smoking.
BMI: body mass index, Hb: Haemoglobin, SD: standard deviation, TIBC: total iron binding capacity, TSF: triceps skinfold, SSF:, Subscapular skinfold, WC: waist circumference, WHR: waist to hip ratio.

Table 3: Comparison of iron indices according to WHR categories

Variable	Normal WHR		High WHR	
	Mean (SE)	***min, max***	***Mean (SE)***	***min, max***
		MEN		
	(WHR≤0.95, n=681, 95.78%)		***(WHR>0.95, n=30, 4.22%)***	
Serum Fe (µmol/l)	16.43 (0.32)	0.74, 63.94	18.40[#] (2.48)	4.73, 73.42
Serum TIBC (µmol/l)	63.94 (0.50)	28.77, 197.22	62.17 (2.53)	42.57, 102.42
Transferrin Saturation (%)	25.71 (0.53)	1.34, 102.22	29.61 (3.26)	7.41, 79.95
Serum Ferritin (µg/l)	100.80 (11.48)	1.00, 2877.17	217.28[*] (111.57)	30.00, 2427.20
Hb (g/dl)	13.29 (0.08)	4.70, 22.90	13.60 (0.51)	9.80, 21.10
		WOMEN		
	(WHR≤0.80, n=697, 73.21%)		***(WHR>0.80, n=255, 26.79%)***	
Serum Fe (µmol/l)	13.26 (0.29)	1.18, 56.30	12.78 (0.45)	0.26, 59.85
Serum TIBC (µmol/l)	69.34 (0.52)	29.13, 171.12	65.21[*#] (0.80)	36.93, 157.26
Transferrin Saturation (%)	19.05 (0.45)	0.74, 85.46	19.54 (0.77)	0.35, 97.03
Serum Ferritin (µg/l)	32.29 (5.44)	0.28, 2678.53	67.86[*#] (11.44)	0.50, 1951.17
Hb (g/dl)	11.90 (0.07)	4.70, 20.50	12.27[*#] (0.16)	5.70, 31.10

*Significant difference between normal and high WHR before adjusting for age, BMI and smoking (p<0.05).
Significant difference between normal and high WHR after adjusting for age, BMI and smoking (p<0.05).
Hb: haemoglobin, SD: standard deviation TIBC: total iron binding capacity, WHR: waist to hip ratio.

Table 4: Comparison of anthropometric indices according to serum ferritin concentration

	Low ferritin group (ferritin<12µg/l)		Normal ferritin group (ferritin 12-150µg/l)		High ferritin group (ferritin>150µg/l)	
Variable	***Mean (SE)***	***min, max***	***Mean (SE)***	***min, max***	***Mean (SE)***	***min, max***
MEN	***(n=23, 3.23%)***		***(n=418, 58.79%)***		***(n=270, 33.97%))***	
BMI (kg/m^2)	20.64 (0.73)	16.42, 31.50	20.29 (0.15)	14.71, 36.91	21.64$^{®}$ (0.31)	13.76, 65.39
WC (cm)	69.08 (1.30)	60.20, 83.40	72.00$^{\#}$ (0.40)	53.60, 114.50	77.52$^{+®\$}$ (0.61)	57.00, 126.20
WHR	0.80 (0.01)	0.71, 0.92	0.82* (0.00)	0.59, 1.05	0.86$^{+®}$ (0.00)	0.71, 1.52
Body fat (%)	19.29 (1.48)	12.53, 36.71	19.97 (0.29)	9.22, 37.15	22.29$^{+®}$ (0.52)	9.17, 39.30
TSF (mm)	7.75 (0.85)	4.90, 18.20	7.09 (0.24)	2.00, 37.30	7.77 (0.32)	1.80, 37.10
SSF (mm)	8.69 (1.01)	5.50, 19.90	9.27 (0.27)	3.40, 40.00	10.32 (0.40)	3.00, 38.10
WOMEN	*(n=155, 16.28%)*		*(n=662, 69.54%)*		*(n=135, 14.18%)*	
BMI (kg/m^2)	24.50 (0.50)	15.45, 45.96	26.40* (0.26)	15.39, 53.64	26.74 (0.64)	14.60, 48.76
WC (cm)	72.47 (0.89)	46.50, 107.50	77.50* (0.50)	54.50, 130.20	81.83$^{+®\$@}$ (1.15)	55.00, 119.60
WHR	0.73 (0.00)	0.57, 0.96	0.75* (0.00)	0.47, 1.00	0.80$^{+®\$@}$ (0.00)	0.64, 0.99
Body fat (%)	45.01 (0.87)	28.17, 68.40	47.81 (0.57)	13.30, 83.79	47.33 (1.42)	28.79, 70.55
TSF (mm)	17.86 (0.80)	4.40, 51.70	19.10 (0.38)	4.40, 50.20	19.12 (1.06)	3.50, 52.10
SSF (mm)	17.15 (0.92)	5.40, 52.90	18.75 (0.51)	4.60, 56.60	20.30 (1.23)	4.40, 60.00

*Significant difference between negative and normal iron balance before adjusting for age, BMI and smoking at p<0.05.

+ significant difference between negative and positive iron balance before adjusting for age, BMI and smoking at p<0.05.

®significant difference between normal and positive before adjusting for age, BMI and smoking at *p*<0.05.

significant difference between negative and normal iron balance after adjusting for age, BMI and smoking at p<0.05.

$ significant difference between negative and positive iron balance group after adjusting for age, BMI and smoking at p<0.05.

@ significant difference between normal and positive iron balance after adjusting for age, BMI and smoking at *p*<0.05.

BMI: body mass index, SD: standard deviation, SSF: Subscapular skinfold, TSF: triceps skinfold, WC: waist circumference, WHR: waist to hip ratio.

Table 5: Comparison of iron indices according to BMI categories

	Underweight (BMI<18.50kg/m^2)		**Normal weight** (BMI 18.50-24.90kg/m^2)		**Overweight** (BMI 25.00-29.90kg/m$^{2)}$)		**Obese** (BMI≥30.00kg/m^2)	
Variable	*Mean (SE)*	*min, max*	*Mean (SE)*	*min, max*	*Mean (SE)*	*min, max*	*Mean (SE)*	*min, max*
MEN	*(n=165, 23.21%)*		*(n=451, 63.43%)*		*(n=69, 9.70%)*		*(n=26, 3.66%)*	
Serum Fe (µmol/l)	16.05 (0.77)	0.74, 73.42	16.75 (0.40)	1.02, 63.94	16.46 (0.91)	3.25, 38.0	15.67 (1.40)	3.62, 36.57
Serum TIBC (µmol/l)	61.62 (0.94)	28.77, 97.77	64.49$^{*\#}$ (0.65)	36.54, 197.22	64.84$^{\$}$ (1.55)	41.64, 109.50	64.89 (15.67)	46.26, 84.48
Transferrin Saturation (%)	26.05 (1.28)	2.20, 97.36	25.97 (0.65)	1.34, 102.22	25.38 (1.37)	5.02, 63.14)	24.15 (2.12)	4.57, 54.91
Serum Ferritin (µg/l)	92.26 (20.90)	2.50, 2387.60	101.59 (16.95)	1.00, 2877.17	134.52 (16.81)	1.54, 807.22	174.02 (46.58)	5.86, 956.88
Hb (g/dl)	13.43 (0.18)	7.40, 22.90	13.22 (0.09)	4.70, 21.10	13.25 (0.21)	8.90, 17.70	13.90 (0.47)	11.00, 21.10
WOMEN	*(n=56, 5.88%)*		*(n=380, 3.99%)*		*(n=242, 25.42%)*		*(n=271, 28.47%)*	
Serum Fe (µmol/l)	11.75 (1.36)	1.85, 51.55	13.12 (0.39)	1.18, 40.96	14.33 (0.46)	2.30, 56.30	12.42$^{\mu\,♀\sigma}$ (0.42)	0.26, 59.85
Serum TIBC (µmol/l)	68.95 (2.83)	29.13, 171.12	68.49 (0.69)	32.97, 120.96	68.46 (0.81)	39.33, 122.82	67.51 (0.82)	36.93, 157.26
Transferrin Saturation (%)	17.04 (2.19)	1.30, 83.96	19.03 (0.60)	0.74, 64.79	20.92 (0.77)	2.35, 85.46	18.35$^{\mu\sigma}$ (0.69)	0.35, 97.03
Serum Ferritin (µg/l)	46.69 (39.16)	1.24, 1951.17	31.60$^{*\#}$ (6.45)	0.28, 1470.70	43.24$^{+\$}$ (12.66)	0.83, 2678.53	47.16$^{®@}$ (6.60)	0.50, 775.58
Hb (g/dl)	12.43 (0.46)	6.67, 31.10	12.05* (0.98)	6.10, 20.90	12.00^{+} (0.12)	4.70, 19.80	11.87$^{®@}$ (0.12)	5.70, 19.60

*Significant difference between underweight and normal weight before adjusting for age and smoking (p<0.05).

+ significant difference between underweight and pre-obese before adjusting for age and smoking (p,0.05).

® significant difference between underweight and obese before adjusting for age and smoking (p<0.05).

♀ significant difference between normal weight and obese before adjusting for age and smoking (p<0.05).

µ significant difference between pre-obese and obese before adjusting for age and smoking (*p*<0.05).

significant difference between underweight and normal weight after adjusting for age and smoking (p<0.05).

$ significant difference between underweight and pre-obese after adjusting for age and smoking (p<0.05).

@ significant difference between underweight and obese after adjusting for age and smoking (p<0.05).

σ significant difference between pre-obese and obese after adjusting for age and smoking (*p*<0.05).

BMI: body mass index, Hb: haemoglobin, SD: standard deviation, TIBC: total iron binding capacity.

Discussion

This is the first study to our knowledge that assessed the relationship between iron indices and anthropometric CVD markers in an African population. This study employed anthropometric measures which have been reported to be associated with CVD risk. These factors include WC, WHR, triceps and subscapular skinfolds (TSF and SSF), body fat and BMI.[15, 24] The results showed that both men and women in the high WHR category had higher ferritin concentration than those in the normal WHR category. A positive association between ferritin and BMI, WC, WHR, body fat and SSF was demonstrated for both men and women; though this disappeared after adjusting for age, BMI and smoking. WC and WHR increased with increasing ferritin concentration in both men and women. Serum iron decreased with increasing BMI in women only.

The results obtained in this study are similar to the work of Gillum[25] who reported that serum ferritin concentration associated positively with WHR and BMI in Mexican American men.

Norwegian men aged 20-49 were also reported to have a mean serum ferritin concentration that related positively to their mean BMI.[26] Additionally, a study conducted on diabetic patients reported that serum ferritin correlated positively with visceral fat area and subcutaneous fat area. This study excluded patients with high C-reactive protein concentration in order to exclude elevation of ferritin that may be caused by inflammation. The authors, therefore, concluded that ferritin may be a useful indicator of systemic fat.[27]

Conversely, Eftekhari *et al*.[28] reported a negative correlation between serum ferritin and BMI in Iranian adolescent girls. This is in contrast to the results of this study that showed a positive correlation between serum ferritin and BMI. The authors attributed the result to the age group of the study population. Adolescence, being a peculiar stage of growth, is characterised by a growth spurt and increased iron requirement. The onset of menstruation in girls makes their iron need greater than that of boys.[29] The present study was composed of men and women across all age groups which makes it less comparable to the Iranian study. Studies have also reported appreciable differences in the prevalence

of obesity among different ethnic groups.[30, 31] Chambers *et al.*[32] reported an inverse relationship between body fat distribution and serum iron in Hispanic women but not in White or African American or Asian women.

In the present study, a lower serum iron concentration in the obese than the normal weight and the pre-obese women was observed. Serum iron in the obese remained significantly lower than in the pre-obese women after adjusting for age and smoking. This is congruent to the findings by Tussing-Humphreys[33] who reported that the prevalence of iron deficiency was higher in overweight girls. Moreover, serum iron was reported to decrease as BMI increased in adolescent Iranian girls.[27] The National Health and Nutrition Examination Survey-I[34] reported that higher BMI was significantly associated with lower serum iron in women. Serum iron is, however, not a sensitive marker of iron status due to diurnal changes.[35]

An understanding of the link between fat deposition and ferritin secretion may offer an explanation to some of these observations. During fat deposition lipid biosynthesis increases which might lead to an increase in iron induced lipid oxidation as result of reactivity of intracellular iron with lipids. There is a probability that ferritin is elevated to act as an iron cytoprotective agent.[36] Thus, increased ferritin concentration may be an adaptive mechanism to reduce iron induced oxidative stress which could explain the positive correlation between ferritin and anthropometric CVD risk factors. Ferritin is an acute phase reactant which increases during inflammation. Since obesity is considered an inflammatory state,[37] it could serve as an additional explanation for the positive association between ferritin and anthropometric indicators. A deficient iron store owing to greater iron requirements in obese adults because of their larger blood volume[38] has also been proposed to be the mechanism involved in the iron deficiency-obesity association. Functional iron deficiency can occur during inflammation (even when iron stores are optimal) as a result of impairment of normal physiological systems for transport of iron to target tissue.[10]

It is not clear which one precedes the other, obesity or iron deficiency. Iron takes part in diverse physiological functions such as transport of oxygen by haemoglobin and

myoglobin, energy metabolism by the haeme-containing proteins of the mitochondria electron transport apparatus and the conversion of ribose to deoxyribose nucleic acids by the iron containing ribonucleotide reductase which is required for the propagation of genetic information.[10] Given the critical dependence of body tissues on iron, it is possible that its deficiency can result in accumulation of fat in body tissues. Impaired fat oxidation has been reported to be a risk factor for excess weight gain in several populations known to be susceptible to obesity.[39, 40] Fat that is not oxidised must be stored which can result in increased fat deposition with time. Iron deficiency has also been linked to problems with work and exercise capacity among adults[41] which may eventually lead to a sedentary lifestyle and weight gain.

Lipid deposition allows efficient storage of maximal calories in adipose tissue located beneath the skin (subcutaneous fat), around internal organs (visceral fat), and in the yellow bone marrow.[42] Subcutaneous fat tissue stores about 80% of all body fat and excess fat is stored in other storage tissues such as the intra-abdominal tissue when the subcutaneous tissue reaches a threshold level.[43] Thus, storage of excess fat in the central region may be a signal for abnormal fat storage which has adverse health implications. It has been found that body fat distribution especially, abdominal fat is more important than total body fat in the etiology of CVDs.[44] William *et al.*[45] identified measurements at or above the waist to be most associated with disease risk for both genders. However, in the present study 79% of men and 60% of women were within optimal cut-off point.[24]

There is speculation that hepcidin, a peptide hormone involved in the regulation of intracellular iron, may be involved in the association of obesity and iron deficiency.[46] Hepcidin is an acute phase reactant which is stimulated in inflammation, including obesity.[47] Moreover, recent discoveries indicated that adipocytes are not just passive organs for fat storage, instead, they are also endocrine organs which play regulatory roles in whole-body physiology.[48] Increased secretion of hepcidin has been reported to inhibit the release of non-haeme iron from macrophages.[49] It is possible that increased expression of hepcidin in obese individuals interfered with iron absorption thereby

resulting to iron deficiency. Unfortunately, hepcidin was not measured in the present study as it might have helped in clarifying the situation.

Gender has been reported to be one of the factors that influence serum ferritin concentration.[50] A significant difference in serum ferritin concentration was demonstrated between men and women in this study. Leggett *et al.*[51] found that the pattern of ferritin increase varied in men and women. Ferritin concentration in women, though increasing, remained low until after menopause while that of men continued to increase reaching a peak in their forth decade when iron stores are expected to be stable following the growth period. This suggests that the observed difference between men and women may be as a result of physiological differences (i.e. menstruation and hormonal secretion) which affect iron storage. Moreover, the pattern of fat distribution in men has been reported to differ from that in women. Women usually show greater lower body fat distribution (gynoid) while men show more upper body fat distribution (android).[42] The present study supports these findings as men had significantly higher WC than women.

Conclusively, this study illustrates that a relationship exists between some measures of iron status and certain anthropometric CVD risk markers, particularly, ferritin and WC or WHR. This implies that individuals who fell within the positive iron balance group, the at risk group for iron overload, may additionally be at a greater risk of developing CVDs in this particular population. However, due to the nature of the design of this study, causality cannot be established.

Implication for health and research

Iron deficiency is the most common micronutrient deficiency in the world[2] and it has been a priority for most countries when addressing issues on health. South Africa is among countries with moderate iron deficiency.[52] The Department of Health in South Africa is using an adapted form of UNICEF's conceptual framework to address malnutrition which means action is being taken at all levels of causation.[53] Strategies employed include provision of iron supplements to pregnant and postpartum women, fortification of several food vehicles and dietary diversification (using the food based dietary guidelines). It has been reported that some indicators are improving while others

are worsening over the years. In addition, reports have shown that nutrition status varies considerably among the nine provinces and possibly within each province.[54, 55]

On the hand, iron overload has increasingly been recognized as a public health issue in African populations, especially Southern Africa where the consumption of traditional beer has been identified as a major contributor.[10] However, the results of the present study suggest that increased abdominal obesity may also be another major contributor to increased iron stores in this population. Therefore, it may be pertinent to scale up interventions to reduce obesity in this population alongside on-going iron intervention programmes. Furthermore, consideration should be given to the use of different and locally relevant strategies for different provinces or probably different municipalities within a province. To enhance further the effort put into improving health and nutrition, different and relevant strategies addressing the various public health concerns of our population will be helpful in arriving at the desired nutrition and health goals.

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Conflict of interest declaration

The authors declare that there are no conflicts of interests.

Authorship responsibilities

(1) design and concept of the study (O.R.A., H.H.V. and P.T.P.)

(2) acquisition of data (H.H.V. and H.S.K.)

(3) data analysis and interpretation (P.T.P., O.R.A. and R.L.M.)

(4) manuscript draft (O.R.A., P.T.P, R.L.M., H.S.K., H.H.V.)

(5) critical revision of the manuscript for important intellectual content (O.R.A., P.T.P, R.L.M., H.S.K., H.H.V.)

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CHAPTER 5

IRON STATUS AND CARDIOVASCULAR DISEASE RISK IN BLACK SOUTH AFRICAN WOMEN: THE PURE STUDY

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CHAPTER 5: IRON STATUS AND CARDIOVASCULAR DISEASE RISK IN BLACK SOUTH AFRICAN WOMEN: THE PURE STUDY

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Running title: Role of iron in health

Abstract

Objective: To examine the associations between measures of iron status and cardiovascular disease (CVD) risk factors in black South African women.

Design: The study was cross-sectional in design. Demographic information and health history were obtained during individual interviews using validated questionnaires. Anthropometric indices, iron indices, blood pressure, blood glucose and lipid profiles were measured using standard procedures. Iron status was assessed using serum concentrations of ferritin, transferrin receptor and transferrin receptor to ferritin ratio.

Setting: North West Province of South Africa.
Subjects: 1262 apparently healthy black South African women (≥35 years).

***Results*:** Associations between iron status parameters and CVD risk factors were generally weak ($r<0.3$, $P<0.01$) and were not retained when adjusting for age, BMI, smoking, alcohol consumption and C-reactive protein. Waist circumference (WC) and waist to hip ratio (WHR) were higher in the fourth quartile of serum ferritin than the third, also, in the third quartile compared to second quartile ($P<0.05$). 31% and 52% of the women had excess abdominal obesity as indicated by WC and WHR respectively. The mean (95% confidence interval) serum transferrin receptor concentration {9.09µg/l(8.77, 9.44)} was high indicating risk of iron deficiency. The mean (95% confidence interval) concentrations of lipids {TC=4.78(4.64, 4.93), HDL-C=1.45(1.39, 1.52), LDL-C=1.65(1.53, 1.78), TG=1.12(1.07, 1.18)mmol/l} were within recommended reference ranges.

***Conclusions*:** No significant association could be demonstrated between iron status parameters and established CVD risk factors. However, excessive abdominal adiposity indicated by high WC and WHR contributes significantly to increased serum ferritin concentration in these African women.

Keywords: Iron stores, cardiovascular disease, African women, PURE study.

Introduction

The role iron plays in the pathogenesis of cardiovascular diseases (CVD) has increasingly been of interest in the field of public health. Excess iron has been proposed to catalyze the conversion of poorly reactive free radicals into highly reactive ones, a process known to predispose to or promote the incidence of CVDs[1]. At the moment, a number of iron supplementation or fortification programmes are on-going in many developed and developing nations. Reports have[2] shown that the percentage of iron replete persons is on the increase in developed nations; reports also exist of iron overload in sub-groups of people in the less developed nations[3]. Moreover, there are speculations that iron overload may become a world epidemic[4].

Most of the cross-sectional studies that examined the link between iron stores and increased CVD risk were conducted in men[5-11]. In a study in women with poor iron status, a strong association was found between iron stores and CVD risk factors[2]. This raises concern about the situation among African women. While undernutrition remains a problem, obesity and associated non-communicable diseases which were thought to be a problem of industrialized countries, are now increasingly becoming prevalent in the less industrialized countries, especially those undergoing transition[12-13]. It has been proposed that iron overload may not be a problem in a healthy population but in persons with increased oxidative stress and obesity, iron overload may pose a greater risk[14-15].

However, studies that have examined the role of iron status in CVD have been inconsistent possibly because of the use of different measures of iron status [5-6,10-11]. Iron status measured as dietary iron, serum iron, serum ferritin and transferrin saturation have limitations because they are influenced by short-term effects such as inflammation, iron intake, blood loss and diurnal variations[16-17]. Soluble transferrin receptor (TfR) concentration has been suggested as an improved measure of iron status[18]. Furthermore, because serum ferritin reflects the storage iron compartment and serum TfR reflects the functional iron compartment, the TfR to ferritin ratio has been suggested as a better estimate of body iron compared to either of the two measured independently[19]. Tuomainen *et al*[8] reported that this ratio is virtually independent of inflammation. In

view of this, the present study is aimed at using concentrations of serum ferritin, TfR and ratio of TfR/ferritin to examine the association between iron status and CVD risk factors.

Methods

Study design, subject selection and organizational procedures

This cross-sectional study was part of the North West Province, South Africa (NWPSA) leg of the 12-year Prospective Urban and Rural Epidemiological (PURE) study. The PURE study investigates the effect of health transition on chronic diseases of lifestyle in urban and rural subjects. All the baseline data used for the present study were collected during 2005 in the North West Province of South Africa. Migration stability was the main selection criterion within the chosen rural and urban communities.

The rural community (A) was identified 450 km west of Potchefstroom on the highway to Botswana. A deep rural community (B), 35 km east from A and only accessible with a gravel road, was also included. Community C was selected from the established part of the Township next to Potchefstroom and D from the informal settlements surrounding community C. A household census regarding number of people, their ages and health profile was randomly done in 6000 houses (1500 in each community).

Every head of household gave signed consent to fill out the questionnaire. If a person refused or was not at home, the next house was taken and a non-complier questionnaire was filled out. From the data obtained a paper selection of possible subjects with no reported chronic diseases of lifestyle, tuberculosis or diagnosed human immunodeficiency virus (HIV) was made. A total of 2010 (35 years and older) apparently healthy African volunteers were recruited. The sample for the study described in this article consisted of only the 1262 women participants. Participants were fasted (10-12 hours) for the baseline blood sampling and other measurements. Trained field workers assisted in providing information to the participants in their language of choice. Participants received feedback regarding their blood pressure, fasting glucose

concentrations and HIV status, and were referred to the clinics or hospital where necessary. Travelling expenses of participants were covered.

Ethical approval was obtained from the Ethics Committee of the North–West University, Potchefstroom, South Africa **(Ethics number: 04M10)** and signed informed consent forms were received from all participants. Permission to conduct the study was obtained from the North West Department of Health, tribal chiefs, community leaders, employers and mayors.

Questionnaires

Structured, validated demographic, socio-economic and lifestyle questionnaires were administered by trained field workers during home visits in the language preferred by the participants. The questionnaires used were adapted and used by all countries participating in the PURE study.

Anthropometric measurements

Anthropometric measurements were done by Biokinetists. Height was measured to the nearest 0.5cm with a stadiometer (Invicta, IP 1465, UK) and weight was determined on a portable electronic scale to the nearest 0.01kg (Precision Health Scale, A & D Company, Japan). All the measurements were done according to the guidelines adopted at the National Institute of Health sponsored Arlie Conference[20]. Body circumferences of participants were measured in light underwear with calibrated instruments (Holtain® unstretchable metal tape; John Bull® calipers). Body mass index (BMI) was calculated by dividing weight in kilograms by height in meter squared. Waist to hip ratio (WHR) was calculated by dividing waist circumference (WC) by hip circumference.

HIV status

Everyone who signed an informed consent form was tested for HIV infection, but was given the choice of knowing her status. Whole blood was used for the determination of HIV status making use of the First Response (PMC Medical, India) rapid HIV card test. If tested positive, the test was repeated with the Pareeshak (BHAT Bio-tech India) card test. A pre-test counselling in groups of 10 persons before the blood sample was taken

and individual post-test counselling was done according to the protocol of the National Department of Health of South Africa.

Blood, serum and plasma samples

A disposable needle was used to draw blood from the ante-cubital vein in the right arm of participants. The blood collection tubes were appropriately filled in order to ensure optimal blood:anticoagulant ratios. Each tube was inverted gently five times for thorough mixing. Samples were place in ice boxes after proper labeling. A new sterile transfer pipette was used for aliquotting blood samples for analysis. Serum was prepared by allowing blood to clot at room temperature for 30minutes; it was then centrifuged at 2000g for 15minutes at 10^0C. Blood was centrifuged within two hours of collection. All blood and serum samples were stored at -70^0C in the laboratory. Plasma samples were collected in ethylenediamine tetra acetic acid (EDTA) tubes, centrifuged at 2000g for 15minutes at 4^0C and transferred to cryo tubes for storage at -70^0C.

Biochemical and physiological analyses

Systolic blood pressure (BP) and diastolic BP were obtained with the validated OMRON HEM-757 device (Omron HEM-757). After a 10 minute rest period BP measurements were performed twice (5 minutes apart) on the right arm (brachial artery), while the participants were seated upright and relaxed with the right arm supported at heart level. Plasma glucose was measured using Vitros DT6011 Chemistry Analyser, an Ortho-Clinical Diagnostics tool (Rochester, New York, USA). Quantitative determination of total cholesterol (TC), high density lipoprotein cholesterol (HDL-C), triglyceride (TG) and high sensitivity C-reactive protein (CRP) concentration in serum was done by Sequential Multiple Analyser Computer (SMAC) using the Konelab^{20iTM} auto analyser (Thermo Fisher Scientific Oy, Vantaa, Finland). Low density lipoprotein cholesterol (LDL-C) was calculated using the Friedewald formula[21]. Serum ferritin and TFR concentration were determined quantitatively using an enzyme immunoassay procedure (Ramco Laboratories, Inc, Stafford Texas). The coefficient of variance (CV) for all assays was <10%.

Statistical analyses

Data were analysed using version 17 of the Statistical Package for Social Sciences (SPSS Inc Chicago III). Data were not normally distributed and were log transformed to improve normality. Data were presented as mean estimates, medians and confidence intervals (CI). Pearson correlation was used to assess the associations between iron status and CVD risk factors. In this study, the following CVD risk factors were examined: BMI, WC, WHR, TC, LDL-C, HDL-C, TG, systolic and diastolic BP, plasma glucose and CRP. Age, BMI, smoking, alcohol consumption and serum CRP were treated as valid confounders. Association was further assessed by a partial correlation adjusting for these confounders. To further assess the relationship between iron status and CVD risk factors, participants were grouped and analysed in quartiles of ferritin, TfR and TfR/ferritin ratio. Multivariate analysis was used to assess significant differences between different quartiles before and after adjusting for valid confounders. A $P<0.05$ was considered statistically significant. HIV status did not confound or modify the associations between iron indices and CVD risk factors; hence, it was not treated as a valid confounder.

Results

Table 1 outlines selected characteristics of women participants in the PURE study. The mean values of variables were within recommended reference ranges[22-26] except for TfR and WHR which were higher, indicating risk for iron deficiency and abdominal obesity respectively in this population.

Table 2 illustrates the proportion (%) of women participants according standard cut-off points for variables and, proportion of self-reported smokers and alcohol consumers. 50% of the women participants were self reported smokers while 33% were alcohol consumers. In addition, 53% were either overweight or obese while 31% had excess WC and 52% had excess WHR. As for iron indices, 56% had high TfR concentration while 33% had excess iron stores as defined by ferritin concentration. Those at risk as defined by biological health markers were 48% for TC, 43% for HDL-C, 27% for LDL-C, 23%

for TG, 27% for glucose and 25% for CRP. Diastolic and systolic BP were high in 57% and 49% of the women respectively.

The correlations between iron indices and CVD risk factors are shown in Table 3. Ferritin correlated positively with TC, HDL-C, LDL-C, TG, diastolic BP, systolic BP, CRP and alcohol consumption before adjusting for valid confounders (age, BMI, smoking, alcohol consumption and CRP) but the correlations were not retained after adjustment. There was a negative correlation between TfR and LDL-C, TG and alcohol consumption while and a positive correlation existed between TfR and WHR. All of these disappeared after adjusting for valid confounders. TfR/ferritin ratio correlated negatively with TC, LDL-C, TG, diastolic BP, systolic BP, CRP and alcohol consumption before adjusting for valid confounders but was not retained after adjustment. The correlations observed between iron indices and CVD risk factors were generally weak ($r < 0.3$).

Table 1 Selected characteristics of women participants in the PURE study (N=1262)

Variable	Mean	Median (95% CI)
Age (years	49.16	47.00 (48.57, 49.76)
Smoking status (cigarette /day)	2.61	4.00 (2.38, 2.85)
Alcohol consumption (g/day)	7.74	13.93(6.59, 8.89)
BMI (kg/m^2)	23.87	25.83 (23.22, 24.54)
WC (cm]	76.38	80.50 (75.16, 77.80)
WHR	0.80	0.79 (0.79, 1.81)
TfR (µg/l)	9.09	8.95 (8.77, 9.44)
Ferritin (µg/l)	94.84	94.41(81.84, 110-15)
TfR/Ferritin ratio	0.25	0.09 (0.19, 0.30)
TC (mmol/l)	4.78	4.93 (4.64, 4.93)
HDL-C (mmol/l)	1.45	1.33 (1.39, 1.52)
TG (mmol/l)	1.12	1.14(1.07,1.18)
LDL-C	1.65	1.80 (1.53, 1.78)
Diastolic BP (mmHg)	85.90	87.49 (84.52, 87.49)
Systolic BP (mmHg)	127.93	129.50 (125.60, 130.31)
Glucose (mmol/l)	5.34	5.45 (5.21, 5.47)
CRP [mg/l)	2.81	3.69 (5.21, 5.47)

N, number; CI, confidence interval; BMI, body mass index; WC, waist circumference; WHR, waist to hip ratio; TfR, transferrin receptor; TC, total cholesterol; HDL-C, high density lipoprotein cholesterol; TG, triglyceride; LDL-C, low density lipoprotein cholesterol; BP, blood pressure; CRP, C-reactive protein.

Table 2 Proportion of women participants in the PURE study according to standard cut-off points, smoking and alcohol consumption status

Variable	Cut off points	N (%)
Smokers Non smokers		437 (50%) 430 (50%)
Alcohol consumers Non alcohol consumers		391 (33%) 787 (67%)
BMI	*18-25kg/m2* *<18kg/m2* *>25kg/m2*	137 (12%) 410 (35%) 641 (53%)
WC	*<88cm* *≥88cm*	808 (69%) 368 (31%)
WHR	*<0.80* *≥0.80*	534 (48%) 578 (52%)
TfR	*<8.5µg/l* *≥8.5µg/l*	462 (44%) 597 (56%)
Ferritin	*<12µg/l* *12-150µg/l* *>150µg/l)*	94 (9%) 610 (58%) 355 (33%)
TC	*≤5mmol/l* *>5mmol/l*	612 (52%) 564 (48%)
HDL-C	*≥1.3mmol/l* *<1.3mmol/l*	666 (57%) 506 (43%)
TG	*≤1.7mmol/l* *>1.7mmol/l*	904 (77%) 265 (23%)
LDL-C	≤3.0mmol/l >3.0mmol/l	754 (73%) 281 (27%)
Diastolic BP	*≤85mmHG* *>85mmHG*	501(43%) 670(57%)
Systolic BP	*≤130mmHg* *>130mmHg*	593 (51%) 578 (49%)
Glucose	*≤6.1mmol/l* *>6.1mmol/l*	851 (73%) 316 (27%)
CRP	≤10mg >10mg/l /l	874 (75%) 296(25%)

N, number; BMI, body mass index; WC, waist circumference; WHR, waist to hip ratio; TfR, transferrin receptor; TC, total cholesterol; HDL-C, high density lipoprotein cholesterol; TG, triglyceride; LDL-C, low density lipoprotein cholesterol; BP, blood pressure; CRP, C-reactive protein.

Table 3 Correlations between iron indices and CVD risk factors of participants

	Ferritin (µg/L)		TfR		TfR-Ferritin ratio	
	r	**r_p**	**r**	**r_p**	**r**	**r_p**
Ferritin (µg/l)	--	--	-.351**	-.079	-.776**	-.705**
TfR (µg/l)	-.351**	-.079	--	--	.511**	.410**
TfR/Ferritin ratio	-.776**	-.705**	.511**	.410**	--	--
BMI (kg/m^2)	-.059	--	.009	---	-.025	--
WC (cm)	.029	-.022	.003	-.019	-.058	.056
WHR	.049	.071	.124**	.014	.017	-.067
TC (mmol/l)	.065*	-.037	-.031	-.091	-.072*	-.017
HDL-C (mmol/l)	.065*	-.046	-.037	.102	-.025	.028
TG (mmol/l)	.187**	.034	-.075*	-.079	-.120**	-.042
LDL-C (mmol/l)	.140**	.012	-.063*	-.157	-.109**	-.053
Diastolic BP (mmHg)	.182**	.068	-.035	-.055	-.139**	-.084
Systolic BP (mmHg)	.169**	.011	-.048	-.097	-.116**	-.086
Glucose (mmol/l)	.019	-.007	.051	.048	.018	-.013
CRP (mg/l)	.158**	--	.029	--	-.133**	--
Smoking (cigarette/day)	.008	--	-.078	--	-.008	--
Alcohol consumption (g/day)	.207**	--	-.109*	--	-.147**	--

r, Pearson correlation; r_p, Partial correlation after adjusting for age, BMI, smoking, alcohol consumption and CRP
BMI, body mass index; WC, waist circumference; WHR, waist to hip ratio; TfR, transferrin receptor; TC, total cholesterol; HDL-C, high density lipoprotein cholesterol; TG, triglyceride; LDL-C, low density lipoprotein cholesterol; BP, blood pressure; CRP, C-reactive protein.
** Correlation coefficient is significant at the 0.01 level (2-tailed).
* Correlation coefficient is significant at the 0.05 level (2-tailed).

Table 4 Mean (95% CI) and median values of CVD risk factors according to ferritin quartiles

Variables	Q_1 *Ferritin<38.2µg/l* *N=277*		Q_2 *Ferritin 38.2-100.3µg/l* *N=272*		Q_3 *Ferritin 100.4-208.2µg/l* *N=251*		Q_4 *Ferritin>208.2µg/l* *N=260*	
	Mean	*Median (CI)*	*Mean*	*Median (CI)*	*Mean*	*Median (CI)*	*Mean*	*Median (CI)*
BMI (kg/m^2)	25.52	25.93 (24.72, 26.36)	26.06	25.75 (25.24, 26.92)	27.10[a]	27.36 (26.24, 28.05)	24.09[abc]	23.52 (23.33, 24.89)
WC (cm)	78.70	78.00 (77.09, 80.35)	79.43	80.40 (77.80, 81.09)	82.03[ab *#]	83.41 (80-35, 83.75)	79.06c[@]	78.38 (77.45, 80.72)
WHR	0.78	0.77 (0.77, 0.79)	0.79	0.78 (77.80, 81.09)	0.80[a#]	0.80 (0.79, 0.82)	0.82[abc@]	0.82 (0.81, 0.83)
TC (mmol/l)	4.86	4.82 (4.69, 5.04)	4.88	4.76 (4.73, 5.06)	5.05	4.96 (4.89, 5.24)	5.15[ab]	5.13 (4.98, 5.33)
HDL-C (mmol/l)	1.39	1.34 (1.31, 1.46)	1.41	1.34 (1.33, 1.48)	1.40	1.32 (1.32, 1.48)	1.51[a]	1.48 (1.43, 1.58)
TG (mmol/l)	1.05	0.98 (0.99, 1.12)	1.13	1.09 (1.07, 1.21)	1.28[ab]	1.23 (1.20, 1.36)	1.33[ab]	1.28 (1.25, 1.41)
LDL-C (mmol/l)	1.63	0.59 (1.49, 1.79)	1.76	0.71(1.61, 1.93)	2.06[ab]	1.12 (1.87, 2.26)	2.13[ab]	1.05 (1.95, 2.34)
D-BP (mmHg)	83.17	82.50 (81.47, 84.72)	86.89[a]	86.49 (85.11, 88.71)	88.10[a]	88.49 (86.29, 89.94)	90.36[ab]	90.74 (88.51, 92.04)
S-BP (mmHg)	125.60	123.99 (122.74, 128.23)	130.91	129.00 (127.93, 133.96)	33.35[a]	132.01(130.31, 136.45)	136.77[ab]	136.99 (133.96, 139.95)
CRP (mg/l)	2.36	2.77 (1.96, 2.85)	3.47[a]	3.99 (1.96, 4.18)	3.81[a]	4.26 (3.13; 4.62)	3.94[a]	4.23 (3.26, 4.76)

Superscript letters indicate significant difference (P<0.05).
[a] vs quartile 1, [b] vs quartile 2, [c] vs quartile 3 (unadjusted).
* vs quartile 1, [#] vs quartile 2, [@] vs quartile 3 (adjusted to age, body mass index ,smoking, alcohol consumption and CRP)

Table 5 Mean (95% CI) and median values of CVD risk factors according to TfR quartiles

Variables	*Q_1 TfR<7.8µg/l N=374*		*Q_2 TfR 7.8-9.7µg/l N=262*		*Q_3 TfR 9.8-11.9µg/l N=216*		*Q_4 TfR>11.9µg/l N=208*	
	Mean	*Median (CI)*	*Mean*	*Median (CI)*	*Mean*	*Median (CI)*	*Mean*	*Median (CI)*
BMI (kg/m^2)	25.29	24.60(24.60, 26.06)	25.82	26.10 (24.94, 26.66)	25.82	26.30 (24.88, 26.79)	25.94	25.06 (25.00, 26.91)
WC (cm)	79.43	78.80 (77.98, 80.90)	79.43	80.85 (77.80, 81.09)	79.98	80.50 (78.16, 81.84)	80.72	78.98 (78.88, 82.60)
WHR	0.80	0.79 (0.79, 0.81)	0.78[a]	0.78 (0.77, 0.79)	0.81[b]	0.80 (0.79, 0.81)	0.80[b]	0.80 (0.79, 0.81)
TC (mmol/l)	5.04	4.99 (4.89, 5.19)	4.88[*]	4.87 (4.72, 5.05)	5.06[*]	4.97 (4.88, 5.27)	4.94	4.86 (4.75, 5.12)
HDL-C (mmol/l)	1.42	1.38 (1.36, 1.48)	1.43	1.36 (1.34, 1.50)	1.46	1.44 (1.38, 1.55)	1.40	1.33 (1.32, 1.48)
TG (mmol/l)	1.27	1.18 (1.21, 1.34)	1.13[a]	1.06 (1.06, 1.20)	1.14[a]	1.14 (1.07, 1.22)	1.19	1.14 (1.11, 1.27)
LDL-C (mmol/l)	2.05	1.93 (1.89, 2.22)	1.71[a]	1.70 (1.56, 1.88)	1.84	1.84 (1.66, 2.04)	1.85	1.80 (1.67, 2.06)
D-BP (mmHg)	86.49	87.49 (85.11, 88.10)	87.49	87.49 (85.70, 89.33)	86.89	86.50 (84.91, 88.92)	87.09[*@]	86.50 (85.11, 89.12)
S-BP (mmHg)	131.52	129.99 (129.12, 134.27)	131.82	128.50 (128.82, 134.89)	131.82	131.49 (128.82, 135.20)	130.61	126.01 (127.35, 133.96)
Glucose(mmol/l)	5.40	5.41 (5.26, 5.54)	5.49	5.44 (5.33, 5.66)	5.38	5.47 (5.19, 5.57)	5.66	5.51 (5.47, 5.86)
CRP (mg/l)	3.13	3.15 (2.66, 3.68)	3.29	3.63 (2.72, 3.98)	3.15	4.12 (5.19, 5.57)	3.89	4.84 (3.15, 4.80)

TfR, transferrin receptor; Q, quartile; N, number; CI, confidence interval; BMI, body mass index; WC, waist circumference; WHR, waist to hip ratio; TfR, transferrin receptor; TC, total cholesterol; HDL-C, high density lipoprotein cholesterol; TG, triglyceride; LDL-C, low density lipoprotein cholesterol; D-BP, diastolic blood pressure; S-BP, systolic blood pressure; CRP,C- reactive protein .

Superscript letters indicate significant difference (P<0.05).

[a] vs quartile 1, [b] vs quartile 2, [c] vs quartile 3 (unadjusted).

* vs quartile 1, [#] vs quartile 2, [@] vs quartile 3 (adjusted to age, body mass index ,smoking, alcohol consumption and CRP).

Table 6 Mean (95% CI) and median values CVD risk factors according to TfR/ferritin ratio quartiles

Variables	Q_1 *TfR/Ferr<0.04* *N=256*		Q_2 *TfR/Ferr 0.04-0.09* *N=263*		Q_3 *TfR/Ferr 0.10-0.25* *N=281*		Q_4 *TfR/Ferr>0.25* *N=260*	
	Mean	*Median (CI)*	*Mean*	*Median (CI)*	*Mean*	*Median (CI) e*	*Mean*	*Median (CI)*
BMI (kg/m^2)	24.09	23.56 (23.33, 24.88)	26.97[a]	27.35 (26.06, 27.86)	26.18[a]	25.96 (25.35, 27.03)	25.52[ab]	25.64 (24.66, 26.36)
WC (cm)	79.06	78.51 (77.44, 80.72)	81.65[a*]	83.10 (79.98, 83.36)	79.61	78.14 (77.98, 81.28)	78.88[b]	78.14 (77.26, 80.72)
WHR	0.82	0.82 (0.81, 0.83)	0.80[a]	0.80 (0.79, 0.81)	0.79[a]	0.78 (0.78, 0.80)	0.78[a]	0.78 (0.77, 0.80)
TC (mmol/l)	5.15	5.06 (4.97, 5.33)	4.97	5.02 (4.80, 5.15)	4.94	4.82 (4.78, 5.11)	4.88[a]	4.85 (0.77, 0.80)
HDL-C (mmol/l)	1.51	1.53 (1.44, 1.60)	1.39[a]	1.33 (1.31, 1.46)	1.39[a]	1.34 (1.32, 1.47)	1.40[a]	1.34 (1.33, 1.48)
TG (mmol/l)	1.37	1.32 (1.32, 1.46)	1.22[a]	1.17 (1.31, 1.46)	1.14[a]	1.07 (1.07, 1.21)	1.06[ab]	1.05 (1.00, 1.13)
LDL-C (mmol/l)	2.18	2.16 (1.99, 2.40)	1.93	1.94 (1.75, 2.12)	1.7[9a]	1.65 (1.64, 1.97)	1.65[ab]	1.70 (1.50, 1.81)
D-BP (mmHg)	90.15	90.99 (88.30, 92.04)	88.10	87.50 (86.29, 89.94)	86.49[a]	86.26 (84.91, 88.30)	83.36[abc]	82.51 (81.65, 85.11)
S-BP (mmHg)	136.14	136.50 (133.35, 139.31)	133.65	131.25 (86.29, 89.94)	130.91[a]	30.02 (128.23, 133.96)	125.60[abc]	123.99 (122.74, 128.52)
Glucose (mmol/l)	5.53	5.68 (5.35, 5.70)	5.38	5.35 (5.22, 5.55)	5.52	5.45 (5.35, 5.68)	5.45	5.40 (5.28, 5.62)
CRP (mg/l)	3.87	3.74 (3.21, 4.68)	3.79	4.63 (3.13, 4.60)	3.40	3.77 (2.83, 4.09)	2.42[abc]	2.80 (2.00, 2.92)

TfR, transferrin receptor; Q, quartile; TfR/Ferr, transferrin receptor to ferritin ratio; N, number; CI, confidence interval; BMI, body mass index; WC, waist circumference; WHR, waist to hip ratio; TfR, transferrin receptor; TC, total cholesterol; HDL-C, high density lipoprotein cholesterol; TG, triglyceride; LDL-C, low density lipoprotein cholesterol; D-BP, diastolic blood pressure; S-BP, systolic blood pressure; CRP,C-reactive protein.

Superscript letters indicate significant difference (P<0.05).

[a] vs quartile 1, [b] vs quartile 2, [c] vs quartile 3 (unadjusted).

* vs quartile 1, [#] vs quartile 2, [@] vs quartile 3 (adjusted to age, body mass index ,smoking, alcohol consumption and CRP).

CVD risk factors are compared according to ferritin quartiles in Table 4. BMI and WC were higher in quartile three than quartile one before adjusting for valid confounders, After adjusting for the valid confounders, WC in the third quartile remained significantly higher than the second and fourth quartile. WHR increased across ferritin quartiles; however, after adjustment of valid confounders quartile three remained higher than quartile two and quartile four remained higher than quartile three. TC, TG, LDL-C, diastolic BP and systolic BP were significantly higher in the fourth quartile than the first two quartiles before adjusting for valid confounders but were not retained after adjustment. A significantly higher HDL-C value was observed in quartile four than quartile one before adjusting for confounders. No significant difference was observed for blood glucose across ferritin quartiles. CRP was higher in quartile two, three and four than quartile one.

Table 5 shows the comparison of CVD risk factors according to TfR quartiles. BMI, WC, HDL-C, systolic BP, glucose and CRP did not vary significantly among the quartiles. WHR was significantly higher in quartile three and four than quartile two before adjusting for age, BMI, smoking, alcohol consumption and CRP. In addition, WHR was higher in quartile two than quartile one before adjusting for valid confounders. A significantly higher diastolic-BP was observed in quartile four than quartile one and three after adjusting for valid confounders. LDL-C was only lower in quartile two than quartile one before adjustment while TC became significantly higher in quartile two and three than quartile one after adjusting for valid confounders.

Table 6 compares CVD risk factors among TfR/Ferritin ratio quartiles. A significantly higher BMI was observed in quartiles two, three and four than quartile one. There were consistently lower values of WHR, TC, HDL-C, TG, LDL-C diastolic BP, systolic BP and CRP in the fourth quartile than the first quartile before adjusting for valid confounders. Only WC in quartile two was significantly higher than quartile one after adjusting for valid confounders.

Discussion

This study examined the associations between iron status and CVD risk factors in black South African women. Ferritin was positively associated with TC, HDL-C, TG, LDL-C, diastolic and systolic BP, CRP and alcohol consumption. TfR/ferritin ratio was negatively associated with the same variables as ferritin except for HDL-C. TfR was negatively associated with TC, LDL-C and alcohol consumption, but positively with WHR. All these associations were not retained after adjusting for age, BMI, smoking, alcohol consumption and CRP. WC and WHR were significantly higher in the fourth quartile of ferritin than the third quartile after adjustment. In addition, WC and WHR in the third quartile of ferritin were higher than the second quartile after adjusting for valid confounders.

The hypothesis that excess iron is causally linked to CVD dates as far back as the 80's when Sullivan[27] suggested that increased incidence of CVDs in men and postmenopausal women was due to increased body iron. Free iron can cause the oxidation of LDL-C thereby making it highly cardiotoxic[28]. Oxidized LDL-C has the ability to cause direct injury to endothelial cells leading to generation of foam cells[28]. However, the results of this study do not support this hypothesis as the observed associations could be attributed to other factors such as age, BMI, smoking, alcohol consumption and infection influencing iron stores. Though very weak, WC, WHR, TC, TG, and diastolic BP were positively associated with ferritin when age, BMI, smoking and CRP only were adjusted for in the analysis. The inclusion of alcohol consumption to the list of valid confounders removed this association. This indicates that alcohol consumption is a strong predictor of iron status in this population as confirmed by the study conducted by Gopane[29] in the same population. Furthermore, this population seem to be at risk of iron deficiency as indicated by high mean serum TfR concentration {9.09µg/l (8.77; 9.44)} which could explain the lack of significant association between measures of iron status and the CVD risk factors in the present study. Additionally, 56% of the participants had a mean serum TfR concentration greater than 8.50µg/l as compared to 33% with hardly increased serum ferritin concentration (150.00µg/l). It is

also possible that the associations were not significant because most of the women were not markedly hypercholesterolaemic (52% had TC<5.00mmol/l).

The findings of the present study do not agree with some studies that have examined the relationship between iron status and CVD risk factors[6,30]. These studies demonstrated a positive association between serum ferritin and TC concentration. Postmenopausal women were used as participants in these studies which make their results less comparable to those of the present study which was composed of adult women both in the pre (76%) and post (24%) menopausal age (>55years). Menopause itself has been reported to result in increased iron storage and lipid production due to the absence of the protective effect of endogenous oestrogen[31]. Additionally, a positive association was found between serum ferritin and TG but not TC, HDL-C and BP in a healthy population of white Danish women aged 40-60[32]. In the present study, TG was positively associated with ferritin only before adjusting for valid confounders. It has been proposed that increased serum TG may cause an increase in the release of small LDL particles through lipid transfer reaction[33]. Small LDL particles are easily oxidized, the initial step in CVD development[34]. Increases in small and dense LDL particles have been linked to increased CVD risk[34].

The findings of the present study are comparable to the observations made by Binkoski *et al*[35] who reported that increased body iron does not increase the susceptibility of LDL to oxidative stress in women with low iron status. A meta-analysis also confirmed that increased iron stores do not play a significant role in the development of CVDs[36]. Additionally, the Atherosclerotic Risk In Communities (ARIC) study[37] reported that there was no evidence of a link between serum ferritin and LDL oxidation parameters in both United States men and women (mean age of 59 years) without existing myocardial infarction or history of ischaemic heart attack.

In the Transition and Health during Urbanization of South Africans (THUSA) study conducted ten years ago in the same population, Aderibigbe *et al*[38] reported that serum ferritin concentration was significantly higher in the high WHR category than the normal WHR category. This observation was retained in women after adjustment was made for age, BMI and smoking. This is congruent to the findings of the present study as WHR

was significantly higher in the fourth quartile of ferritin than the third quartile. Similarly, WHR in the third quartile of ferritin was higher than the second quartile after adjusting for valid confounders. The same trend was observed for WC in the present study. This suggests that central obesity contributes significantly to increased serum ferritin concentration of women in this population.

Ferritin, an acute phase reactant, is elevated during inflammation[15]. Obesity, now recognized as an inflammatory condition[39], may contribute to the elevated ferritin levels observed in this population. Hepcidin, the hormone that inhibits release of iron from the stores and reduces absorption of iron has been shown to be elevated in obese individuals[15]. However, in the present study obese women did not have lower iron status. Adiposity, especially centralized adipose tissue, has been reported to promote atherogenetic reactions by interacting with other CVD risk factors[14]. Crist *et al*[5] reported that markers of lipid damages such as oxidized low density lipoprotein (oxLDL) and 15-isoprostane $F_{2\alpha}$ (PGF_2) and not markers of DNA or protein damage contribute significantly to the development of CVDs. This supports the role of obesity in the pathogenesis of CVDs[40]. A high WC has been linked to increased oxLDL in both men and women[41]. In the present study, 31% of participants exceeded the recommended limit for WC (>88cm) while 52% exceeded the reference limits for WHR (<0.80). It has been hypothesized that iron overload may not be a problem in a healthy population but in a population with obesity and related non-communicable diseases, iron overload may pose a major threat[14].

No significant correlation was observed between serum ferritin and TfR concentration after adjusting for valid confounders. This implies that one of these iron parameters was influenced by other factors affecting iron status e.g. ferritin is affected by alcohol consumption and inflammation[42]. In the present study, the mean serum ferritin concentration of drinkers (260.42µg/l) doubled that in non-drinkers (122.58µg/l). The mean self-reported alcohol consumption in this population was (7.74g/d) which falls within reference range for moderate drinking[43]. However, the mean self-reported alcohol intake of women drinkers was (23.30g/d) which is way above reference range for moderate drinking[43-44].

The mechanism by which alcohol increases serum ferritin concentration is not fully understood[45]. One of the speculated mechanisms is the leakage of intracellular ferritin by cell necrosis[45]. Serum ferritin, under normal circumstances, is in equilibrium with intracellular ferritin[25]. Alcohol is thought to influence this equilibrium[45]. This is supported by the observation that serum ferritin in alcoholics correlates with increased transaminase levels which were normalized when alcohol was withdrawn[46]. Alcohol may also have a direct effect on ferritin synthesis[45]. Alcohol induces ferritin synthesis in rat hepatocyte primary cultures at low concentrations, which can easily be achieved in alcoholism[45].

It was formerly thought that iron accumulation has to be severe to cause oxidative stress but research findings suggest that moderately excessive serum iron may contribute to formation of oxygen radical which can initiate oxidation of LDL[47]. Oxidized LDL is a scavenger receptor and when taken up by the macrophages can lead to the formation of foam cells, the most essential cells of the fatty streak[14]. A mean serum ferritin concentration of 80µg/l has been associated with significant increases in incidence of heart disease for both genders[27]. In the present study, mean serum ferritin concentration was 94.84µg/l. It is believed that oxidative stress itself can release the iron needed for production of reactive species. Superoxide radical have been observed to liberate iron from ferritin thereby inducing lipid peroxidation. It has been proposed that the role of iron in CVD involves the conversion of poorly reactive free radicals to highly reactive ones[28].

In conclusion, this population-based study suggests no significant association between iron status and CVD risk factors, with the exception of ferritin, which increases with increasing abdominal obesity as defined by WHR and WC. This may pose a greater threat for cardiovascular disease as the proportion of iron replete individuals increase.

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CHAPTER 6

GENERAL SUMMARY, DISCUSSIONS, RECOMMENDATIONS AND CONCLUSIONS

CHAPTER 6: GENERAL SUMMARY, DISCUSSION, RECOMMENDATIONS AND CONCLUSIONS

6.1 Introduction

In this chapter a summary of the main findings from the Transition and Health during Urbanisation of South Africans (THUSA) and the Prospective Urban and Rural Epidemiological (PURE) studies reported in this thesis will be given. The recommendations made in this thesis were based on these findings.

Additionally, the results from the two studies are integrated, discussed and interpreted, focusing on the compatibility of the main findings and the recommendations.

6.2 Main findings

The salient observations of the studies reported in this thesis were:

In the THUSA study, waist circumference (WC) and waist to hip ratio (WHR) were positively associated with serum ferritin concentration in both men and women. In a similar cross-sectional, population based study (PURE) conducted seven years after in the same localities as the THUSA study, WC and WHR increased with increasing ferritin concentration in the women as well. This confirms the positive association between abdominal obesity as indicated by excess WC and WHR, and ferritin concentration in this population.

Additionally, women showed a significant decrease in serum iron concentration with increasing body mass index (BMI) in the THUSA study. The results from the THUSA study indicate that the mean concentration of iron indices {haemoglobin (Hb), total iron binding capacity (TIBC), ferritin, and serum iron} were within optimal ranges for both men and women except for percentage transferrin saturation which was lower in women. This is an indication for risk of iron deficiency in the women. Unfortunately, serum

transferrin receptor (TfR), an improved measure of iron deficiency (Kohgo *et al.,* 1986) was not measured in the THUSA study. In the PURE study where TfR concentration was measured, the presence of iron deficiency risk was confirmed as TfR concentration was within ranges classified as iron deficiency state.

As for the PURE study, the associations observed between iron indices and cardiovascular disease (CVD) risk factors were weak. These associations were not retained when valid confounders (age, BMI, smoking, C-reactive protein and alcohol consumption) were adjusted for in the analysis. This suggests that the observed association could be attributed to other factors influencing either/both iron status parameters and CVD risk factors. The lack of significant association between TfR and ferritin concentration when alcohol was included to the list of valid confounders confirms that other factors could have affected the iron status parameters. Alcohol consumption has been shown to affect serum ferritin concentration while having little or no effect on TfR concentration (Cylwik *et al*., 2010; Gopane *et al*., 2010; Pisa *et al.,* 2010).

6.3 Public health perspective

In these population-based studies, serum ferritin concentration associated positively with WC and WHR but not with other risk factors for CVDs. These results support the potential role of iron in the pathogenesis of CVD. Abnormal body fat distribution may lead to abnormal iron status which is a culprit in the initiation of oxidative reactions leading to damage of body molecules (Sempos *et al.*, 2000). South Africa, like many other developing countries, has employed several strategies to combat iron deficiency. This includes provision of iron supplements to pregnant and postpartum women, fortification of several food vehicles and dietary diversification (using the food based dietary guidelines). Most of these strategies were developed without paying special attention to the possible effect of body fat distribution/adiposity on iron status. As iron intervention programmes continue along with the increasing prevalence of overweight and obesity, it is important to continue to investigate the association between different

iron indices and CVD risk factors so as to possibly elucidate whether iron is linked to CVD.

6.4 Recommendations and conclusions

This study concludes that serum ferritin concentration was positively associated with WC and WHR but not with other CVD risk factors. This can be interpreted as an indication of the possible role of iron obesity and thus in the development of CVDs.

6.4.1 Scaling up obesity intervention programmes

As it has been shown that WC and WHR were positively associated with serum ferritin concentration and excess serum ferritin concentration being the culprit in the pathogenesis of CVDs (Sempos *et al.*, 2000), it may be important to scale up interventions targeted at reducing the prevalence of obesity in this population. Abdominal obesity seems to be more important than total body obesity as WC and WHR showed stronger positive association with serum ferritin than BMI. The causes of obesity are diverse. As illustrated by the UNICEF conceptual framework, obesity could be caused by direct factors (lifestyle factors), underlying factors (infrastructure and support systems) or basic factors (regulations and policies) (Gibney *et al*., 2004). Interventions should address all levels of causation in order to achieve desirable results.

6.4.2 Identification and assessment of high risk groups for iron inventions

Women and children are the most vulnerable to iron deficiency and these group of people should be targeted for iron interventions. Interventions targeted at the entire population may have adverse effect as the iron status varies by gender, age and province. Therefore, consideration should be given to the use of different and specifically relevant strategies as determined by the prevailing health need of the individual or group.

6.4.3 Proper evaluation and monitoring of intervention programmes

Although iron deficiency is prevalent in this population, progression into an iron overload state is not desirable. Proper evaluation and monitoring of intervention programmes should be ensured in order to know when the goal of an intervention programme has been achieved and not allow development into an excessive stage that has its own consequences.

6.4.4 Addressing other influencing factors

The results of this thesis have additionally shown that there are other factors that may influence the relationship between iron status, obesity and CVDs risk factors. These factors include alcohol consumption, infection and diet. Special attention should be given to these factors when developing interventions for either iron deficiency or obesity.

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ADDENDA

ADDENDA: THUSA study

ADDENDUM 1: Recruitment and informed consent form

THUSA PROJECT : PU FOR CHE

RECRUITMENT AND INFORMED CONSENT FORM

Title of the project: Nutritional and health status of Africans in transition

Name: ..... No.....

Address: .....

Tel no:.....

Age: .....

Are you pregnant? .....

Are you lactating? .....

Do you suffer from diabetes? hypertension? Other disease?.....

When did you have your last meal? .....

or anything but water to drink? .....

INFORMED CONSENT

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.

.....

Signature of volunteer

Witnesses

....

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

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

1, ... (full names) the parent/legal guardian of the person named above, hereby consent that he/she may participate in the THUSA project.

Signature ..... Date .....

Relationship .....

ADDENDUM 2: Anthropometry form

ADDENDUM 2: Anthropometry form

THUSA PROJECT – SOUTH AFRICA 1998
ANTHROPOMETRY

Subject ID#

Gender (1 = M, 2 = F)

Projection box + constant

		.	

Skinfolds

Triceps			.				.					.			
Subscapular			.				.					.			
Illiac Crest			.				.					.			
Supraspinale			.				.					.			
Abdominal			.				.					.			
Front Thigh			.				.					.			
Medial Calf			.				.					.			

Girths

Arm - Relaxed			.				.					.			
Arm - Fully flexed/Tensed			.				.					.			
Forearm - Maximum			.				.					.			
Waist - Minimum			.				.					.			
Hip (Gluteal) - Maximum			.				.					.			
Thigh - 1cm below gluteal fold			.				.					.			
Thigh - Mid trio-tib lat			.				.					.			
Calf - Maximum			.				.					.			

Breadths

Humerus (cm)			.				.								
Wrist (cm)			.				.					.			
Femur (cm)			.				.					.			
Ankle (cm)			.				.					.			

Other

Mass (kg)				.	
Stature - Stretched (cm)				.	

ADDENDUM 3: Demographic questionnaire

ADDENDUM 3: Demographic questionnaire

Potchefstroomse Universiteit
vir Christelike Hoër Onderwys

Subject number			
Date	D	M	Y
Place			
Interviewer			

Home address _____

_____

_____

_____

Sex	Male	1
	Female	2

Age			
Date of birth	D	M	Y

First Language	Tswana	1
	Afr	2
	Eng	3
	Xhosa	4
	Zulu	5
	Other	6

Second Language	Tswana	1
	Afr	2
	Eng	3
	Xhosa	4
	Zulu	5
	Other	6

What is your marital status?	Never married	1
	Married	2
	Divorced	3
	Widowed	4

Do you suffer from:	High blood	Yes	1
		No	2
	Diabetes	Yes	1
		No	2
	CHD	Yes	1
		No	2
	Stroke	Yes	1
		No	2

Does anyone in your family suffer from:	High blood	Yes	1
		No	2
	Diabetes	Yes	1
		No	2
	CHD	Yes	1
		No	2
	Stroke	Yes	1
		No	2

Do you take medicine regularly?	Yes	1
	No	2
If yes - what do you take?		

Do you snuff?	Yes	1
	No	2
Do you smoke?	Yes	1
	No	2
If no - have you smoked regularly before?	Yes	1
	No	2

If yes - what do you smoke?	Cigarettes	1
	Tobacco/pipe	2
	Other	3

If other - describe

How much do you smoke?	per day	
	per week	
For how long have you been smoking (years)		
Calculate pack years		

What is your highest qualification?	None	1
	< St.6	2
	St. 6-8	3
	St. 6-8 + trade	4
	St. 9-10	5
	St. 9-10 + trade	6
	St. 9-10 + academic	7

What is your occupation?	

Do you have a job at the moment?	Yes	1
	No	2

If yes - what kind of job?	

On which days of the week do you work?	Irregular (piece work)	1
	Part time (1-4 days)	2
	Full time (5-6 days)	3

How much money do you earn? Is it between:	R0-100	
	R101-500	
	R501-1000	
	R1000-2000	
	R2000-3000	
	R3000+	

What is the source of this income?	

Do you receive any additional pensions?	Yes	1
	No	2

How much pension do you receive per month?

Interviewer - *Re-evaluate final income category*	R0-100	1
	R101-500	2
	R501-1000	3
	R1000-2000	4
	R2000-3000	5
	R3000+	6

Who else contributes money to your household? How much?

Yes	1
No	2

Who else contributes other resources eg. food,

sharing work/chors to your household? - specify!

Yes	1
No	2

Does any member of your household have the right to use any property as his/her own?	Yes	1
	No	2

What type of property?	

How do you use it?	

Please name the members of your household

Member	Age	Education	Present job

What type of house do you live in?	Traditional	1
	Mokuku	2
	Brick house	3
	Other	4
Specify other		

Do you share a toilet with other households?	Yes	1
	No	2

What type of toilet do you have?	Communal	1
	None	2
	Bucket system	3
	Outside longdrop	4
	Outside chemical	5
	Outside water flush	6
	Inside water flush	7

Where do you get your drinking water from?	Fountain, river	1
	Communal tap	2
	Tap on premises	3
	Tap in house	4
	Other	5
If other specify		

Do you have access to electricity inside your house?	Yes	1
	No	2

What type of stove do you have?	None	1
	Coal/wood	2
	Gas or parrafin	3
	Electric	4

What type of fridge do you have?	None	1
	Parraffin	2
	Gas	3
	Electric	4

How long have you been living here? (years)	

Where did you live before coming here?	Rural area	1
	Farm	2
	Squatter camp	3
	Township	4

ADDENDUM 4: Quantitative food frequency questionnaire

ADDENDUM 4: Quantitative food frequency questionnaire

THUSA Quantitative Food Frequency Questionnaire

Subject ID Initials **Subject**

☐☐	☐☐☐	☐☐☐	☐☐	☐☐☐

Centre # **Community #** **Household #** **Subject #**

M L
Today's date: ☐☐☐☐ ☐☐ ☐☐
year *month* *day*

1. Name: _____
_____

2. Not applicable in South Africa

3. National identity # or equivalent _____ N/A ☐

4. DOB: ☐☐☐☐ ☐☐ ☐☐ **OR Age** ☐☐ **years**

5. Sex: ☐ Female ☐ Male

Please think carefully about the food and drink you have consumed during the ***past month*** (four weeks). I will 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 everyday, 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 the sizes or bigger than the pictures.

There are no right or wrong answers.

Everything you tell me is confidential. Only your subject number appears on the form.

Is there anything you want to ask now?

Are you willing to go on with the questions?

FOOD FREQUENCY QUESTIONNAIRE

INSTRUCTIONS: 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.

FOOD	DESCRIPTION	AMOUNT	TIMES EATEN				CODE	AMOUNT / DAY
			Per day	Per week	Per month	Seldom / Never		
PORRIDGE AND BREAKFAST CEREALS AND OTHER STARCH								
Maize-meal porridge	Stiff (pap)						3400	
Maize-meal porridge	Soft (slappap)						3399	
Maize-meal porridge	Crumbly (phutu)						3401	
Ting								
Mabella	Stiff						3437	
Mabella	Soft							
Oats							3239	
Other cooked porridge	Type: _____							
Breakfast cereals	Brand name of cereals at home now: _____ _____ _____ _____							
Do you pour milk on your porridge or cereal? **1** Yes **2** No								
If yes, what type of milk (whole fresh, sour, 1%, fat free, milk blend, etc) _____								
If yes, how much milk								
Do you put sugar on your porridge or cereal? **1** Yes **2** No								
If yes, how much							3989	

FOOD	DESCRIPTION	AMOUNT	TIMES EATEN				CODE	AMOUNT / DAY
			Per day	Per week	Per month	Seldom / Never		
sugar							3989	
							3989	
Samp	Bought Self ground						3250	
Samp and beans	Give ratio of samp:beans						3402 (1:1)	
Samp and peanuts	Give ratio of samp:peanuts						3250 (samp)	
Rice	White						3247	
	Brown						3315	
	Maize Rice						3250	
Pasta	Macaroni Spaghetti Other specify: _____ _____						3262	
Pizza	Home made: Specify topping _____ _____						3353 (base+ch)	
	Bought: Specify topping _____ _____						3353 (base+ch)	

FOOD	DESCRIPTION	AMOUNT	TIMES EATEN				CODE	AMOUNT / DAY
			Per day	Per week	Per month	Seldom / Never		
You are being very helpful. Can I now ask you about meat? **CHICKEN, MEAT, FISH** ***How many times do you eat meat (beef, mutton, pork, chicken, fish) per week?*** _____								
Chicken (codes with skin)	Boiled						2926	
	Fried: in batter/crumbs Eg Kentucky						3018	
	Fried: Not coated							
	Bought: Chicken Licken						2925	
	Bought: Nando's							
	Roasted / Grilled						2925	
	Other: _____							
Do you eat chicken skin?	Always		Sometimes		**3**	Never		
Chicken bones stew								
Chicken feet							2997	
Chicken offal								
Red meat	How do you like meat? With fat Fat trimmed							
Red meat	Fried							
	Stewed							
	Mince with tomato and onion						2987	

FOOD	DESCRIPTION	AMOUNT	TIMES EATEN				CODE	AMOUNT / DAY
			Per day	Per week	Per month	Seldom / Never		
	Other:							
Beef Offal	Intestines: boiled nothing added						3003	
	Stewed with vegetables							
	Liver						2920	
	Kidney						2923	
	Other: Specify _____ _____							
Goat meat	Boiled						4281	
	Stewed with vegetables							
	Grilled / Roasted						4281	
What type of vegetables is usually put into meat stews? _____								
Wors / Sausage							2931	
Bacon							2906	
Cold meats	Polony						2919	
	Ham						2967	
	Vienna						2936	

FOOD	DESCRIPTION	AMOUNT	TIMES EATEN				CODE	AMOUNT / DAY
			Per day	Per week	Per month	Seldom / Never		
	Other: Specify _____ _____ _____							
Canned meat	Bully beef							
	Other: Specify _____ _____							
Meat pie	Beef						2939	
	Steak and kidney						2957	
	Cornish						2953	
	Chicken						2954	
	Other							
Hamburger	Bought							
Dried beans/peas/lentils	Soup						3145	
	Salad							
Soya products eg. Toppers	Brands at home now: _____ _____						3196 (Toppers)	
Pilchards in tomato/chilli/brine	Whole						3102	
	Mashed with fried onion							
Fried fish	With batter/crumbs							

FOOD	DESCRIPTION	AMOUNT	TIMES EATEN				CODE	AMOUNT / DAY
			Per day	Per week	Per month	Seldom / Never		
	Without batter/crumbs							
Other canned fish	Tuna						3056 (oil)	
	Pickled fish							
	Other: Specify _____							
Fish cakes	Bought: Fried						3080	
	Home made with potato						3098	
Fish fingers	Bought						3081	
Eggs	Boiled/poached						2867	
	Scrambled: milk + fat							
	Fried: Fat							

Now we come to vegetables and fruit

VEGETABLES AND FRUIT

Cabbage	How do you cook cabbage?							
	Boiled, nothing added						3756	
	Boiled with potato and onion and fat							
	Fried, nothing added Fried in ……							
	Boiled, then fried with potato, onion							
	Other:							
	Don't know							
Spinach/morogo/ beetroot leaves other green leafy	How do you cook spinach?							
	Boiled, nothing added						3913	
	Boiled with fat added Type of fat ……..							
	With onion, tomato, potato							

FOOD	DESCRIPTION	AMOUNT	TIMES EATEN				CODE	AMOUNT / DAY
			Per day	Per week	Per month	Seldom / Never		
	With peanuts							
	Other:							
	Don't know							
Tomato and onion gravy	Home made with fat Type of fat ……..							
	Without fat						3925	
	Canned						4192	
Pumpkin (yellow)	How do you cook pumpkin?							
	Boiled, nothing added						4164	
	Cooked in fat and sugar Fat ……							
	Other							
	Don't know							
Carrots	How do you cook carrots?							
	Boiled, nothing added						3757	
	Boiled, sugar and fat Fat ……							
	With potato and onion: Fat							
	Raw, salad						3709	
	Chakalaka							
	Other							
	Don't know							
Mealies/ Sweet corn	How do you eat mealies?							
	On cob – fat added Fat ……							
	On cob – no fat added						3725	
	Creamed sweet corn / canned						3726	
	Whole kernel/canned						3942	
Beetroot	Salad						3699	
	Boiled, nothing added						3698	
Potatoes	How do you cook potatoes?							

FOOD	DESCRIPTION	AMOUNT	TIMES EATEN				CODE	AMOUNT / DAY
			Per day	Per week	Per month	Seldom / Never		
	Boiled/baked with skin						4155	
	Boiled/baked without skin						3737	
	Mashed							
	Roasted Fat ……							
	French fries (chips)						3740	
Sweet potatoes	How do you cook sweet potatoes?							
	Boiled/baked with skin						3748	
	Boiled/baked without skin						3903	
	Mashed							
	Other: _____ _							
	Don't know							
Salad vegetables	Mixed salad: tomato, lettuce and cucumber					`	3921	
	Raw tomato						3750	
	Other salad vegetables: _____ _____							
Other vegetables, specify + preparation	_____ _____ _____							
Do you like fruit?		**1** Yes		**2** No				

FOOD	DESCRIPTION	AMOUNT	TIMES EATEN				CODE	AMOUNT / DAY
			Per day	Per week	Per month	Seldom / Never		
Apples							3592	
Pears							3582	
Oranges							3560	
Naartjie							3558	
Grapes							3550	
Peaches	Fresh						3565	
	Canned						3567	
Apricots	Fresh						3534	
	Canned						3535	
Mangoes							3556	
Guavas	Fresh						3551	
	Canned						3553	
Avocado							3656	
Wild fruit/berries	Specify type: _____							
Dried fruit	Types: _____							
Other fruit	_____ _____							
If subject eats canned fruit: Do you have custard with the canned fruit?			Yes		**2**	No		
Custard	Home made: Milk							
	Commercial eg Ultramel						2716	

BREAD AND BREAD SPREADS

FOOD	DESCRIPTION	AMOUNT	TIMES EATEN				CODE	AMOUNT / DAY
			Per day	Per week	Per month	Seldom / Never		
Bread / Bread rolls	White						3210	
	Brown						3211	
	Whole wheat						3212	
Do you spread anything on the bread?		**1** Always		**2** Sometimes		**3** Never		
Margarine	What brand do you have at home now?							
	Don't know _____							
Peanut butter							3485	
Jam/syrup/honey							3985	
Marmite / Fray bentos / Oxo							4058	
Fish/meat paste							3109	
Cheese	Type: _____ _____ _____							
Achaar								
Other spreads	Specify: _____ _____							

FOOD	DESCRIPTION	AMOUNT	TIMES EATEN				CODE	AMOUNT / DAY
			Per day	Per week	Per month	Seldom / Never		
Dumpling								
Vetkoek	White flour						3257	
	Whole wheat flour						3324	
Provita, crackers, etc							3235	
Mayonnaise / salad dressing	Mayonnaise						3488	
	Other: Specify _____							
DRINKS								
Tea	English (normal)						4038	
	Rooibos						4054	
Coffee							4037	
Sugar/cup tea or coffee	Tea:						3989	
	Coffee:						3989	
Milk/cup tea or coffee	What type of milk do you use in tea and coffee?							
	Fresh/long life: whole/full						2718	
	Fresh/long life: 2%/low fat						2772	
	Fresh/long life: fat free						2775	
	Whole milk powder Brand: _____						2721 (powder)	
	Low fat milk powder Brand: _____						2825 (powder)	

FOOD	DESCRIPTION	AMOUNT	TIMES EATEN				CODE	AMOUNT / DAY
			Per day	Per week	Per month	Seldom / Never		
	Skimmed milk powder Brand: _____						2825 (powder)	
	Milk blend Brand: _____						2770 (powder)	
	Whitener: type _____ _____							
	Condensed milk						2714	
	Evaporated milk						2715	
	None							
Milk as such	What type of milk do you drink milk as such?							
	Fresh/long life: whole/full						2718	
	Fresh/long life: 2%/low fat						2772	
	Fresh/long life: fat free						2775	
	Condensed milk						2714	
	Sour/maas						2787	
	Other: _____ _____							

FOOD	DESCRIPTION	AMOUNT	TIMES EATEN				CODE	AMOUNT / DAY
			Per day	Per week	Per month	Seldom / Never		
Milk drinks	Nestle: _____							
	Milo: _____							
	Flavoured milk: _____							
	Other:							
Yoghurt	Drinking yoghurt						2756	
	Thick yoghurt						2734	
	Low fat sweetened with fruit						2732	
Squash	Sweet O						4027	
	Six O							
	Oros/Lecol – with sugar						3982	
	- artificially sweetener						3990	
	KoolAid						4027	
	Other: _____ _____							
Fruit juice	Fresh/Liquifruit/Ceres						2866	
	Tropica (Dairy –fruit juice mix)						2791	

FOOD	DESCRIPTION	AMOUNT	TIMES EATEN				CODE	AMOUNT / DAY
			Per day	Per week	Per month	Seldom / Never		
	Other: _____ _____ _____							
Fizzy drinks	Sweetened						3981	
Coke, fanta, etc	Diet							
Maueu/Motogo							4056	
Home brew								
Tlokwe							4039	
Beer							4031	
Spirits							4035	
Wine red							4033	
Wine White							4033	
Other specify	_____ _____ _____							
SNACKS AND SWEETS								
Potato crisps							3417	
Peanuts	Raw						4285	
	Roasted						3458	

FOOD	DESCRIPTION	AMOUNT	TIMES EATEN				CODE	AMOUNT / DAY
			Per day	Per week	Per month	Seldom / Never		
Cheese curls, Niknaks, etc							3267	
Raisins							3552	
Peanuts and raisins								
Chocolates	Name: _____ _____ _____							
Candies	Sugus, gums, hard sweets, etc						4000	
Sweets	Toffees, fudge, caramels						3991	
Biscuits/cookies	Type: _____ _____ _____							
Cakes and tarts	Type: _____ _____ _____							
Scones								
Rusks	Type: _____							

FOOD	DESCRIPTION	AMOUNT	TIMES EATEN				CODE	AMOUNT / DAY
			Per day	Per week	Per month	Seldom / Never		
	_____							
Savouries	Sausage rolls						2939	
	Samoosas: Meat filling						3355	
	Samoosas: Vegetable filling						3414	
	Biscuits eg bacon kips							
	Other specify: _____ _____							
Jelly							3983	
Baked pudding	Type: _____							
Instant pudding	Milk type: _____							
Ice cream							3483	
Sorbet							3491	
Other specify	_____ _____ _____							

FOOD	DESCRIPTION	AMOUNT	TIMES EATEN				CODE	AMOUNT / DAY
			Per day	Per week	Per month	Seldom / Never		
SAUCES, GRAVIES AND CONDIMENTS								
Tomato sauce / Worcester sauce							3139	
Chutney							3168	
Pickles							3866	
Packet soups							3165	
Other:	_____ _____							
WILD BIRDS, ANIMALS OR INCECTS (hunted in rural areas or on farms)								
Wild fruit								
MISCELLANEOUS: Please mention any other foods used more than once/two times a week which we have talked about:								

FOOD	DESCRIPTION	AMOUNT	TIMES EATEN				CODE	AMOUNT / DAY
			Per day	Per week	Per month	Seldom / Never		
INDIGENOUS/TRADITIONAL FOODS/PLANTS/ANIMALS **Please tell me if you use any indigenous plants OR other indigenous foods like mopani worms, locusts ect to eat**								
Specify								

ADDENDA

ADDENDA: PURE study

ADDENDUM 1: Appointment letter

ADDEDUM 1: APPOINTMENT LETTER

POTCHEFSTROOM CAMPUS: FACULTY OF HEALTH SCIENCES

PURE-SA Project (Prospective Urban and Rural Epidemiology)

APPOINTMENT LETTER

Dear Participant

Thank you for being willing to help us in this very important project. We are sure that the project will contribute to improve health of all the people of the North West Province.

The aim of the project is to get enough information regarding the development of chronic diseases like Diabetes, Stroke, Lung disease and Heart disease with urbanisation to plan appropriate health and nutrition intervention strategies. At the time you receive this letter you would have been visited by a fieldworker and you already have filled out several questionnaires and signed consent to give a blood sample. This letter serves to inform you of the date and time the blood sample and other measurements will be done at the premises of the North-West University on the Potchefstroom Campus.

IMPORTANT INFORMATION

1. You will be picked up by a taxi accompanied by Ms Susan Legwete on **……** by 0….h00. Susan will tell you the place where you will be picked up.
2. You **MUST NOT EAT OR DRINK** anything after ten o'clock of the previous night (10 pm of the night before). This is necessary for the glucose test to be accurate.
3. You **MUST BRING YOUR ID DOCUMENT** with you
4. Your taxi fare will be paid by us and you will receive food after the blood sample is taken.
5. If you are employed, please show this letter to your employer.

Dear Employer

This serves to ask you to give one day's paid leave to……. in order to allow him/her to attend his appointment with the research team of the PURE-SA study at the North-West University.

Thank you for your cooperation. For any further information please contact Dr A Kruger at 082 7715778

A Kruger (project leader)

ADDENDUM 2: Recruitment and informed consent form

ADDENDUM 2: Recruitment and informed consent

POTCHEFSTROOM CAMPUS

PURE-SA Project

INFORMED CONSENT FORM

Title of the project: PURE-Project (Prospective Urban and Rural Epidemiology)

INFORMED CONSENT (Phase 1)

I, the undersigned ……(full names)

understand that the only information that will asked from me is the family census and household questionnaires. I understand that a field worker from the PURE-study will ask me the questions and that all the information gained from me will kept confidential.

I indemnify the University, also any employee or student of the University, of any liability against myself, which may arise during the course of the project.

I will not submit any claims against the University regarding personal detrimental effects due to the project, due to negligence by the University, its employees or students, or any other subjects.

……..

(Signature of the subject)

Signed at .....on ……..

Witnesses

1. .....

2. . .....

Signed at ..... on ……

PURE-SA Project

INFORMED CONSENT FORM

PURE-Project (Prospective Urban and Rural Epidemiology)

INFORMED CONSENT

I, the undersigned ……(full names)

read/listened to the information on the project in PART 1 and PART 2 of this document and I declare that I understand the information. I had the opportunity to discuss aspects of the project with the project leader and I declare that I participate in the project as a volunteer. I hereby give my consent to be a subject in this project.

I indemnify the University, also any employee or student of the University, of any liability against myself, which may arise during the course of the project.

I will not submit any claims against the University regarding personal detrimental effects due to the project, due to negligence by the University, its employees or students, or any other subjects.

I agree to be tested for HIV :	YES	NO
I want to know my HIV-status:	YES	NO
I agree to give a blood sample	YES	NO

(The HIV testing and other measurements will only be done during September-December 2005. You have the right to change your mind and at that time you will be asked to sign an inform consent again on HIV testing)

……..
(Signature of the subject)

Signed at .....on ……..

Witnesses

1. .....

2. . .....

Signed at ..... on ……

POTCHEFSTROOM CAMPUS

PURE-SA Project (Prospective Urban and Rural Epidemiology)

INFORMED CONSENT FORM (including the PRIMER-study)

I, the undersigned …….....……(full names) read / listened to the information on the project in PART 1 and PART 2 of this document and I declare that I understand the information. I had the opportunity to discuss aspects of the project with the project leader and I declare that I participate in the project as a volunteer. I hereby give my consent to be a subject in this project.

I agree to be tested for HIV	Yes	No
I want to know my HIV-status	Yes	No
I agree to give a blood sample	Yes	No

I hereby also declare that I am aware that:

this blood sample will be used for the purpose of

a. Isolating DNA to look at genetic factors that are currently associated with Type 2 Diabetes (i.e. the Calpain10, Adiponectin, Leptin and Leptin Receptor genes), or genetic factors that may be associated with Non Communicable diseases in the future. We give the assurance that all genetic tests and experiments will only focus on genotypes suspected to contribute to an increased risk of non communicable diseases of lifestyle.
b. Testing for liver function by determining liver enzymes such as AST, GGT,
c. Analyses of other than genetic parameters for Diabetes Mellitus such as HbA_1C, Blood glucose and Insulin
d. Analyses of clotting factors and hypertension markers
e. Analyses of bone health, iron and nutrition status
f. And may be stored until such time as the above measurements/analyses will be done.

2. A two hour glucose tolerance test will be done
3. Body measurements such as height, weight, skinfold thicknesses, arm and leg circumferences will be taken
4. Electrocardiograph be taken
5. Blood pressure to be taken
6. Pulse wave velocity measurements will be made
7. A urine sample to be collected to analyse for the presence of heavy metals such as lead and mercury,
8. A Spirometer test to be performed to determine lung function
9. A handgrip test to be performed to test muscle strength
10. A hair sample to be taken to test for fumonisin mycotoxins.

…….....……..
(Signature of the subject)
Signed at **... Potchefstroom / Ganyesa ...** (delete not applicable option) on **……/……/ 2005**

Witnesses

1. ..... 2. .....……

Signed at **... Potchefstroom / Ganyesa ...** (delete not applicable option) on **……/……/ 2005**

PART 1

1. School/Institute:

Faculty of Health Sciences, North-West University

2. Title of project/trial:
PURE: Prospective Urban and Rural Epidemiological study

3. Full names, surname and qualifications of project leader:
Dr. Annamarie Kruger, Ph.D. (Nutrition)

4. Rank/position of project leader:
Research Manager

5.. Aim of this project

PURE's aim is that understanding the different lifestyle and health transitions of individuals in response to societal changes will elucidate societal and individual adaptive strategies that could diminish the adverse health effects of industrialization and urbanization on health, while retaining its benefits.

6. Explanation of the nature of all procedures, including identification of new procedures:

Each participant will have to fill in a number of questionnaires (Adult questionnaire, Physical activity questionnaire, Food frequency questionnaire, Health questionnaire) with the help of field workers. A blood and urine sample will be taken. Physical measures will be performed, including anthropometric measures (such as weight, height, and waist circumference), blood pressure, lung capacity and lung volume and an ECG will be performed.

7. Description of the nature of discomfort or hazards of probable permanent consequences for the subjects which may be associated with the project: (Including possible side-effects of and interactions between drugs or radio-active isotopes which may be used.)

It will take each participant quite a while (about two hours) to complete all the tests and discomfort may be experienced with the taking of blood samples. No measures will have permanent damage or consequences for the participants.

8. Precautions taken to protect the subjects:

The research nurse will be present at all times, and will be responsible for the blood sampling. She is very experienced and has performed these procedures numerous times in previous studies.

9. Description of the benefits which may be expected from this project:

When measures with immediate results are taken, such as blood glucose levels or blood pressure, the information will be communicated to the individual to seek professional help. Since this study is a longitudinal study, subjects that are high at risk will be identified from the dataset and personal feedback will be given.

10. Alternative procedures which may be beneficial to the subjects:

There will be tested for HIV/AIDS, therefore pre-test counselling will be given. If the subject wants to know his/her status and he/her tests positive, post counselling will also be given.

PART 2

To the subject signing the consent:

You are invited to participate in a research project. It is important that you read/listen to and understand the following general principles, which apply to all participants in our research project:

1. Participation in this project is voluntary.

2. It is possible that you personally will not derive any benefit from participation in this project, although the knowledge obtained from the results may be beneficial to other people.

3. You will be free to withdraw from the project at any stage without having to explain the reasons for your withdrawal. However, we would like to request that you would rather not withdraw without a thorough consideration of your decision, since it may have an effect on the statistical reliability of the results of the project.

4. The nature of the project, possible risk factors, factors which may cause discomfort, the expected benefits to the subjects and the known and the most probable permanent consequences which may follow from your participation in this project, are discussed in Part 1 of this document.

5. We encourage you to ask questions at any stage about the project and procedures to the project leader or the personnel, who will readily give more information. They will discuss all procedures with you.

6. The University staff will use standardised procedures and take all possible precaution to protect the subject from risks.

7. All information will be kept CONFIDENTIAL and no personal information will be published without my consent.

Dr ANNAMARIE KRUGER
Contact details: 082 771 5778 / 018 299 4037(Office)

Potchefstroom Campus

The PURE project
Information to communities

Dear Participant

Thank you for being willing to help us in this very important project. We are sure that the project will contribute to improve health of all the people of the North West Province.

The aim of the project is to get enough information regarding the development of chronic diseases like Diabetes, Stroke, Lung disease and heart disease with urbanisation to plan appropriate health and nutrition intervention strategies.

For this study we need 2 000 subjects whom we can follow for 12 years. The baseline survey will be done from April 2005 to November 2005. The subjects must be from rural as well as urban communities. Therefore, 500 subjects from 4 different levels of urbanisation will be needed. Ganyesa and Tlakgameng were chosen for the rural and semi-rural areas because they are still under tribal law with a good infra structure and stability. We also spoke to Chief M. Letlhogile and the mayor Mr E. Tladinyane and both gentlemen gave us permission to do the research in these two communities. Ikageng and the informal Ikageng were chosen as it is convenient and near the University. Cllr GG Megalanyane and Cllr Mahesh Roopa are informed about the study.

All the questionnaires will be filled out at your houses by trained research field workers who are from your communities. After a household survey and a family census on most of the households in your community to give us an overview of the total community, 250 men and 250 women from all four sites (Ganyesa, Tlakgameng, Ikageng, and the Informal Ikageng) will be asked to proceed with the study. These subjects should be

- Older than 35 years
- Healthy – which means that they must not be aware of any disease and do not take any chronic medication

These 2 000 subjects will be asked to fill out the adult questionnaire, the food frequency questionnaire, the health questionnaire and the physical activity questionnaire. We will also make an appointment with each subject to take some measurements such as weight, height, skinfold thicknesses, ECG (test for heart abnormalities), lung functions, blood pressure, blood glucose, blood samples and a urine sample.

It is very important that we gather quality data and knowledge. Because HIV/AIDS is such a devastating illness and affects almost all aspects of health, it is necessary to know if HIV is absent before we analyse the data. Therefore we will ask questions about your HIV status which you are allow not to answer.

It is also very important to us that you feel free to participate in this study and that you understand what the study is all about. The fieldworker will ask you to sign this form after you have read and understood it.

Kind regards

Dr ANNAMARIE KRUGER
Contact details: 082 7715778 / 018 2994037(W) / 018 2907024(H)

ADDENDUM 3: Referral letter

ADDENDUM 3: Referral letter

POTCHEFSTROOM CAMPUS: FACULTY OF HEALTH SCIENCES

PURE-SA Project (Prospective Urban and Rural Epidemiology)

REFERRAL LETTER

To whom it may concern

Dear Doctor/Sister
Mr/Ms ……participated in a project of our research group on ……

His/her fasted/random blood glucose was ……..mmol/L

His/her resting blood pressure was …….mmHg

Will you please be so kind to attend to this patient?
Thank you and warm regards

…….
Dr A Kruger (project leader)
Contact details: 082 7715778

ADDENDUM 4: Quantitative food frequency questionnaire

ADDENDUM 4: Quantitative food frequency questionnaire

PURE Quantitative Food Frequency Questionnaire

Subject ID **Subject Initials**

☐☐	☐☐☐	☐☐☐	☐☐	☐☐☐
Centre #	**Community #**	**Household #**		**Subject #**

F M L

Today's date: ☐☐☐☐ ☐☐ ☐☐
year *month* *day*

1. Name: _____
_____

2. Not applicable in South Africa

3. National identity # or equivalent _____ **N/A** ☐

4. DOB: ☐☐☐☐ ☐☐ ☐☐ **OR Age** ☐☐ **years**

5. Sex: ☐ Female ☐ Male

Please think carefully about the food and drink you have consumed during the ***past month*** (four weeks). I will 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 everyday, 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 the sizes or bigger than the pictures.

There are no right or wrong answers.

Everything you tell me is confidential. Only your subject number appears on the form.

Is there anything you want to ask now?

Are you willing to go on with the questions?

FOOD FREQUENCY QUESTIONNAIRE

INSTRUCTIONS: 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.

FOOD	DESCRIPTION	AMOUNT	TIMES EATEN				CODE	AMOUNT / DAY
			Per day	Per week	Per month	Seldom / Never		
PORRIDGE AND BREAKFAST CEREALS AND OTHER STARCH								
Maize-meal porridge	Stiff (pap)						3400	
Maize-meal porridge	Soft (slappap)						3399	
Maize-meal porridge	Crumbly (phutu)						3401	
Ting								
Mabella	Stiff						3437	
Mabella	Soft							
Oats							3239	
Other cooked porridge	Type: _____							
Breakfast cereals	Brand name of cereals at home now: _____ _____ _____ _____							

Do you pour milk on your porridge or cereal? 1 Yes 2 No If yes, what type of milk (whole fresh, sour, 1%, fat free, milk blend, etc) _____								
If yes, how much milk								
Do you put sugar on your porridge or cereal? Yes No								
If yes, how much sugar							3989	
							3989	
							3989	
Samp	Bought Self ground						3250	
Samp and beans	Give ratio of samp:beans						3402 (1:1)	
Samp and peanuts	Give ratio of samp:peanuts						3250 (samp)	
Rice	White						3247	
	Brown						3315	
	Maize Rice						3250	
Pasta	Macaroni Spaghetti Other specify: _____ _____						3262	

Pizza	Home made: Specify topping _____ _____						3353 (base+ch)	
	Bought: Specify topping _____ _____						3353 (base+ch)	

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

CHICKEN, MEAT, FISH

How many times do you eat meat (beef, mutton, pork, chicken, fish) per week?

_____

Chicken (codes with skin)	Boiled						2926	
	Fried: in batter/crumbs Eg Kentucky						3018	
	Fried: Not coated							
	Bought: Chicken Licken						2925	
	Bought: Nando's							
	Roasted / Grilled						2925	
	Other: _____							
Do you eat chicken skin?		**1** Always		**2** Sometimes		**3** Never		
Chicken bones stew								
Chicken feet							2997	

Chicken offal								
Red meat	How do you like meat? With fat Fat trimmed							
Red meat	Fried							
	Stewed							
	Mince with tomato and onion						2987	
	Other:							
Beef Offal	Intestines: boiled nothing added						3003	
	Stewed with vegetables							
	Liver						2920	
	Kidney						2923	
	Other: Specify _____ _____							
Goat meat	Boiled						4281	
	Stewed with vegetables							
	Grilled / Roasted						4281	
What type of vegetables is usually put into meat stews? _____								
Wors / Sausage							2931	

Bacon							2906	
Cold meats	Polony						2919	
	Ham						2967	
	Vienna						2936	
	Other: Specify _____ _____ _____							
Canned meat	Bully beef							
	Other: Specify _____ _____							
Meat pie	Beef						2939	
	Steak and kidney						2957	
	Cornish						2953	
	Chicken						2954	
	Other							
Hamburger	Bought							
Dried beans/peas/lentils	Soup						3145	
	Salad							
Soya products eg. Toppers	Brands at home now: _____ _____						3196 (Toppers)	
Pilchards in tomato/chilli/brine	Whole						3102	
	Mashed with fried onion							

Fried fish	With batter/crumbs							
	Without batter/crumbs							
Other canned fish	Tuna						3056 (oil)	
	Pickled fish							
	Other: Specify _____							
Fish cakes	Bought: Fried						3080	
	Home made with potato						3098	
Fish fingers	Bought						3081	
Eggs	Boiled/poached						2867	
	Scrambled: milk + fat							
	Fried: Fat							

Now we come to vegetables and fruit

VEGETABLES AND FRUIT

Cabbage	How do you cook cabbage?							
	Boiled, nothing added						3756	
	Boiled with potato and onion and fat							
	Fried, nothing added Fried in ……							
	Boiled, then fried with potato, onion							
	Other:							
	Don't know							

Spinach/morogo/ beetroot leaves other green leafy	How do you cook spinach?							
	Boiled, nothing added						3913	
	Boiled with fat added Type of fat ……..							
	With onion, tomato, potato							
	With peanuts							
	Other:							
	Don't know							
Tomato and onion gravy	Home made with fat Type of fat ……..							
	Without fat						3925	
	Canned						4192	
Pumpkin (yellow)	How do you cook pumpkin?							
	Boiled, nothing added						4164	
	Cooked in fat and sugar Fat ……							
	Boiled, little sugar and fat Fat ……							
	Other							
	Don't know							
Carrots	How do you cook carrots?							
	Boiled, nothing added						3757	
	Boiled, sugar and fat Fat ……							
	With potato and onion: Fat							
	Raw, salad						3709	
	Chakalaka							
	Other							
	Don't know							
Mealies/	How do you eat mealies?							

Sweet corn	On cob – fat added Fat ……							
	On cob – no fat added						3725	
	Creamed sweet corn / canned						3726	
	Whole kernel/canned						3942	
Beetroot	Salad						3699	
	Boiled, nothing added						3698	
Potatoes	How do you cook potatoes?							
	Boiled/baked with skin						4155	
	Boiled/baked without skin						3737	
	Mashed							
	Roasted Fat ……							
	French fries (chips)						3740	
Sweet potatoes	How do you cook sweet potatoes?							
	Boiled/baked with skin						3748	
	Boiled/baked without skin						3903	
	Mashed							
	Other: _____							
	Don't know							
Salad vegetables	Mixed salad: tomato, lettuce and cucumber						3921	
	Raw tomato						3750	
	Other salad vegetables: _____ _____							
Other vegetables, specify + preparation	_____ _____ _____							

Do you like fruit?		**1** Yes		**2** No				
Apples							3592	
Pears							3582	
Oranges							3560	
Naartjie							3558	
Grapes							3550	
Peaches	Fresh						3565	
	Canned						3567	
Apricots	Fresh						3534	
	Canned						3535	
Mangoes							3556	
Guavas	Fresh						3551	
	Canned						3553	
Avocado							3656	
Wild fruit/berries	Specify type: _____							
Dried fruit	Types: _____							
Other fruit	_____ _____							
If subject eats canned fruit: Do you have custard with the canned fruit?				Yes		**2** No		
Custard	Home made: Milk							
	Commercial eg Ultramel						2716	
BREAD AND BREAD SPREADS								
Bread / Bread rolls	White						3210	
	Brown						3211	

	Whole wheat						3212	
Do you spread anything on the bread?		Always		Sometimes		Never		
Margarine	What brand do you have at home now?							
	Don't know _____							
Peanut butter							3485	
Jam/syrup/honey							3985	
Marmite / Fray bentos / Oxo							4058	
Fish/meat paste							3109	
Cheese	Type: _____ _____ _____							
Achaar								
Other spreads	Specify: _____ _____							
Dumpling								

Vetkoek	White flour						3257	
	Whole wheat flour						3324	
Provita, crackers, etc							3235	
Mayonnaise / salad dressing	Mayonnaise						3488	
	Other: Specify _____							
DRINKS								
Tea	English (normal)						4038	
	Rooibos						4054	
Coffee							4037	
Sugar/cup tea or coffee	Tea:						3989	
	Coffee:						3989	
Milk/cup tea or coffee	What type of milk do you use in tea and coffee?							
	Fresh/long life: whole/full						2718	
	Fresh/long life: 2%/low fat						2772	
	Fresh/long life: fat free						2775	

	Whole milk powder Brand: _____						2721 (powder)	
	Low fat milk powder Brand: _____						2825 (powder)	
	Skimmed milk powder Brand: _____						2825 (powder)	
	Milk blend Brand: _____						2770 (powder)	
	Whitener: type _____ _____							
	Condensed milk						2714	
	Evaporated milk						2715	
	None							
Milk as such	What type of milk do you drink milk as such?							
	Fresh/long life: whole/full						2718	
	Fresh/long life: 2%/low fat						2772	
	Fresh/long life: fat free						2775	

	Condensed milk						2714	
	Sour/maas						2787	
	Other: _____ _____							
Milk drinks	Nestle: _____							
	Milo: _____							
	Flavoured milk: _____							
	Other:							
Yoghurt	Drinking yoghurt						2756	
	Thick yoghurt						2734	
	Low fat sweetened with fruit						2732	
Squash	Sweet O						4027	
	Six O							
	Oros/Lecol – with sugar						3982	
	- artificially sweetener						3990	
	KoolAid						4027	

	Other: _____ _____							
Fruit juice	Fresh/Liquifruit/Ceres						2866	
	Tropica (Dairy –fruit juice mix)						2791	
	Other: _____ _____ _____							
Fizzy drinks **Coke, fanta, etc**	Sweetened						3981	
	Diet							
Maueu/Motogo							4056	
Home brew								
Tlokwe							4039	
Beer							4031	
Spirits							4035	
Wine red							4033	
Wine White							4033	
Other specify	_____ _____ _____							

SNACKS AND SWEETS

Potato crisps							3417	
Peanuts	Raw						4285	
	Roasted						3458	
Cheese curls, Niknaks, etc							3267	
Raisins							3552	
Peanuts and raisins								
Chocolates	Name: _____ _____ _____							
Candies	Sugus, gums, hard sweets, etc						4000	
Sweets	Toffees, fudge, caramels						3991	
Biscuits/cookies	Type: _____ _____ _____							
Cakes and tarts	Type: _____ _____ _____							

Scones								
Rusks	Type: _____ _____							
Savouries	Sausage rolls						2939	
	Samoosas: Meat filling						3355	
	Samoosas: Vegetable filling						3414	
	Biscuits eg bacon kips							
	Other specify: _____ _____							
Jelly							3983	
Baked pudding	Type: _____							
Instant pudding	Milk type: _____							
Ice cream							3483	
Sorbet							3491	
Other specify	_____ _____ _____							

SAUCES, GRAVIES AND CONDIMENTS

Tomato sauce / Worcester sauce							3139	
Chutney							3168	
Pickles							3866	
Packet soups							3165	
Other:	_____ _____							
<u>WILD BIRDS, ANIMALS OR INCECTS</u> (hunted in rural areas or on farms)								
Wild fruit								
<u>MISCELLANEOUS:</u> Please mention any other foods used more than once/two times a week which we have talked about:								
INDIGENOUS/TRADITIONAL FOODS/PLANTS/ANIMALS **Please tell me if you use any indigenous plants OR other indigenous foods like mopani worms, locusts ect to eat**								
Specify								

ADDENDUM 5: Pure 24 hour recall dietary intake

ADDENDUM 5: Pure 24 hour recall dietary intake

PURE 24-hour recall dietary intake

Subject ID **Subject Initials**

☐☐ ☐☐☐ ☐☐☐ ☐☐ ☐☐☐

Centre # **Community #** **Household #** **Subject #**

F M L

☐☐☐☐ ☐☐ ☐☐

Today's date: *year* *month* *day*

1. Name: _____
_____

2. Not applicable in South Africa

3. National identity # or equivalent _____ **N/A** ☐

4. DOB: ☐☐☐☐ ☐☐ ☐☐ **OR Age** ☐☐ **years**

5. Sex: ☐ Female ☐ Male

6. What day was yesterday? (tick correct one)

Monday	Tuesday	Wednesday	Thursday	Friday	Saturday	Sunday

7. Would you describe the food that you ate yesterday as typical of your usual food intake?

Yes	1	No	2

Greetings!

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 are eat and drink. This information is important to know as it will tell us if people are eating enough and if they are healthy.

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?

I want to first ask you a few general questions about your food intake, the preparation of food and the type of food that you use in your home.

Instruction

Circle the subject's answer.

8. What type of pot do you usually use to prepare food in? (may answer more than one)

Iron pot …… ☐ **1**

Stainless steel pot …… ☐ **2**

Aluminium pot …… ☐ **3**

Glass ware …… ☐ 4

Other (specify) …… ☐ **5**

9. Do you eat maize meal porridge? **1** Yes **2** No

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

Brand name:

_____

Don't know: _____ **2**

Grind self: _____ **3**

If brand name is given, do you usually use this brand? **1** Yes **2** No **3** Don't know

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**

10. Do you eat fat/margarine or use it in the preparation of food? **1** Yes **2** No

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

Brand name:

_____

Don't know: _____ **2**

If brand name is given, do you usually use this brand? **1** Yes | No | Don't know

11. Do you use oil in the preparation of food? Yes | No

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

Brand name:

_____

Don't know: _____ **2**

If brand name is given, do you usually use this brand? **1** Yes **2** No | Don't know

What type of oil do you buy for deep frying?

Brand name:

_____

Do you use the same oil more than once? Yes | No

If yes, how many times will you use the same oil?

_____

12. What type of salt do you use?

Give brand names

_____

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, chips, fritos, biltong, dried sausage, etc

Very much	Like it	Not at all
1	2	3

13. Do you use any of the following:

	Name of product	Amount per day
Vitamins/vitamins and minerals		
Tonics		
Health foods		
Body building preparations		
Dietary fibre supplement		
Other: Specify _____ _____ _____		

I want to find out about everything you ate or drank yesterday, including water or food you pick from the veld. Please tell me everything you ate from the time you woke up yesterday up to the time you went to sleep. I will also ask you where you ate the food and how much you ate.

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

Time	Place	Description of food and preparation method	Amount	Amount in gram	Code (office use)