A COMPARISON BETWEEN THE EFFECTS OF BLACK TEA AND ROOIBOS ON THE IRON STATUS OF PRIMARY SCHOOL CHILDREN

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PREFACE

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ABSTRACT

Background: Clinical studies have shown that tea consumption leads to decreased iron absorption. This finding is however, not supported by epidemiological studies, where no relationship between an increased tea consumption and a lower iron status in a population at risk of iron depletion has been found.

Objectives: The main aim of this study was to compare the effects of black tea and Rooibos consumption on the iron status of primary school children in a rural setting in Potchefstroom, South Africa.

Methods: One hundred and seventy five children, aged six to fifteen years, participated in this single blind, randomised, parallel intervention trial. Subjects were randomly allocated to receive two 200ml servings of either black tea or Rooibos with milk and sugar. These beverages were consumed during breaks and at the same time as the food from the school-feeding scheme. The trial proceeded for sixteen weeks. The children received antihelminthic treatment (500mg mebendazole) at baseline. Haemoglobin, haematocrit, serum iron, ferritin and transferrin were measured and total iron binding capacity and transferrin saturation were calculated. Trained fieldworkers measured dietary intakes by means of 24-hour dietary recalls and anthropometrists took anthropometric measurements. All the above mentioned data were gathered at the beginning and at the end of the intervention period.

Results: Measurements indicated a study population that is malnourished in terms of anthropometrical indices and nutrient intakes. Biochemical markers of iron status also indicated that the population could be at risk of iron depletion. Changes in red blood cell count, haemoglobin, haematocrit, mean corpuscular haemoglobin (MCH), mean corpuscular volume (MCV), serum iron, transferrin, transferrin saturation, ferritin and total iron binding capacity (TIBC) did not differ significantly between the two groups. Mean red blood cell count, haematocrit, MCV, transferrin and TIBC increased significantly from baseline to end in both groups (all $p<0.0001$) and MCH decreased significantly ($p<0.0001$). Mean haemoglobin increased significantly with black tea consumption ($p=0.002$), although not with the consumption of Rooibos ($p=0.073$).

Conclusion: Black tea or Rooibos consumption has similar effects on the iron status of primary school children. Iron status was not compromised by black tea in comparison with Rooibos. This questions the proposed limitation of black tea consumption as a public health strategy in order to combat iron deficiency in a population with marginal iron status.

Key words: Black tea, Rooibos, iron, haemoglobin, ferritin, red blood cell count, haematocrit, mean corpuscular volume, mean corpuscular haemoglobin, serum iron, transferrin, transferrin saturation, total iron binding capacity.

OPSOMMING

Agtergrond: Kliniese studies het getoon dat tee-inname tot verlaagde ysterabsorpsie lei. Hierdie bevinding word egter nie deur epidemiologiese studies ondersteun nie. Laasgenoemde studies kon geen verwantskap vind tussen verhoogde tee-inname en 'n laer ysterstatus onder 'n populasie met 'n hoë risiko vir ysteruitputting nie.

Doelwitte: Die hoofdoel van hierdie studie was om die effekte van swart tee en Rooibos met mekaar te vergelyk met betrekking tot die ysterstatus van laerskoolkinders in 'n plattelandse opset in Potchefstroom, Suid-Afrika.

Metodes: Een-honderd-vyf-en-sewentig kinders, met ouderdomme wisselend van ses to vyftien jaar, het deelgeneem aan hierdie gerandomiseerde, paralelle intervensiestudie. Die kinders was ewekansig verdeel in twee groepe, waarvan elk twee maal per dag 200ml onderskeidelik swarttee of Rooibos met melk en suiker ontvang het. Hierdie drankies was aangebied tydens pouses tesame met die voedsel soos bepaal vir die skoolvoedingskema. Die intervensie het sestien weke geduur. Die kinders het ontwurmingsmiddels (500mg mebendasool) ontvang by aanvang van die studie. Hemoglobin, hematokrit, serumyster, ferritien en transferrien is gemeet en totale ysterbindingskapasiteit en transferrienversadiging is bereken. Opgeleide veldwerkers het dieetopnames gedoen by wyse van die 24 uur-herroepmetode. Antropometriese status is bepaal deur gekwalifiseerde antropometriste. Al bogenoemde veranderlikes is bepaal by aanvang sowel as by afsluiting van die intervensie.

Resultate: Opnames het 'n wangevoede populasie in terme van antropometriese veranderlikes en nutrientinnames, getoon. Biochemiese merkers van ysterstatus het ook getoon dat die populasie 'n risiko het vir ysteruitputting. Veranderinge in rooibloedseltelling, hemoglobien, hematokrit, gemiddelde korpuskulêre volume (GKV), gemiddelde korpuskulêre hemoglobien (GKH), serumyster, transferrien, transferrienversadiging, ferritien en totale ysterbindingskapasiteit het nie merkwaardig verskil tussen die twee groepe nie. Gemiddelde rooibloedseltellings, hematokrit, GKV, transferrien en totale ysterbindingskapasiteit het betekenisvol toegeneem in die tydperk onder beide groepe ($p<0.0001$) en GKH het betekenisvol verlaag ($p<0.0001$). Gemiddelde hemoglobien het betekenisvol toegeneem onder die swarttee drinkers ($p=0.002$), maar nie onder die Rooibosdrinkers nie ($p=0.073$).

Gevolgtrekking: Die inname van swarttee of Rooibos het soortgelyke effekte op die ysterstatus van laerskoolkinders gehad. Ysterstatus is nie nadelig beïnvloed deur swarttee-inname waneer dit vergelyk word met Rooibos nie. Hierdie bevinding bevraagteken die voorgestelde inperking van swarttee-inname om ystertekort binne 'n populasie met marginale ysterstatus te bekamp.

Trefwoorde: Swarttee, Rooibos, yster, hemoglobien, ferritien, rooibloedseltelling, hematokrit, gemiddelde korpuskulêre volume, gemiddelde korpuskulêre hemoglobien, serumyster, transferrien, transferrienversadiging, totale ysterbindingskapasiteit

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LIST OF ABBREVIATIONS

CDC	Centers for Disease Control
CI	Confidence interval
H:A	Height for age
Hb	Haemoglobin
Hct	Haematocrit
IDA	Iron deficiency anaemia
kJ	Kilojoule
MCH	Mean corpuscular haemoglobin
MCHC	Mean corpuscular haemoglobin count
MCV	Mean corpuscular volume
NFCS	National Food Consumption Survey
NCHS	National Center for Health Statistics
P	Statistical significance
pH	percentage hydrogen
RBC	Red blood cell
RDA	Recommended daily allowance
RDW	Red cell distribution width
RE	Retinol equivalents
SAVACG	South African Vitamin A Consultative Group
TFN	Transferrin
THUSA BANA	Transition and Health during Urbanisation in South Africa in Children (Bana;Setswana word for children
TIBC	Total iron binding capacity
TS	Transferrin saturation
TSF	Transferrin
UNICEF	United Nations Children's Fund
UNU	United Nations University
W:A	Weight for age
WHO	World Health Organisation

CHAPTER 1: Introduction

1.1 Background

Black tea derived from the dried leaves of the plant *Camelia sinensis*, is a popular drink consumed daily by millions of people across the world and was first discovered in China (Higdon & Frei, 2003). Rooibos, on the other hand, originates from the leaves and stems of the indigenous South African plant *Aspalathus linearis* (Bramati *et al.*, 2002). Anti-oxidative activity had been attributed to these two beverages, based on their flavonoid contents, yielding beneficial health effects that have been demonstrated in both animal and human studies (Yang *et al.*, 2000; Bramati *et al.*, 2002; Cabrera *et al.*, 2003; Higdon & Frei, 2003).

Black tea contains tannins, which forms part of the polyphenol group. Tannins have been demonstrated to form insoluble complexes with non-haem iron within the gastro-intestinal tract, rendering the non-haem iron unavailable for absorption. Polyphenols, therefore impair the bioavailability of non-haem iron when consumed with meals (Brune *et al.*, 1989; Hurrell *et al.*, 1999). This finding is supported by many clinical studies (Disler *et al.*, 1975; Hallberg & Hulten., 2000; Reddy et al., 2000; Dufresne & Furnworth, 2001), but other studies had conflicting results (Farkas & Le Riche, 1987; Powel, 1994; Hollman *et al.*, 1997; Trevisanato & Kim, 2000).

Rooibos is caffeine-free and has a low tannin content. These characteristics of the beverage have increased the popularity of Rooibos as a health beverage (Bramati *et al.*, 2002). Furthermore, it has been reported that Rooibos does not have a deleterious effect on iron absorption, when compared to black tea (Hesseling *et al.*, 1979).

The aim of this study was, therefore, to determine the effects of regular black tea or Rooibos consumption on the iron status of primary school children at risk of iron deficiency.

1.2 Problem statement

Results from the Thusa Bana study indicated that school children between the ages 10 and 15 years consumed approximately 350ml black tea per day (Kruger, 2003). It has been shown that tea consumption inhibits iron absorption, although the practical significance of the effect on iron status is not known. Vorster *et al.* (1997) have reported low total dietary intakes of iron among South African school children. The National Food Consumption Survey (Labaradarios *et al.*, 1999) pointed out that at national level, 25-37% of South African children had an intake of less than half of the recommended level of iron intake, whereas children having an iron intake less than two-thirds of the recommended

daily allowance, ranged between 36 and 57%. As quoted by Soekarjo *et al.* (2001), iron deficiency is associated with impaired psychomotor development and cognitive function and may lead to a deficit in work performance. It is, therefore, necessary to identify factors contributing to the development of iron deficiency among children and to detect and correct these factors where possible.

1.3 Objectives

The main aim of the study was to compare the effects of black tea and Rooibos consumption on the iron status of primary school children in a rural school in South Africa. Specific objectives were:

- Assessment of the iron status of the participants at baseline and after 4 months of the consumption of 400ml black tea or Rooibos, respectively, in a randomised, parallel study
- Assessment of the anthropometric nutritional status of the children
- Assessment of the nutrient intakes of the children during the study period

1.4 Structure of the dissertation

The effects of black tea and Rooibos consumption on the iron status of school children is compared and explored in this dissertation.

In Chapter 2, micronutrient deficiencies, especially with regard to iron and iron metabolism; an introduction to black tea and Rooibos; the claimed health effects of tea and the association between tea consumption and iron status are examined in a literature review. Chapter 3 provides a detailed explanation of the methodology that was used in this study. The results obtained are presented in Chapter 4 and the discussion in Chapter 5. The conclusion and recommendations follow in Chapter 6

A complete reference list is provided in alphabetical order and all questionnaires used to obtain information during the course of the study are included in the Addendum section.

CHAPTER 2
Tea consumption and iron status: A review of the literature

2.1 The concern of micro nutrient deficiencies

According to the World Health Organisation (1994), one of the most common nutritional deficiencies in the world is that of iron. Iron deficiency and anaemia affect over 3.5 billion people in the developing world (UNICEF/UNU/WHO/MI, 1998). Such a deficiency has been shown to prejudice cognitive ability, the immune system, physical activity and work capacity. The South African Vitamin A Consultative Group (SAVACG) conducted a survey in 1995 and found that 21% of South African children younger than 6 years suffer from anaemia. In terms of iron status, one out of ten children were iron deficient or depleted and one out of twenty had iron deficiency anaemia. Anaemia was more prevalent in the urban areas.

The National Food Consumption Survey (Labadarios *et al.*, 1999) included South African children between the ages of 1 and 9 years. It was concluded that at least one out of three children had an intake of approximately less than half of the recommended level for a number of important nutrients. Inadequate intakes of energy, calcium, iron, zinc, selenium, vitamins A, D, C and E, riboflavin, niacin and vitamin B6 were found. At national level, 25-37% of children had an iron intake of less than half of the recommended level. The corresponding percentage range for children having an iron intake of less than two thirds of the RDA was 36-57%. In the North West Province, more than 50% of the children aged 4-6 years had an iron intake less than two thirds of the RDA, whereas more than 60% of children aged 7-9 years had iron intakes lower than two thirds of the RDA.

2.2 The importance of an adequate iron intake

Iron plays a variety of roles in mammalian metabolism including functions such as serving as a metal co-factor for many enzymes. These enzymes are involved in biological functions such as:

- reversible oxygen binding to the haem-containing proteins of haemoglobin and myoglobin (which are involved in iron transport and storage)
- the stepwise release of energy by the haem-containing proteins of the mitochondrial electron transport apparatus
- the conversion of certain acids for the propagation of genetic information and the controlled interactions with molecular oxygen by haem-iron proteins
- non-haem iron-containing oxygenases (Cook & Skikne., 1992; Ponka, 1999).

These are all mechanisms in which iron plays an essential role and iron is, therefore, a very important micronutrient.

The major portion of body iron is contained in circulating red cells, therefore, anaemia is considered the major liability of iron deficiency. However, even in developing countries, anaemia severe enough to produce symptoms such as weakness, shortness of breath and dizziness, is relatively uncommon (Cook *et al.*, 1997).

Several stages of iron depletion lead to an incremental involvement of different body systems. These include some cerebral changes soon after the reserve of iron is depleted and a decreased activity in nutrient-dependent enzymes. This can be followed by constraints in motor development and physical activity, secondary to a drop in the oxygen supply to muscle fibers. Finally, severe anaemia would alter higher cognitive functions (Pollitt, 2001).

Anaemia has been associated with numerous detrimental effects on physical and mental development of young children and is irreversible when iron therapy is started after the critical period for development has elapsed (Kretchner *et al.*, 1996). Iron deficiency, with or without anaemia, interferes with learning capacity and school achievement, growth and appetite in older children and adolescents (Soekarjo *et al.*, 2001). In a review of studies, as reported by Grantham-McGregor & Ani (2001), longitudinal observation studies showed that haemoglobin levels in early childhood were linked to cognitive development or school achievement in later childhood. Formerly anaemic children continued to be at a developmental disadvantage at one or more of the follow-up assessments.

Pre-school skills, fine and gross motor skills and visual-motor integration were also affected by a poor iron status. Anaemic children had worse performances, but came from more deprived environments than the non-anaemic children. These differences were reduced to writing, reading, arithmetic, motorskills, spatial memory and, in the older children, selective attention. Thus, children anaemic in childhood continue to have poor cognitive and motor development and school achievement into middle childhood. An increased mortality accompanied by an impaired immune function is also attributed to anaemia (Fishman, 2000). Through these effects, anaemia has a major hampering effect on the economic growth of many developing countries (Soekarjo *et al.*, 2001).

Experimental and clinical studies suggest an increased risk of infection during iron deficiency. Iron is essential for proper cell differentiation and cell growth. In addition, iron is a component of many enzymes that are critical for the proper enzymatic functioning of immune cells (Hershko, 1996). *In vitro* studies show an unfavourable effect of iron deficiency on human T-cell and phagocyte function (Walter *et al.*, 1997). Iron deficiency from dietary restriction reduces the T-lymphocyte proportion in the blood, although the absolute number of T-cells can be either reduced or unchanged (Vyclyborets,

2000). Iron deficiency due to blood loss does not reduce the proportion of T-cells, but does decrease the total T-cell numbers. The discrepancy between the two types of iron deficiencies is probably related to the long period of time required for the development of iron deficiency by dietary restriction as compared with blood loss (Beard, 2001). Additionally, bacteria require iron for growth and increased iron availability enhances bacterial virulence. Less iron is available for bacteria during infection, due to the iron shift that occurs as part of the acute-phase reaction. The shift involves rapid decrease in serum iron, with a fall in transferrin saturation. The latter could compete for available iron sources and, therefore, contribute to an inhibition of microbial growth and a decreased virulence (Walter *et al.*, 1997).

2.3 Sources of dietary iron

Dietary iron is available in two valence states, namely Fe^{2+} (ferrous) and Fe^{3+} (ferric). Ferrous iron is found mostly in haem iron, while the ferric iron is found in non-haem iron (Cook, 1990; Lopez *et al.*, 2002).

Haem iron is found in meat products as it is present in the haemoglobin and myoglobin of animals. This form of iron is relatively available and 20-30% of haem iron is absorbed from the diet. The level of haem iron absorption can, however, rise to 40% in the case of iron deficiency. Haem iron absorption is relatively unaffected by other dietary factors (Lopez *et al.*, 2002).

Non-haem iron is found in plant foods such as cereals, vegetable pulses, dried fruit etc. and compared to haem iron, it is typically absorbed less than 10% and often less than 5% (Cook, 1990; Lopez *et al.*, 2002).

2.4 Cellular iron metabolism

The major processes responsible for modulating iron homeostasis are intestinal absorption, interorgan transport and uptake and cellular utilisation. A number of proteins facilitate the transport, uptake, use and storage of iron (Eisenstein & Blemings, 1998).

Once absorbed from the bowel, iron is transported across the mucosal cell to the blood, where it is transported by the protein transferrin to developing red cells in the bone marrow. Iron is stored mostly in the liver as ferritin, a reliable and accessible source of iron (Frewin *et al.*, 1997). Therefore, decreased iron stores are reflected by decreased serum concentration of ferritin (Uthman, 1998). If the storage capacity of iron in ferritin is exceeded, a complex of iron with phosphate and hydroxide forms, namely hemosiderin, which is physiologically available (Baggott & Dennis, 1997).

Figure 2.1 gives a schematic diagram of the intestinal absorption of iron. Dietary iron is absorbed across the mucosa mainly in the ferrous form (Ponka, 1999; Anderson, 2000). This reduction is favoured by the low pH (percentage hydrogen) and is important because ferrous iron dissociates more easily from ligands than ferric iron (Baggott & Dennis, 1997). Once entering the alkaline rich cytosol of the duodenum, the ferrous iron is enzymatically removed from the ferro-porphyrin complex. The free iron combines immediately with the protein apo-ferritin to form ferritin. The latter is an intracellular store, which also carries bound iron from the mucosa to the basolateral membrane of the absorbing cell by means of an active transport mechanism. The iron is released into the bloodstream (Ponka, 1999; Anderson, 2000) where it is re-oxidised to the ferrous form by the enzyme ferroxidase II (Baggott & Dennis, 1997; Uthman, 1998).

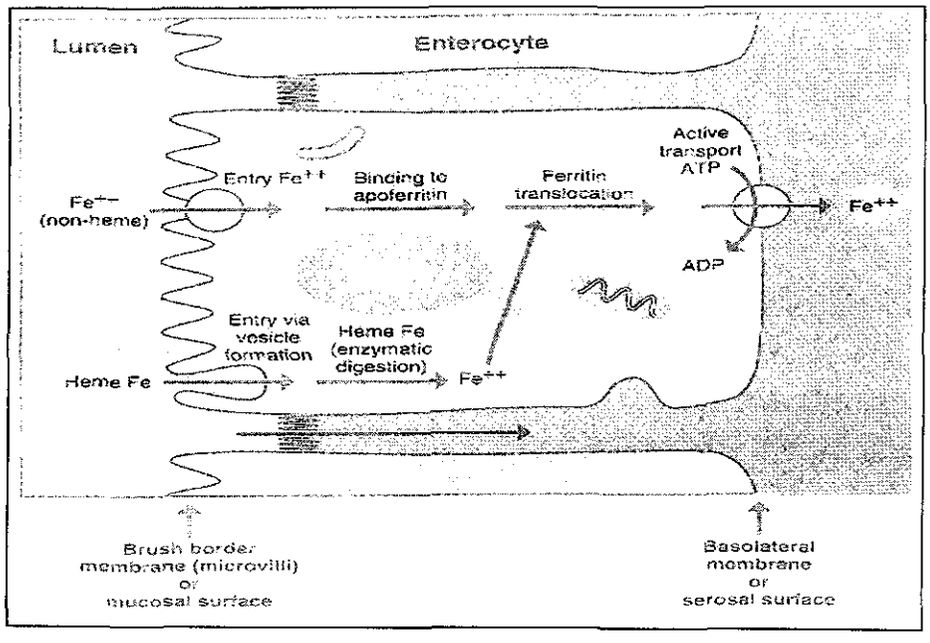


Figure 2.1 Diagram of the intestinal absorption of iron (Anderson, 2000)

Once in the blood, iron is transported bound to transferrin to the various areas of use, as illustrated in Figure 2.2 (Anderson, 2000). Iron can be stored in the muscle as myoglobin, used in the bone marrow for erythropoiesis or stored in the liver, spleen or bone marrow by means of the reticulo-endothelial system. Haemoglobin is formed in the process of erythropoiesis. This haemoglobin is then used mainly for either cellular functions, such as cell respiration; in oxygen transport in the muscle via myoglobin or stored in the liver, spleen, bone marrow or the R-E system. Iron loss occurs in urine, sweat, bile, and faeces or due to haemorrhage. Part of the synthesis of haemoglobin includes protoporphyrin, as it is a precursor of haem. It accumulates in the form of free erythrocyte protoporphyrin when the amount of haem that can be produced is limited by iron deficiency. An increase in the concentration of this variable occurs just before the onset of anaemia and is more sensitive as an early sign of iron depletion than haemoglobin (Lee & Nieman, 1993; Deakin, 2000).

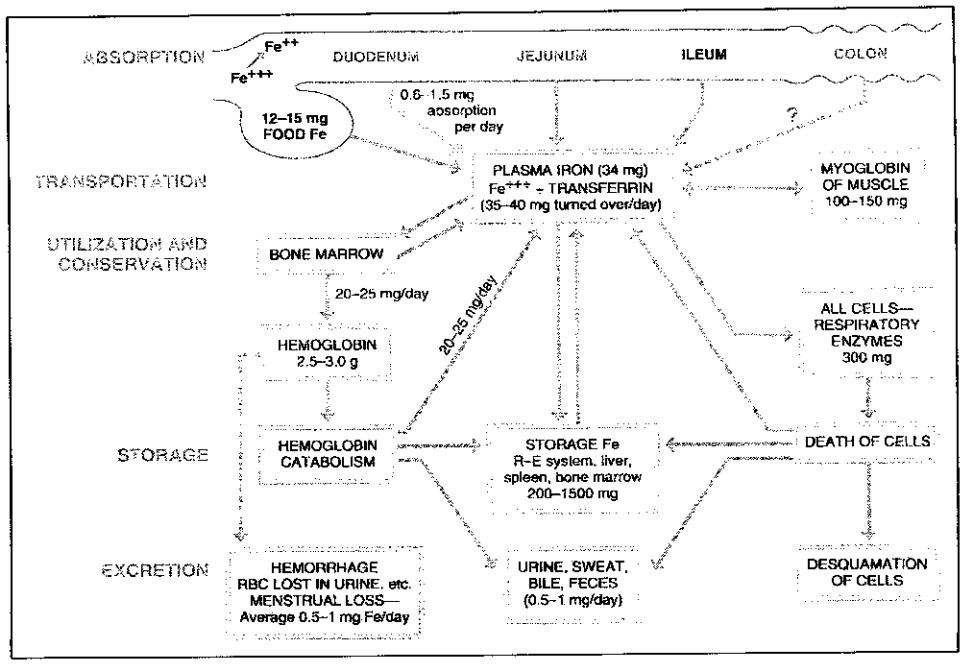


Figure 2.2 Schematic diagram of iron metabolism (Anderson, 2000)

2.5 Manifestations of iron deficiency

An inadequate diet may cause anaemia, low serum ferritin levels and low mean cell volume. Low haemoglobin levels combined with normal or high serum ferritin concentration is indicative of anaemia of chronic disease. Serum transferrin can distinguish between iron deficiency anaemia and anaemia of chronic disease and is useful in determining the true prevalence of iron deficiency anaemia (Bijlsma, 2001).

Iron deficiency can be defined as an insufficient supply for optimal physiological production and function of iron dependant cellular components, where transferrin saturation and ferritin are measurable variables for recognition (WHO, 2001). Iron deficiency represents a spectrum ranging from iron depletion to iron deficiency anaemia. Iron deficiency leads to impaired haemoglobin production that leads to iron deficiency anaemia (WHO, 2001). Iron deficiency occurs in three stages:

1. Decreased iron stores without effecting essential body iron. This is marked by low ferritin levels. Iron depletion is characterised by a reduction in the amount of stored iron, as measured by serum ferritin (CDC, 1998). In this stage, functional iron may not be affected and no physiological impairments are caused, but a person has no iron stores to mobilise if the body needs more iron

2. When the stored iron is depleted and iron supply to the transport protein, apo-transferrin is compromised, inadequate iron is available to erythroid bone marrow and tissue for normal biochemistry and functions. This is known as iron deficiency erythropoesis. Abnormalities in

physiological functions may occur (such as lowered work capacity). This stage is characterised by low ferritin, reduced transferrin saturation, increased transferrin, high levels of erythropoetin and an increased transferrin receptor count. Haemoglobin may also be reduced (mild anaemia) and increased mycrocytic cells may be present (Lee & Nieman, 1993). The second stage of iron deficiency erythyropoeisis indicates iron deficiency or functional iron deficiency. In early stages iron deficiency erythropoiesis is difficult to detect as traditional laboratory parameters may not have changed significantly and there's an overlap in values between normal and iron deficient subjects (CDC, 1998; WHO 2001)

3. In iron deficiency anaemia, there is a significant reduction in haemoglobin and a decrease in Mean corpuscular volume (MCV). Microcytic, hypochromic red blood cells occur. Functioning of several organ systems are affected. This is the most severe form of iron deficiency, where iron shortage leads to underproduction of mycrocytic, hypochromic red blood cells (CDC, 1998).

Several indices should be used to determine iron status (Lee & Nieman, 1993; Bijlsma, 2001). Suggested variable interpretations used in previous studies, are the following:

- Moderate to severe iron deficiency anaemia can easily be identified in children by a low MCV, a reduced serum ferritin level, a reduced serum iron, an increased total iron binding capacity, an increase in red cell distribution width values and an increase in haemoglobin after the institution of iron therapy (Oski, 1993)

- Lee & Nieman (1993) reported the use of a four-variable model, where serum ferritin or MCV and one of MCV, serum ferritin, erythrocyte protoporphyrin or transferrin saturation, are abnormal according to the cut-off values

- A decreased haemoglobin and red blood cell (RBC) production can be seen in anaemia and will be accompanied by an increased platelet count (Monteleone, 1998). The Mentzer index can distinguish between iron deficiency and thalassemia. It is calculated by dividing MCV/RBC values. If the quotient is <13, thalassemia is apparent and if it is >13, iron deficiency is more likely

- Cook & Skikne (1992) reported that iron deficiency without anaemia is most likely when serum ferritin, serum iron and transferrin saturation is low and erythrocyte protoporphyrin is increased.

Iron deficiency can, therefore, be diagnosed in various ways, depending on the available data. It is recommended to use two or three biochemical markers of iron status to diagnose iron deficiency rather than interpretation on the basis of a single indicator. Acute phase response should be eliminated during diagnosis, as it can lower certain biochemical markers (NCHS, 1991).

2.6 The absorption of dietary iron

An individual's iron status and several dietary factors can also influence dietary iron absorption (Cook, 1990; Lopez *et al.*, 2002). Figure 2.3 illustrates some of the features of iron metabolism and erythropoiesis, with emphasis on the points in the process at which certain vitamins may influence iron deficiency and anaemia. Vitamins such as vitamin A, folic acid, vitamin B12, riboflavin and vitamin B6 are essential for the production of RBC, while vitamins C and E have protecting effects on mature RBC from premature destruction by free radical oxidation. Riboflavin, vitamin A and vitamin C may also improve intestinal iron absorption and facilitate its mobilisation from body stores, thereby preventing anaemia (Fishman *et al.*, 2000). A more in depth discussion on the influence of vitamins on iron status will follow.

Haem iron is protected within the porphyrin complex while in the lumen of the gut. Therefore, the type of meal has no influence on its absorption. Non-haem iron enters the intestinal mucosa in the form of a reduced ionic iron. The physio-chemical properties of the metal determine the availability of this iron pool, especially the degree in which it remains soluble within the intestinal lumen (Galàn *et al.*, 1989). Whereas haem iron is poorly affected by other dietary components, non-haem iron is absorbed in ionic form by receptors on the mucosa cells and its bio-availability varies, depending on the iron status of the subjects as well as the nature of the meal (Lopez *et al.*, 2002). It is a well-known fact that meat, poultry, fish and other seafood enhance the absorption of non-haem iron. Other factors influencing iron absorption from a meal are the rate of gastric emptying, the volume of the meal and its fat content as well as body size (Hallberg & Hylthen, 2000).

Intestinal absorption is the main and complex stage for maintaining normal mineral homeostasis. Several mechanisms exist that affect mineral absorption from the gastrointestinal tract. The bioavailability of a metal ion for mucosal absorption is dependent upon the pH of the intestinal tract. The more alkaline the lumen, the lower the rate of absorption of most minerals. It is, however, not the only interfering factor. Other nutrients, such as vitamins can have equally significant influences on mineral absorption (Lopez *et al.*, 2002).

As quoted by Ahmed *et al.* (1996), vitamin A deficiency has been associated with an impaired ability to utilise endogenous iron stores. Subjects with vitamin A deficiencies have been found to be unresponsive to dietary supplementation with iron. A study by Northrop-Clewes *et al.* (1996) showed that vitamin A may have a protective effect during iron supplementation. Garcia-Casal *et al.* (1997) and Lopez *et al.* (2002) stated that vitamin A and ß-carotene may form a complex with iron, keeping it soluble in the intestinal lumen and preventing the deleterious effect of phytates and polyphenols on

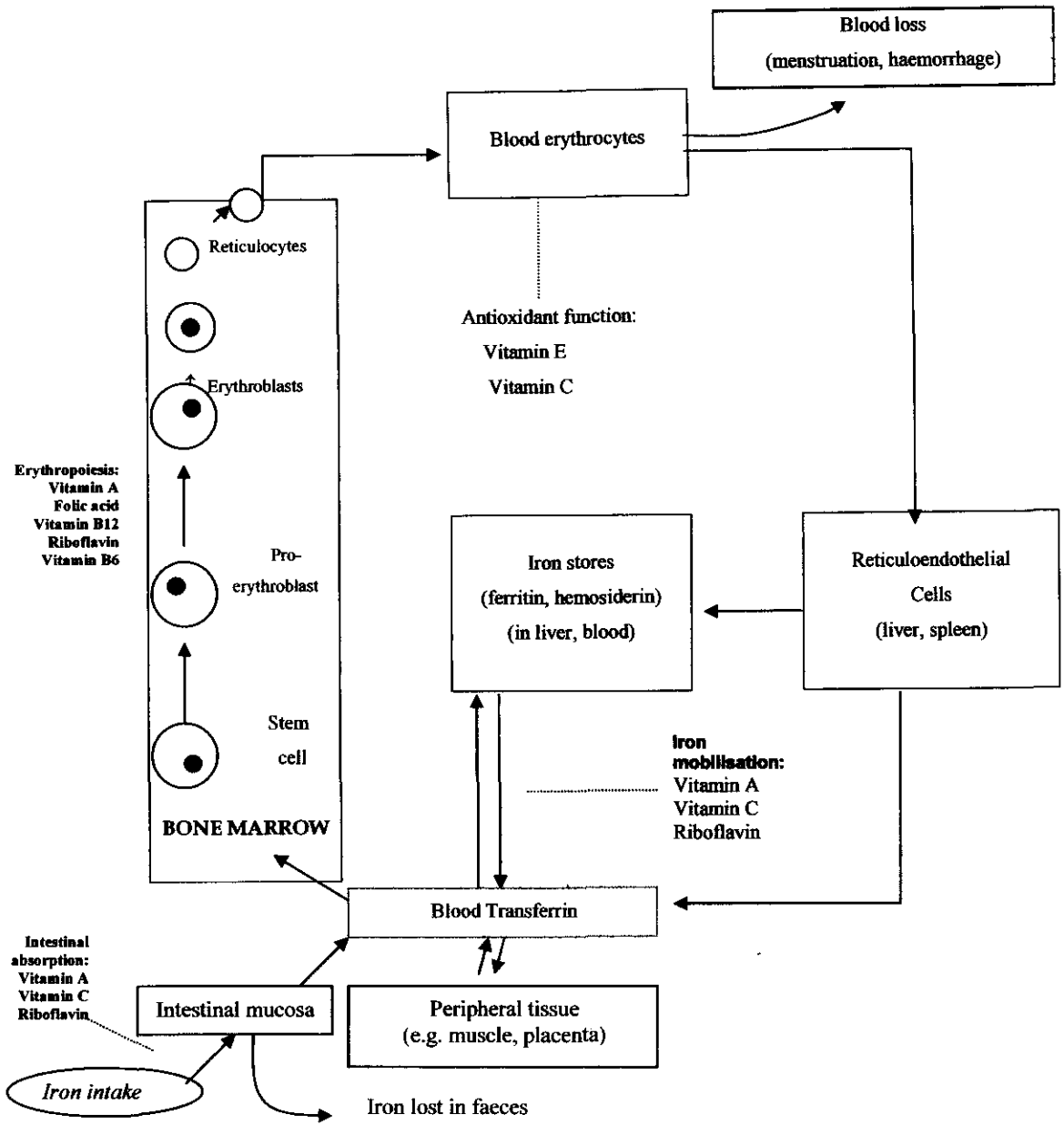


Figure 2.3 Role of vitamins in iron metabolism and erythropoiesis (Fishman *et al.*, 2000)

iron absorption. In the SAVACG study (1995), it was found that, as a group, the children with a marginal or deficient vitamin A status were at a significantly higher risk of being anaemic as well as of having iron deficiency anaemia.

The presence of ascorbic acid has an inhibiting effect on the chelating activity of tea, due to influence on the pH when adding a slice of lemon to the tea. (Trevisanato & Kim, 2000; Lopez *et al.*, 2002). Ascorbic acid (vitamin C) is found in various fruits and vegetables, such as citrus, guavas, berries, tomatoes and fresh potatoes. Even in the absence of inhibitors, ascorbic acid increases non-haem iron

absorption, although this effect is even greater the more phytate or iron-binding polyphenols are present (Hallberg & Huthen, 2000). In the aqueous acid solution of the stomach and the upper part of the duodenum, ascorbic acid reduces ferric iron to ferrous iron, which is soluble in the wide range of a pH between 2 and 11. South and Miller (1998) found that *in vitro*, the absorption reduction only took place when ascorbic acid was combined with iron before mixing with tannic acid. In addition, it also protects iron in the ferrous form of being oxidised back to the ferric form. The ferrous form forms a more stable complex (South & Miller, 1998). This iron absorption enhancing effect is dependent on the dosage and also varies with the phytic acid content of the meal (Lopez *et al.*, 2002).

Organic acids such as citric acid and lactic acid, as found in fermented products such as sauerkraut, have been found to enhance non-haem iron absorption. The lower pH in the duodenum caused by these acids helps to activate phytase in these products. Phytase is the enzyme responsible for the breakdown of phytate (Lopez *et al.*, 2002).

Phytic acid is another dietary factor with significant adverse effects on iron absorption. This compound is present in the bran of wheat, oats, maize and other cereal. In general, the hydrolysis of phytic acid increases mineral absorption. The phosphates from this hydrolysis can, however, react with iron to form insoluble precipitates (Lopez *et al.*, 2002). Hallber and Hulthen (2000) reported that iron absorption was inhibited by 18-82 % when 2mg and 250mg phytate were added to wheat rolls. Other intervention studies (Rossander-Hulten *et al.*, 1990; Siegenberg *et al.*, 1991; Brune *et al.*, 1992) also concluded that iron absorption was inhibited when phytate was added to test meals.

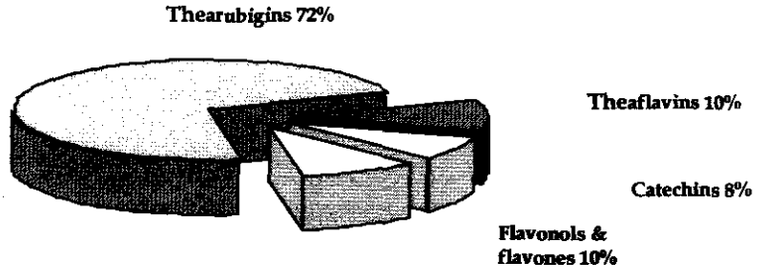
The mechanism of inhibition of iron absorption by calcium is still unclear. It was suggested that a complex formation with iron and phytate takes place. This formation may, however, rather enhance than inhibit calcium absorption by forming a calcium phytate complex (Van der Vijver *et al.*, 1999). Hallberg and Hulthen(2000) argued that, as both haem and non-haem iron absorption are inhibited, the mechanism must involve inhibition of iron extrusion from the enterocyte. Other studies suggest that calcium competes for iron-binding sites on the intestinal iron-binding protein mobilferrin (Van der Vijver *et al.*, 1999). A calcium intake of less than 40mg did not exhibit an inhibiting effect and greater than 300mg did not further decrease iron absorption (Hallberg, 1998).

It is clear that the absorption of non-haem dietary iron can be influenced by other dietary factors, including other micronutrients. Haem iron absorption is, however, relatively unaffected by other dietary factors (Lopez *et al.*, 2002).

2.7 An introduction to black tea and Rooibos

Tea is a very popular drink worldwide. Black tea is manufactured when tea leaves are withered and rolled and left to ferment. During this process, polyphenol oxidase catalyse oxidation, leading to polymerisation of catechins. Theaflavins and other undefined flavonoids are formed, providing the distinct colour and taste to the beverage (Wiseman *et al.*, 1997; Yang *et al.*, 2000; Higdon & Frei, 2003). The distribution ratio of flavonols, catechins, theaflavins and thearubugins in black tea reported differ among the studies, but it is apparent that thearubigins are the most prevalent flavonoid in the beverage, followed by catechins and theaflavins (Lakenbrink *et al.*, 2000; Arts *et al.*, 2002). Figure 2.4 illustrates the flavonoid distribution per 235ml black tea (2min brew time) (Lakenbrink *et al.*, 2000).

Figure 2.4 Flavonoid distribution of a 235ml cup of black tea (Lakenbrink *et al.*, 2000).



Tea flavonoids are polyphenols containing two aromatic rings as functional groups with two or more hydroxyl groups. Figure 2.5 illustrates the basic structure of flavonoids. Molecules with aromatic rings with two hydroxyl

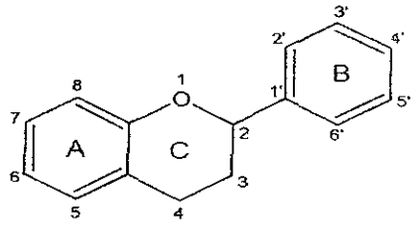


Figure 2.5 The basic structure of flavonoids (Zijp *et al.*, 2002)

groups (catechol) or three hydroxyl groups (galloyl) positioned at adjacent carbon atoms, have an *in vitro* iron binding capacity (Zijp *et al.*, 2000). Because of the strong binding affinity of tea polyphenols to metal ions, the possible effects of tea on the absorption of these nutrients is of importance.

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Rooibos (*Aspalathus linearis*) is a leguminous shrub native to the North Western Cape Province in South Africa (Morton, 1983). The leaves and fine stems of the plant are cut into 3-4 mm lengths, rolled, fermented by leaf enzymes and sun dried for the production of Rooibos tea. Rooibos tea is rich in volatile compounds, polyphenols and minerals, but is caffeine free and has a low tannin content (gallic acid) (Joubert, 1988).

2.8 Health benefits of tea

Tea contains a large amount of catechins, which is a very biologically active group of flavonoids. These are suggested to possess great anti-oxidant power, protecting cells against the adverse effects of reactive oxygen species (Dufresne *et al.*, 2001). Binding of metals ions *in vivo* is an antioxidant action preventing metal ion catalysed generation of reactive species (Halliwell, 1996). A number of studies (Lin *et al.*, 1996; Miller *et al.*, 1996 & Paganga *et al.*, 1996) reported that tea polyphenolics's (also known as flavonoids) anti-oxidative character depends on the polyphenolic compounds, the number and location of the hydroxyl groups and the presence of the galloyl moiety. This anti-oxidative character of tea polyphenols has been studied in relation to various health effects, especially in regard to cardiovascular disease and cancer risk.

Flavonoids constitute the majority of the compounds (36%) of tea leaves from the *Camellia sinensis* bush. The flavonoids can have preventative effects on cardiovascular diseases, involving homocysteine, cholesterol, artherogenesis and the protection of low-density lipoproteins (LDL) against oxidation. It enters the digestive tract, then the cardiovascular system and finally diffuses into several tissues in the human body (Keli *et al.*, 1996).

Arteriosclerosis is caused by constriction of the arteries and/or arterioles. This is achieved by several mechanisms. The role of antioxidants in these mechanisms are (Trevisanato & Kim, 2000):

1. Anti-oxidants block free radicals, preventing proliferation of smooth muscle cells that repair damage caused by free radicals
2. Free radicals also lead to damage of low-density lipoproteins, which in turn leads to fatty streaks in the blood vessel. Antioxidants prevent this low-density lipoprotein damage, minimising fatty streak formation

3. The third mechanism of arteriosclerosis involves cytotoxic activity of oxidised low-density lipoproteins, which increases platelet adherence, promoting hyperproliferation of smooth muscle cells in the vessel. This cytotoxic activity is also inhibited by anti-oxidants.

Two epidemiological studies found inverse associations (Hertog *et al.*, 1993; Nakachi *et al.*, 2000) between tea consumption and cardiovascular disease. One case-control study from Boston (Sesso *et al.*, 1999) matched controls for a significant inverse association. The risk for stroke had also been reported to be significantly lowered when more than 4.7 cups of black tea per day were consumed (Keli *et al.*, 1996). There are, however, other studies that found no significant protective effects of tea consumption on coronary heart disease mortality or stroke (Rimm *et al.*, 1996; Woodward & Turnstall-Pedoe, 1999 & Hirvonen *et al*, 2000).

Some epidemiological studies showed that tea has protective effects against certain cancers (Blot *et al.*, 1996; Kohlmeier *et al.*, 1997). Kinlen *et al.* (1988) found positive associations between tea consumption and all cancers, while Klatsky *et al.* (1993) reported no significant effects. Non-significant effects on various cancers were reported by several studies (Goldbohm *et al.*, 1996; Nagano *et al.*, 2000) and various studies reported inverse effects (Shibata *et al.*, 1994; Knekt *et al.*, 1997; Zeegers *et al.*, 2001). Mernewick *et al.* (2000) reported a weak relationship with respect to the polyphenol content of tea and the protective effect against the direct-acting mutagens. The mechanism of action of flavonoids on the digestive system can be related to the absorption of the flavonoids into the mucosal lining of the gastrointestinal tract. This could account for the blocking of heterocyclic aromatic amines from promoting gastric and colorectal carcinogenesis, therefore, protecting against the development of these cancers (Trevisanato *et al.*, 2000).

Other health effects have been studied in regard to the association between tea and osteoporosis (Kanis *et al.*, 1999, Krall & Dawson-Hughe, 1999), dental caries (Rasheed & Haider, 1998; Trevisanato & Kim, 2000) and kidney stones (Curhan *et al.*, 1996; Borghi *et al.*, 1999). Overall studies do not provide conclusive evidence for these possible health effects of black tea consumption, therefore, larger human studies are needed to examine this field.

2.9 Tea drinking and dietary iron

The inhibitory effects of tea drinking on iron absorption was first reported by Disler *et al.* (1975) in a study that used test meals fed under experimental conditions. Because of this strong inhibiting effect on non-haem iron absorption, it is expected to be associated with a poor iron status. A number of epidemiological studies have attempted to quantify the effect of tea consumption on non-haem iron absorption (Merhav *et al.*, 1985; Razagni, 1991; Metha *et al.*, 1992). The results showed that in populations with inadequate diets or higher iron needs (such as children and pregnant women) tea may

reduce non-haem iron availability to a significant extent, especially as part of a diet containing inadequate amounts of ascorbic acid. In intervention trials on humans, iron absorption from test meals was inhibited 60-70% in response to simultaneous consumption of one cup of weak, normal or strong tea (Disler *et al.*, 1975; Morck *et al.*, 1983; Brune *et al.*, 1989; Reddy & Cook., 1991). The same results were found in rat studies (Disler *et al.*, 1975; South *et al.*, 1997), where tea had a strong inhibiting effect when consumed with a meal, but had weaker negative effects when consumed in between meals. Reddy and Cook(1991) stated that a rat intestine generally absorbs more iron compared to that of a human and is, therefore, not a suitable model for extrapolating results to humans. It has, however, been proposed that human and animal studies may not reflect the role of tea when consumed as part of a complex, normal diet (Powell, 1994).

In the THUSA study (Jerling *et al.*, 2001) in South Africa, the relationship between black tea consumption and markers of iron status were investigated in a representative sample of black Africans at epidemiological level. There was no significant correlation between tea consumption and the biochemical parameters.

A major cause of iron deficiency is impaired absorption of non-haem iron in many plant foods, due to the presence of inhibitors such as phytic acid or polyphenol compounds (Hurrell *et al.*, 1999). These polyphenol compounds are widely present in fruits, vegetables, spices, pulses and cereals and they are highly prevalent in tea, coffee, red wine, cocoa and the different herb teas. These compounds retain their biological activity when immersed in hot water and readily become soluble therein (Trevisanato & Kim., 2000).

Black tea consumption has shown to inhibit iron absorption strongly from composite meals. Polyphenolic compounds are released from the food or beverage during digestion. The functional group of polyphenol compounds is an aromatic structure with one or more hydroxyl groups. Due to their different structures, they could be expected to have the most effective binding capacity with iron (Dufresne & Fernworth, 2001), especially ferric iron in the intestinal lumen and influence iron absorption in different ways. A likely metal binding site for flavonoids is the *o*-3'4'-OH group on the B ring (see Figure 2.4) (Higdon & Frei, 2003). Insoluble complexes with iron are, therefore, formed within the gastro-intestinal tract, rendering it unavailable for absorption (Hurrel *et al.*, 1999; Dufresne & Fernworth, 2001). This inhibitory effect is higher in black tea than in herb teas, cocao or wine, probably due to a higher content of galloyl esters (Hurrell *et al.*, 1999; Hallberg *et al.*, 2000).

It has been reported that the consumption of appreciable amounts of protein can largely assist in overcoming the apparent negative effects of tannins on dietary protein (Wang & Kies, 1991). The inhibiting effect of tea is reported to only be found when tea is consumed simultaneously with foods containing non-haem iron (Derman *et al.*, 1977; Lena *et al.*, 1979; Gillooly *et al.*, 1983). This issue,

however, only concerns people who consume little or no meat products who are at risk for iron deficiency anaemia (vegetarians, infants, young children, pregnant women) (Trevisanato & Kim, 2000). It has also been reported that when a long term diet low in non-haem bio-available iron content is consumed, partial adaptation occurs to maintain homeostatically body iron reserves, serum ferritin levels and a moderate faecal ferritin content. The presence of ascorbic acid can also play a role to overcome the influence of polyphenols on non-haem iron (Dufresne *et al.*, 2000).

On the other hand, some cross sectional studies found no significant association between iron consumption and ferritin or haemoglobin levels (Root *et al.*, 1999; Van der Vijver *et al.*, 1999; Cowin, 2001), even when adjustments were made for animal proteins, pulses, coffee, fruits and vegetables (Soustre *et al.*, 1986). Pate *et al.* (1986) reported a negative association between ferritin and tea consumption ($r=-0.19$). In a study conducted among the elderly, Doyle *et al.* (1999) also reported a negative association between tea consumption and haemoglobin in men. No significant association was however, found with ferritin. Both these cross sectional studies were adjusted for bioavailability factors.

As quoted by Hesseling *et al.* (1979), the absorption of cooked haem iron is not depressed by the consumption of black tea. The tannins in the tea form unabsorbable complexes in the intestines with non-haem iron. Rooibos (*Aspalathus linearis*) contains only 4.4% tannins in dilution, where black tea has 11.5 – 17.5 % tannins (Hesseling *et al.*, 1979). It was found that mean iron absorption after Rooibos, black tea and water consumption was 7.25%, 1.70% and 9.34%, respectively (Hesseling *et al.*, 1979). Rooibos, in contrast to ordinary tea ($P<0.001$), did not affect iron absorption significantly. Bearing in mind that there are no other studies to back these results, a conclusion on the effect of Rooibos on iron status can, therefore, not be drawn.

When tea without milk is consumed, the tannin combines with proteins of undigested foods in the stomach. The formed tannate is digested and the tannin is liberated when the partly digested food passes into the smaller intestine where alkaline tannates are formed. With the addition of milk to tea, the casein tannates are intact at the point of digestion, where the tannins are thought to form complexes with the non-haem iron. Alkaline tannates would not be formed, increasing the bio-availability of non-haem iron (Farkas & Le Riche, 1987).

Hurrel *et al.* (1999) stated that it had been hypothesised that some polyphenols are well known to bind strongly to certain proteins, preventing polyphenols forming complexes with iron. Their study, however, concluded that little or no improvement was found in iron absorption from the bread meal with tea when milk was added. Absorption increased only slightly from 0.71% when tea was given without milk to 0.96% when tea with milk was given. The absorption ratio was 1.04 ($P>0.05$). The explanation given is that milk is very rich in minerals. Tea would first bind to the milk-derived ions,

saturating the chelating activity, leading to dismissal of sequestration of iron from food (Trevisanato & Kim. 2000)

Hollman *et al.* (1997) reported that, although polyphenols have a strong affinity for proline rich proteins such as casein, milk, gelatine and saliva, the consumption of milk and black tea did not affect the polyphenol concentration in the blood, indicating that milk does not reduce polyphenol bio-availability. It has also been reported that the polyphenols of black tea have undergone oxidation to thearubingins and theaflavins (Wang & Kies, 1991), which do not form stable complexes with proteins. This shows that polyphenols may not affect the digestion and absorption of proteins.

Despite these differences, Hurrell *et al.* (1999) showed that any beverage containing 20-50mg total polyphenols would reduce iron absorption from a bread meal by 50-70%. Beverages containing 100-400mg total polyphenols would reduce iron absorption by 60-90%. A study conducted by Hallberg and Hulthen (2000) reported a reduction of iron absorption up to 75-80% when a 200ml serving is served with a meal containing non-haem iron. When tea was served with a meal containing 100g meat, a 50% reduction in the inhibition of iron was found.

The National Diet Nutrition Survey, conducted in London, collected information by social class, thereby showing differences in dietary intakes. It was concluded that there was no clear evidence that higher levels of tea consumption could specifically be associated with poorer iron status. Further studies in this area are required before any firm conclusions can be made (Nelson, 1994).

2.10 Conclusion

Dietary iron absorption is influenced by a number of factors including the kind of iron consumed, gastric emptying rate, body size (Hallberg & Hulthen, 2000), iron status (Lopez *et al.*, 2002) and other dietary components such as vitamin C (Fishman, 2000).

It is unlikely that tea consumption will result in iron deficiency for healthy individuals consuming a balanced diet (Hamdauoui, 1995). There is, however, clearly a lot of controversy in respect of the relation between tea consumption and iron status. Therefore, more studies in this field are needed.

CHAPTER 3: Methods

3.1 Introduction

Tea is a very popular drink among the black South Africans in the North West Province. The tea study was conducted by the School for Physiology, Nutrition and Consumer Science within the Faculty of Health Sciences of the Potchefstroom University. Demographic, anthropometric and dietary intake data were gathered and blood samples were taken to evaluate the effect of tea consumption on the iron status of primary school children and whether there are any significant differences between black tea and Rooibos in this regard. These results could be used to substantiate practical advice on drinking tea sensibly in relation to iron status.

3.2 Study design and ethical aspects

The participants were randomly assigned in this intervention study. A parallel design was chosen, as there were no specific guidelines on the wash out period needed for a cross-over tea intervention. The research was done during school hours at a rural primary school in the North West Province. The study focused on the black African population.

The school was visited during the weeks preceding the collection of data, in order to obtain permission from the principal and school governing body. Visits to the school included an explanation of the protocol to the teachers and to obtain informed consent from the parents of the children. The study was approved by the Ethics Committee of the Potchefstroom University (project number 01M07).

3.3 Subjects and enrollment

A convenience sample of all scholars 6 to 15 years old from a black rural primary school outside Potchefstroom in the North West Province, South Africa, was included. One hundred and seventy five children participated at baseline (103 boys and 72 girls), of which twenty five had dropped out to the end of the intervention, due to either leaving the school (n=8) or not being able to provide a blood sample (n=17). Ninety four boys and fifty six girls completed the study protocol. Antihelminthic tablets (500mg mebendazole) were administered to all children before commencement of the study.

3.4 Intervention

The children consumed 400ml tea daily in two 200ml-servings, during first and second break when the school feeding scheme foods were served. This feeding scheme is designed to provide 25% of the recommended daily allowances of macro and micro nutrients. It consisted of a cold drink (which was

replaced by tea during the study) and two slices of brown bread with peanut butter. The children were randomly allocated to an intervention group receiving black tea, and a control group receiving Rooibos. Rooibos has been found not to affect iron absorption significantly (Hesseling, 1979). Fresh full cream milk (40ml) and sugar (8g) was added to the drinks as this is the traditional way to drink tea. Compliance with the protocol was checked by a school teacher (recruited as a study co-worker) and students. Children received antihelmintic treatment (500mg mebendazole) at the beginning of the study.

3.5 Collection of data

Data collection took place during normal school hours at the beginning of the school year (baseline) and after 4 months' intervention. The dates of data collection were arranged with the school in advance to ensure that the children were ready when the research team arrived on the allocated day. These pre-arranged dates also served the purpose to not impact negatively on the educative programme of the school. Questionnaires were developed and different sets of fieldworkers were trained to complete the appropriate questionnaires. Each of the research teams involved in the study were responsible for training their own set of fieldworkers as used at the different stations. Each respondent received a control card at the onset of the day of data collection. These control cards were signed at each station after completion and controlled at the end of the day to ensure that all the required data were captured. The following stations were included:

- Dietary data collection (24-hour recall)
- Demographic data collection
- Anthropometry
- Blood sampling
- Motor development (reported by a M.Sc. student from the Sport Science department at the Potchefstroom University).

On the research days, the researcher was involved with the recruitment and allocation of children into their age groups and moving the children between stations and also with some of the data collection on the dietary recalls. The researcher was also responsible for overseeing the collection of dietary data and ensuring that procedures were followed that the data were complete and accurate and to provide help in this regard. For the purpose of this study, only the demographic data, dietary data and blood sampling data collected will be reported. A brief discussion on the anthromopetric data will also be included to complete the nutritional status evaluation.

3.5.1 Dietary data

The measurement of dietary data will always be accompanied by some degree of error (Lee & Nieman, 2003). In this study, dietary intakes were gathered by means of the 24-hour recall method. These dietary recalls included the consumption of the school feeding scheme foods. Trained field workers who had worked in previous epidemiological studies, administered the questionnaires after follow-up training. A 24-hour recall (Addendum A) was given by each child in the survey at two visits. A food portion photograph book previously validated for adults and food models were used to estimate portion sizes. The common 24-hour recall includes the foods and beverages consumed the previous day from awakening throughout the day in question until bedtime. Inquiries about the time of consumption and the respondent's activities associated with eating and drinking were also done as they may aid in recalling of food intakes. The method of 24-hour recall dietary assessment was used, as it is the most practical method for assessing average intakes for a large group (Young, 1981). It provides detailed information on food, requires only short-term memory and is easy to use with illiterate respondents and children.

The multiple-pass method (Jonnalagadda *et al.*, 2000) was used during the interviews. The participant was asked to provide a quick list of foods consumed during the past 24 hours (pass 1). Starting with the first food item listed, the interviewer then probed for a more detailed description of the food consumed, including amount, type, preparation method and any additional substances or sauces used (pass 2). The third and final pass of the interview included a review of the listed foods, the details of the foods and the amounts consumed. This method was used to give the interviewer an opportunity to correct any data that had been inaccurate. For double control, the researcher oversaw each questionnaire for accuracy and clarity in order to clarify any information that seemed inaccurate or incomplete, in which case the respondent was asked for elucidation.

Data were coded and computerized by the researcher alone to ensure consistency. Household portion sizes were converted to weights manually. The data were then computerized onto a nutrient analyses computer programme (Food Finder 2) based on South African food composition tables (Langenhoven *et al.*, 1993). Each food item was checked again for correct coding as it was entered onto the computer database. The data were compared to the Recommended Dietary Allowances (Food and Nutrition Board, 1989) to evaluate adequacy. Only the associated nutrients were included for this part, to limit unnecessary data. The purpose of the dietary data was to describe the iron intakes of the subjects and to compare the dietary intakes of the intervention and control groups.

3.5.2. Demographic data

A structured questionnaire (see Addendum B) was developed to obtain information regarding the family and circumstances of each of the participants. Information gathered included age, gender, home language, education level, type of housing, accessibility to water and electricity, occupation of breadwinner and other socio-economic factors. The data were used to describe the study sample, since socio-economic status may have an effect on general health and development of children.

3.5.3 Anthropometry

To standardize descriptions of position, direction and relationships of body positions, the standard and accepted anatomical body position was used throughout the anthropometric measurements. This is known as anatomical position, which in terms refers to a person standing in an erect position with the arms at the sides, hand palms and feet to the front. The anthropometry data form used in this study can be seen in Addendum C.

In measurements of height, the head had to be kept in the Frankfort plane. This position is achieved when the lower edge of the eye socket (orbital) is in the same horizontal plane as the notch superior to the tragus of the ear (the tragion).

Two anatomical landmarks were used to determine the midpoint of the upper-arm where the triceps skin fold thickness is measured. These include the acromiale and the radial. The acromiale landmark refers to the point on the superior-lateral border of the proximal head of the radius. The radial is the point at the proximal and lateral border of the head of the radius. Both anatomical locations are found when the subject assumes a relaxed position with the arms hanging by the sides (Gniadek, 2001).

Percentiles for height-for-age, weight-for-age and triceps skin fold thickness defined anthropometrical nutritional status. The purpose of anthropometric measurements was to describe the nutritional status of the subjects and to check for differences between the intervention and control groups.

Body weight

Trained Nutrition and Human Movement Science students weighed the subjects in their undergarments. Weight was measured to the nearest 0.1kg on a portable electronic scale (Precision, A & D Company, Japan). The subject measured stood up straight on the center of the scale without support and with the weight evenly distributed between both feet.

Stature

Stature was measured with a portable stadiometer to the nearest 0.5cm. The aim with this reading was to determine maximum standing height. The subject had to stand barefoot in an upright position, with the feet together and the heels, buttocks and upper part of the body touching the stadiometer. Body weight had to be evenly distributed between both feet and arms had to hang relaxed along the sides. The head, as positioned in the Frankfort plane, need not touch the scale (Gniadek, 2001).

The subject was instructed to take a deep breath while stretching up as far as possible without raising the feet from the floor. Measurement was taken at the end of the deep breath.

Triceps skin fold thickness

Triceps skin fold thickness was measured in triplicate to the nearest 0.1mm using a John Bull caliper (British Indicators, London). The triceps skin fold (Figure 3.1) is parallel to the long axis of the arm, midway between the acromiale and radial landmarks on the posterior area of the upper arm.

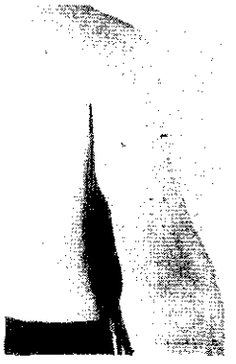

Figure 3.1 The triceps skin fold

The skin fold was grasped and lifted so that the double fold of skin and underlying subcutaneous tissue was held between the thumb and index finger. The nearest edge of the contact faces of the caliper were placed 1 cm away from the edge of the thumb and forefinger, as too deep or too shallow placement may lead to incorrect recording. Measurement was recorded two seconds after the pressure of the full pressure of the caliper was applied. The mean of three measurements per individual were used to describe the anthropometric status for each participant.

3.5.4 Blood sampling and analysis

Venous blood samples (10 ml) were drawn in the morning from each of the subjects. A qualified nursing sister, using a sterile butterfly infusion set (Johnson and Johnson, 21G, 19mm) and syringes performed this procedure. Blood samples were kept on ice during transportation to the university's laboratory. All samples were analysed at the end of the study in one batch.

For plasma, 5 ml blood was divided into EDTA-treated evacuated tubes (BD Vacutaine® SST®), and for serum, a further 5 ml blood was divided to clot in 10 ml glass tubes (Vacutaine® SST®) for no longer than two hours. The blood samples were separated by centrifugation in an Universal 16R™, HETTICH at 10°C for 15 minutes at 2000g and transferred into 1.5ml plastic microtubes (2x 0.5 ml per subject) that were stored frozen at -82°C in a Nuaire™ bio-freezer until further analyzed. Samples were analyzed for red blood cells, haemoglobin, haematocrit, serum ferritin, serum transferrin, mean corpuscular volume, mean corpuscular haemoglobin, mean corpuscular haemoglobin count, red cell distribution width, serum iron and calculation of total iron binding capacity and transferrin saturation.

Whole blood was analyzed within 2 hours of blood sampling with the use of Beckman Coulter $A^C.T^{TM}$ 5 diff Cap Pierce Hematology Analyzer (Beckham Coulter Company, USA). The machine was calibrated with $A^C.T^{TM}$ 5 diff Cal Calibrator before analysis of the samples, to ensure accurate measurements. Parameters estimated by this procedure were red blood cells, haemoglobin, haematocrit, mean corpuscular volume, mean corpuscular haemoglobin, mean corpuscular hemoglobin concentration and red cell distribution width.

Serum iron (S-Fe) was measured on a Vitros DTSC II Module and DT60 II Chemistry System (Ortho-Clinical Diagnostics, USA). A Vitros Chemistry Products DT Calibrator Kit was used to calibrate the machine before usage.

Frozen serum samples were transported in dry ice to the Clinical Research Unit at the Institute for Pathology at the University of Pretoria, where serum ferritin and transferrin were measured by means of the Beckman Coulter Synchron Clinical System LX®20 Hematology Analyser. Total iron binding capacity (TIBC) and transferrin saturation (TS) were calculated according to guidelines used by the Institute for Pathology at the University of Pretoria.

3.6. Statistical evaluation

The Statistica Programme (Version 6) was used for the statistical analyses of the data. Scatterplots were drawn to identify possible outliers. The distribution of each set of data was tested for normality before analysis, using the Kolmogorov-Smirnov & Lilliefors test for normality. Where necessary, data

were normalized using log transformations. Differences between means of variables for the two groups were assessed using appropriate parametric or non-parametric statistical methods, depending on the distribution of the data. Descriptive statistics (means, confidence intervals, medians and 25^{th} and 75^{th} percentiles) were used to present the iron status and dietary intake of the subjects. Statistical significance was reached at the 95% level ($p< 0.05$). Standard multiple regression tests were done to determine whether there was significant association between the biochemical parameters of iron status and several demographical and dietary variables and to describe variances.

3.7 Summary

In this randomized intervention which continued for 16 weeks, black tea and Rooibos consumption were compared in regard to the effects on the iron status of primary school children. A multi-disciplinary team was used to gather demographic, dietary, anthropometric and biochemical data at baseline as well as and the end of the study. The results will be presented in the following chapter.

CHAPTER 4: Research Results

4.1. Introduction

In this chapter, the research results from the performed study will be presented and discussed. As indicated in Chapter 3, the results were obtained from a convenience sample of scholars 6 to 15 years old from a rural primary school outside Potchefstroom in the North West Province, South Africa. Both genders of this mainly black ethnic group were included in the study. The initial number of participants (n=175) declined to the end of the study, due to children either leaving the school (n=8) or not being able to provide a blood sample (n=17), resulting in a final sample of 150 children. The final sample thus comprised 94 black males and 56 females.

In the first part of this chapter, the descriptive statistics of the demographic, dietary data, anthropometry and blood parameters are given. All the results and relationships between the various variables as determinants of iron status are discussed in the second part of the chapter. As there were no significant differences found between the biochemical outcomes of the two genders, results are reported without comparing the genders. Only differences between the two study groups are discussed.

4.2 Research results

4.2.1. Demographic profile of the study group

The study group consisted of school children of both genders aged 6 to 15 years, attending the Bert's Bricks Primary School in the North West Province. The sample comprised children from mainly the black ethnic group, settled in a rural and informal environment, as people from the population lived either on farms or plots of land or in areas with self-fabricated houses, mainly made from corrugated iron and wood. Not all families involved had access to running water and electricity.

Table 4.1 presents a summary of the demographic information of the children at baseline (n=175). The frequencies and percentages are also indicated. The population consisted mainly of Setswana speaking children (n=133; 87%). A great part of the population had access to water and electricity at home, although flush toilets were not readily available for all families of the group. The majority of children (n=149) reported to have a stove at home, but a large part of the group (n=100; 67%) reported not to have refrigeration facilities at home. Only eight of the involved participants reported unemployed breadwinners at home.

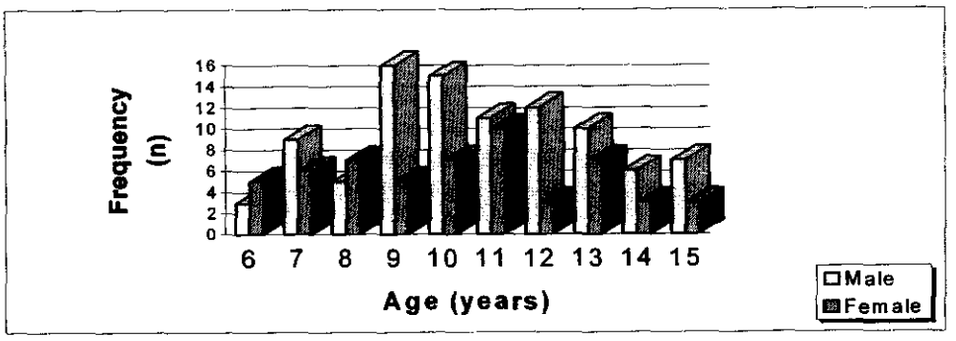

Figure 4.1 Age distribution of the study group

Table 4.1. Demographic information of the children

FAMILY ENVIRONMENT		TOTAL SAMPLE (N=150) Frequency (n)	%
First language	Setswana	133	87
	Xhosa	7	5
	Afrikaans, English, Zulu	10	7
Girls menstruating		6	11% of the 56 girls
Parental occupation	Professional	2	1
	Business/self employed/taxi	6	4
	Domestic worker/contract	135	90
	None	8	5
Type of housing	Traditional hut	2	1
	Mekhuku*	61	41
	Brick house	85	57
	Tent	2	1
Toilet facilities	Flush toilets	68	45
	No flush toilets	82	55
Water supply	Tap on premises or in house	90	60
	No tap on premises or in house	60	40
Electricity supply	Yes	90	60
	No	60	40
Type of fuel used for food preparation	None	1	1
	Coal/wood/gas/paraffin	91	61
	Electric	58	39
Refrigeration facilities	None	100	67
	Paraffin/gas	1	1
	Electric	49	33

**Mekhukhu = shack of fabricated house made mostly of corrugated iron; "tin house"*

4.2.2 Dietary information

A summary of the dietary intakes of the study group can be seen in Table 4.2. The dietary intakes of both groups showed non-significant ($p>0.05$) decreases in total iron, vitamin E and vitamin B_6, and non-significant increases in folate intakes in both groups. Vitamins C and A intakes in both groups

decreased significantly from baseline to end (both vitamins for both groups ($p < 0.0001$). Non-significant increases for intakes of haem iron in the Rooibos group and significant increases in the

Table 4.2 Mean daily dietary intakes at baseline and at the end of the study.

Variables		BLACK TEA			ROOIBOS			
		P	Median	Percentiles 25;75	P	Median	Percentiles 25;75	P variance
Energy (kJ)	B	0.161	6477	4971; 7851	0.592	6126.00	4714.0; 7557.0	0.606
	E		6078	5254; 7542		5887.00	5137.0; 7113.0	
	Δ		-521	-2111; 1241		-152.00	-1897.0; 1969.0	
Iron (mg)	B	0.083	5.05	4.00; 6.70	0.959	4.60	3.30; 6.30	0.202
	E		4.65	3.35; 5.80		4.60	3.10; 6.30	
	Δ		-3.55	-2.10; 0.95		0.15	-1.50; 1.70	
Haem iron (mg)	B	*0.017*	0.20	0.00; 0.40	0.133	0.20	0.00; 0.30	0.285
	E		0.25	0.10; 0.70		0.20	0.10; 0.40	
	Δ		0.10	-0.05; 0.35		0.10	-0.10; 0.20	
Non-haem iron (mg)	B	0.349	1.10	0.70; 2.15	0.499	0.90	0.40; 1.90	0.189
	E		1.00	0.50; 2.10		0.85	0.50; 2.10	
	Δ		-0.10	-1.30; 0.60		0.15	-1.0; 0.90	
Vit. A (RE)	B	*0.0001*	389	310; 457	*0.0001*	388	306; 479	0.488
	E		141	50.0; 275		156	56.0; 259	
	Δ		-233.5	-362; -47.0		-190	-311; -32.0	
Vit. C (mg)	B	*0.0001*	49.0	46.0; 55.5	*0.0001*	48.5	46.0; 57.0	0.372
	E		3.00	1.00; 9.00		4.00	1.0; 9.0	
	Δ		-46.0	-51.0; -38.5		-43.5	-51.0; -20.0	
Vit. E (mg)	B	0.0891	2.48	1.74; 4.26	0.216	2.42	1.51; 4.20	0.693
	E		2.18	1.66; 3.01		2.17	1.59; 3.23	
	Δ		-0.20	-2.04; 0.79		-0.20	-1.40; 0.83	
Vitamin B6 (mg)	B	0.0891	0.52	0.40; 0.68	0.974	0.45	0.35; 0.67	0.167
	E		0.48	0.35; 0.63		0.45	0.34; 0.60	
	Δ		-0.05	-0.25; 0.12		0.00	-0.17; 0.18	
Vitamin B12 (g)	B	*0.013*	1.00	0.30; 1.95	*0.009*	0.85	0.40; 1.70	0.864
	E		1.30	0.50; 2.50		1.30	0.50; 2.20	
	Δ		0.40	-0.60; 1.55		0.35	-0.20; 1.10	
Folate (g)	B	0.871	106.0	85.5; 165	0.320	97.5	72.0; 129	0.552
	E		116.5	82.0; 156		101	68.0; 157	
	Δ		0.000	-57.0; 42.0		5.50	-48.0; 56.0	
		P	Mean	±95 CI	P	Mean	±95 CI	P variance
Riboflavin (mg)	B	*0.0001*	0.73	0.74; 0.91	*0.001*	0.826	0.73; 0.92	0.804
	E		0.62	0.59; 0.72		0.835	0.45; 1.22	
	Δ		-0.14	-0.27; -0.08		0.009	-0.39; 0.41	

**CI = confidence intervals; B= baseline values; E = end values; Δ= difference between baseline and end; P=changes from baseline to end; P variances = differences between changes in the two groups. kJ = kilojoule; RE = retinol equivalents*

black tea group were found. Significant dietary increases for vitamin B_{12} in both groups and riboflavin in the Rooibos group and significant decreases for the riboflavin intake in the black tea group were seen from baseline to end. Dietary intakes between the two groups did, however, not differ significantly either at baseline or at the end of the study.

The recommended daily allowances (RDA's) are used as goals for individual intakes and are set to meet the needs of people in different age groups. When a person has an intake lower than 67% of the RDA, it can be classified as a deficient intake of the nutrient. Table 4.3 summarises the mean dietary intakes of the subjects compared to RDA's for ages 4-15 years. More than 50% of the children had deficient intakes of vitamins E, B_6 and B_{12} and folic acid at the start of the intervention. Overall these low dietary intakes showed a worsening trend, resulting in more deficient intakes of the micro nutrients and the end of the study. For all vitamins except vitamin B_{12} and riboflavin, more than 60% of the children had deficient micro nutrient intakes.

Table 4.3 Percentage of children with deficient micro nutrient intakes (<67% RDA) for their age groups *(Food and Nutrition Board, 2003)*

Micro nutrient	67% RDA	% Children consuming <67% RDA		Micro nutrient	67% RDA	% Children consuming <67% RDA	
		Baseline	*End*			*Baseline*	*End*
Iron (mg/d)				Vitamin B6 (mg/d)			
4-8 y *	6.7	37%	74%	4-8 y	0.402	31%	49%
9-15 y*	5.4	39%	99%	9-15 y	0.67	77%	77%
Vitamin A (g/d)				Vitamin B12 (g/d)			
4-8 y	268	22%	74%	4-8 y	0.804	49%	46%
9-15 y	402	57%	88%	9-15 y	1.206	61%	43%
Vitamin C (mg/d)				Riboflavin (mg/d)			
4-8 y	16.75	20%	80%	4-8 y	0.402	11%	29%
9-15 y	30.15	17%	96%	9-15 y	0.603	29%	48%
Vitamin E (mg/d)				Folate (g/d)			
4-8 y	4.69	86%	91%	4-8 y	134	77%	74%
9-15 y	7.37	91%	95%	9-15 y	201	87%	86%

**4-8 years (n=35); 9-13 years (n=115)*
RDA = Recommended daily allowance; mg/d = milligram per day; g/d = grams per day

4.2.3. Anthropometric information

Weight for age and height for age is widely used to assess protein-energy malnutrition and over nutrition. Figures 4.2 and 4.3 illustrate the weight for age (W:A), height for age (H:A) and triceps skin fold (TSF) indices of the population compared to normal percentiles as developed by the National Centre for Health Statistics in collaboration with the National Center for Chronic Disease Prevention and Health Promotion (2000).

From the figures it can be seen that a large part of the study group had low weight for age indices with 36% of the subjects in the black tea group and 47% of the subjects in the Rooibos group having weights beneath the 10^{th} percentile. The height for age measurements showed 32% of the children

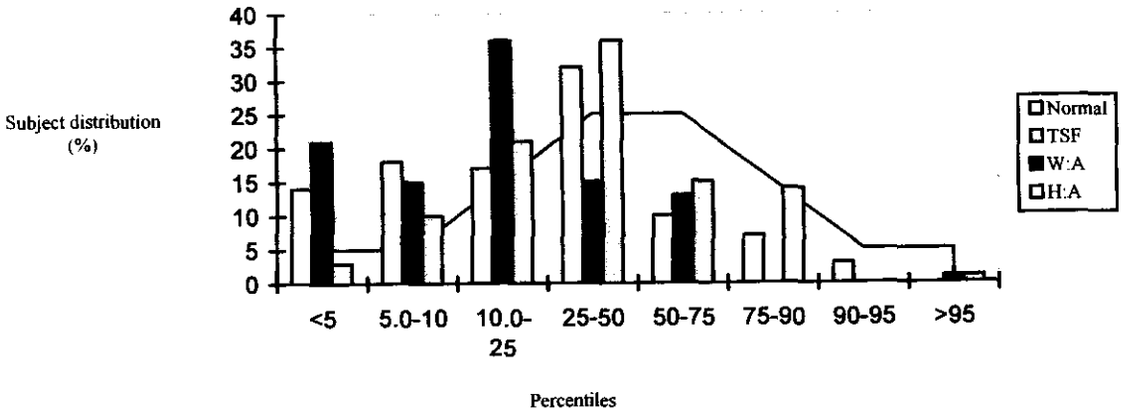

Figure 4.2 Anthropometric distribution of the black tea group

TSF = Triceps skin fold; W:A = Weight foe age; H:A = Height for age

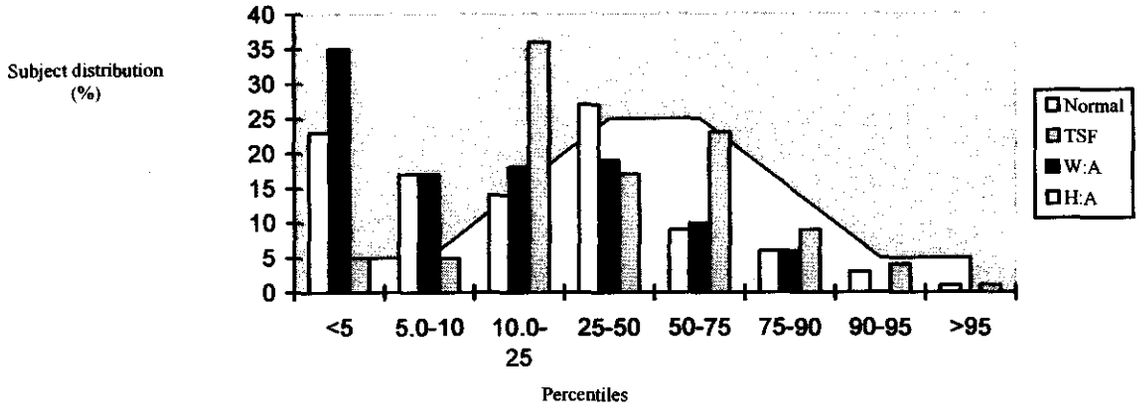

Figure 4.3 Anthropometric distribution of the Rooibos group

TSF = Triceps skin fold; W:A = Weight foe age; H:A = Height for age

consuming black tea and 40% of the Rooibos consumption group, having heights for age below the 10th percentile. Triceps skin folds were more or less normally distributed, except for the 25th to 50th percentile which was represented by 36% of the black tea group and the 10th to 25th percentile that was also represented by 36% (compared to 15% normality) of the Rooibos subjects.

4.2.4. Biochemical parameters

Table 4.4 summarises the biochemical parameters of iron status from baseline to the end of the study and shows a trend of increases for most of the variables in both groups. The increases for serum iron and transferrin saturation in both groups and haemoglobin of the Rooibos group (p=0.073), as well as

decreases in the ferritin for both groups were all non-significant within the two groups respectively. There were significant increases ($p<0.001$) in both groups in RBC count, MCV, serum transferrin , TIBC, haematocrit and in the haemoglobin of black tea consumers ($p=0.002$). Significant decreases ($p<0.0001$) in MCH and mean corpuscular haemoglobin count were found in both groups. Changes in the above mentioned serum concentrations did not differ significantly between the two groups, as can be seen in Table 4.4. Figures 4.4, 4.5, 4.6 and 4.7 present the distribution of children from the two study groups respectively, who had a combination of both low haemoglobin and low ferritin serum and blood parameters.

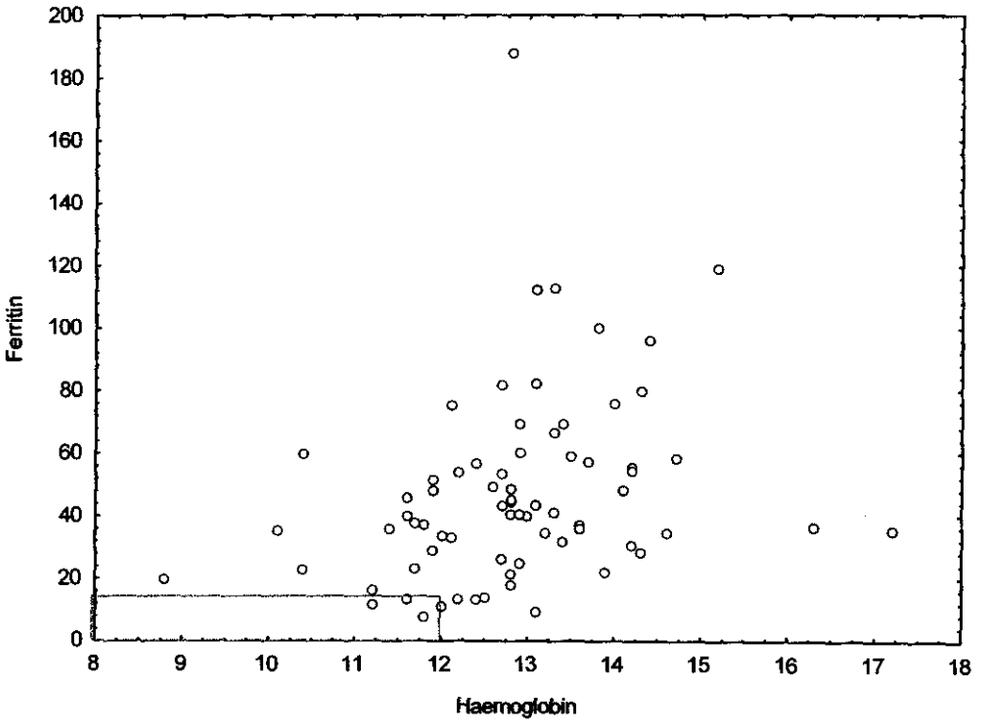

Fig. 4.4 Representation of individuals in the black tea group with a combination of low haemoglobin and low ferritin values at baseline.

Table 4.4 Serum and blood parameters at baseline and at the end of the study.

Variables		BLACK TEA (n=72)			ROOIBOS (n=78)			P	Normal	Iron	Children with deficient values
		Mean	CI ±95	P-value	Mean	CI ±95	P-value	Variance	Range	deficient	
Red blood cell (RBC) ($10^6/\mu L$)	B	4.62	4.51; 4.73	*0.0001*	4.62	4.49; 4.74	*0.0001*	0.908	4.0-5.2	#	
	E	5.26	5.14; 5.39		5.25	5.11; 5.40					
	Δ	0.64	0.50; 0.79		0.63	0.46; 0.81					
Haemoglobin (Hb) (g/dL)	B	12.8	12.5; 13.2	*0.002*	12.8	12.5; 13.1	0.073	0.601	11-16	5-11yr:<11.5 12-15yr: <12	Black tea at baseline, 13% Black tea at end stage, 1% Rooibos at baseline, 15% Rooibos at end, 8%
	E	13.2	13.0; 13.4		13.1	12.8;13.3					
	Δ	0.39	0.15; 0.64		0.29	-0.03; 0.60					
Haematocrit (Hct) (%)	B	34.9	34.1; 35.8	*0.0001*	34.8	33.9; 35.7	*0.0001*	0.945	31-43	<34	Black tea at baseline, 39% Black tea at end stage, 36% Rooibos at baseline, 44% Rooibos at end stage,46%
	E	40.5	39.5; 41.5		40.3	39.1; 41.5					
	Δ	5.55	4.40; 6.70		5.49	4.08; 6.89					
Mean Corpuscular volume (MCV) (fL)	B	75.7	74.7; 76.7	*0.0001*	75.4	74.6; 76.2	*0.0001*	0.640	80-95	<80	Black tea at baseline, 83% Black tea at end stage, 61% Rooibos at baseline, 90% Rooibos at end stage, 71%
	E	77.2	76.2; 78.2		76.8	76.0; 77.7					
	Δ	1.50	0.96; 2.04		1.66	1.25; 2.06					
Mean corpuscular haemoglobin (MCH) (pg)	B	27.8	27.5; 28.1	*0.0001*	27.7	27.4; 28.0	*0.0001*	0.603	27-31	#	
	E	24.7	24.7; 24.3		24.6	24.2; 24.9					
	Δ	-3.15	-3.15; -3.27		-3.101	-3.24; -2.96					
Mean corpuscular haemoglobin *count* (MCHC) (g/dL)	B	36.8	36.5; 36.9	*0.0001*	36.8	36.6; 37.0	*0.0001*	0.947	32-36	#	
	E	31.9	31.8; 32.1		32.0	31.8; 32.2					
	Δ	-4.81	-5.10; -4.52		-4.797	-5.02; -4.58					
Serum Iron (S-Fe) (µmol/l)	B	12.9	11.2; 14.6	0.294	13.1	11.8; 14.4	0.106	0.982	13-31	#	
	E	14.4	12.0; 16.9		14.5	13.0; 15.9					
	Δ	1.55	-1.37; 4.47		1.378	-0.30; 3.06					
Red cell distribution width (RDW) (%)	B	13.6	13.3; 14.0	0.488	13.7	13.4; 14.1	0.130	0.589		>16	Black tea at baseline, 3% Black tea at end stage, 3% Rooibos at baseline, 3% Rooibos at end stage, 4%
	E	13.4	13.2; 13.6		13.3	13.0; 13.6					
	Δ	-0.17	-0.67; 0.32		-0.357	-0.82; 0.11					
Transferrin Saturation (TS) (%)	B	22.5	19.3; 25.7	0.654	22.4	20.3; 24.6	0.408	0.059	30-40	<16	Black tea at baseline, 39% Black tea at end stage, 28% Rooibos ate baseline, 28% Rooibos at end stage, 26%
	E	23.6	19.5; 27.7		23.6	21.2; 26.1					
	Δ	1.10	-3.79; 5.99		1.17	-1.62; 3.96					
		Median	**Percentiles 25;75**		**Mean**	**Percentiles 25;75**					
Transferrin (TFN) (g/l)	B	2.58	2.41; 2.80	*0.0001*	2.54	2.36; 2.91	*0.0001*	0.574	2-9	<2.03	Black tea:No deficiencies Rooibos at baseline, 1% Rooibos at end stage, 1%
	E	2.71	2.54; 2.99		2.72	2.50; 3.06					
	Δ	0.18	0.04; 0.31		0.18	0.003; 0.28					
Ferritin (S-Fer) (µg/l)	B	40.5	28.7; 57.8	0.095	41.8	26.9; 62.8	0.499	0.466	15-200	≤15	Black tea at baseline, 11% Black tea at end, 10% Rooibos at baseline, 12% Rooibos at end, 12%
	E	38.2	23.4; 59.1		39.0	25.9; 52.9					
	Δ	-2.20	-12.7; 5.20		-0.20	-7.80; 6.70					
Total iron binding capacity (TIBC) (µmol/l)	B	57.7	53.9; 62.8	*0.0001*	56.7	52.8; 65.1	*0.0001*	0.557	45-73	>76.1	Black tea at baseline, 4% Black tea at end stage, 7% Rooibos at baseline, 9% Rooibos at end stage, 9%
	E	60.6	56.8; 67.0		60.9	56.2; 68.9					
	Δ	3.92	1.01; 6.83		3.92	0.45; 6.27					

 # No standard cut-off values for children available, CI = Confidence interval; P value = changes from base to end within groups; P variance = changes between groups

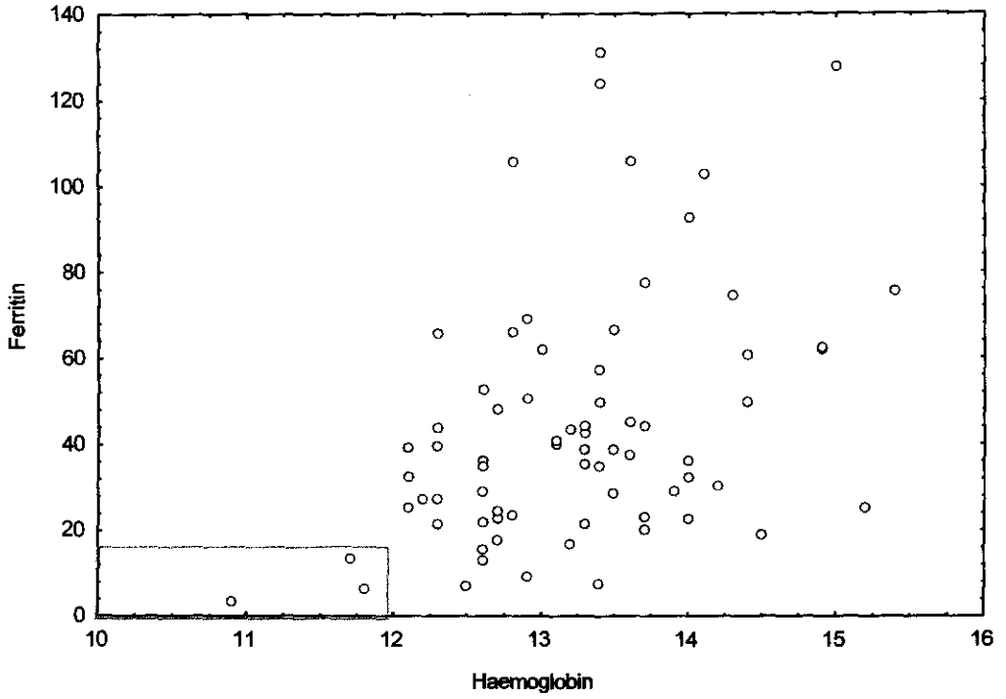

Figure 4.5. Representation of individuals in the black tea group with a combination of low haemoglobin and low ferritin values at end stage.

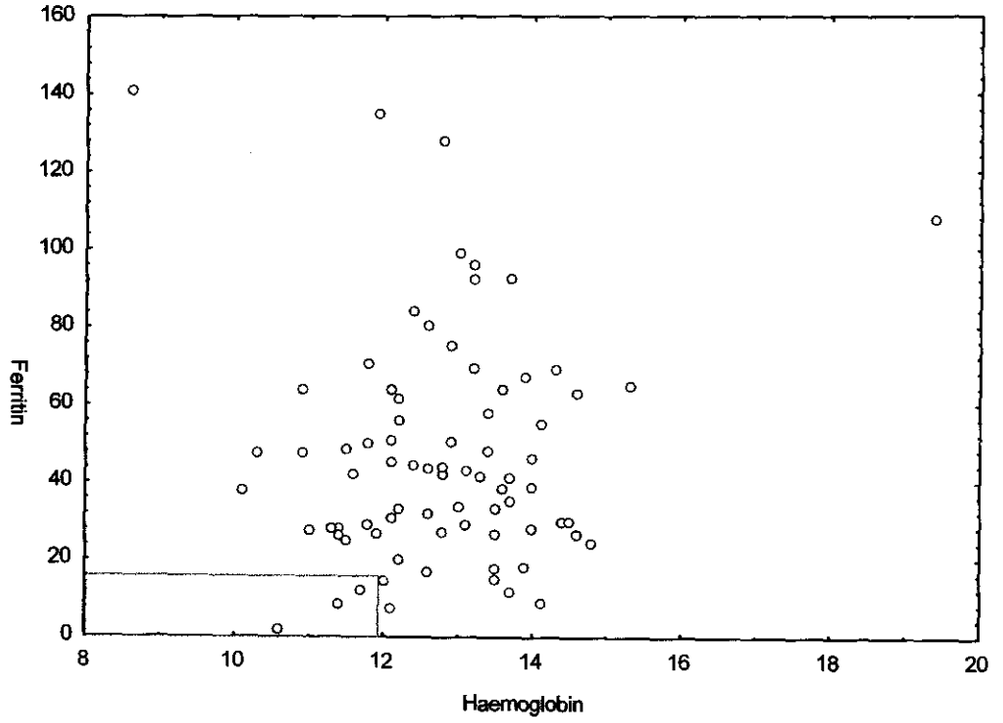

Figure 4.6 Representation of individuals in the Rooibos group with a combination of low haemoglobin and low ferritin values at baseline.

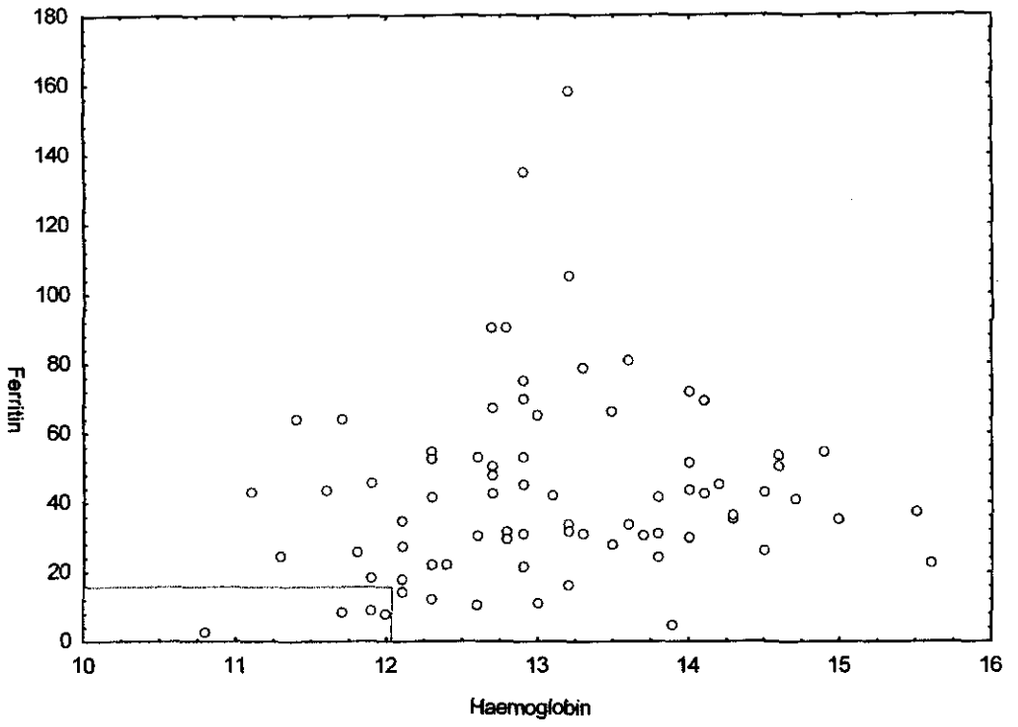

Figure 4.7 Representation of individuals in the Rooibos group with a combination of low haemoglobin and low ferritin values at end stage.

From the figures it can be seen that in the black tea group (n=72), four of the participants had low values of haemoglobin and ferritin. This number decreased to three children with low haemoglobin and low ferritin values at the end of the intervention. In the Rooibos group (n=78), there were five children with low haemoglobin and low ferritin values at baseline and four children with low haemoglobin and ferritin values at the end of the intervention.

4.2.5 Regression summary

Regression analysis of the association between iron status and demographic and dietary variables were done to determine to what extent demographic and dietary variables could predict ferritin, haemoglobin and transferrin saturation. The results are summarised in Table 4.5. Gender, age, water access and electricity were the most apparent demographic data to influence the blood parameters of this population, although still to a small extent. The beta coefficients were reviewed in order to evaluate the relative contribution of each predictor to the overall prediction of the dependant variable. The regression coefficients of water and electricity were negative, indicating that the less water and electricity were available, the greater the serum ferritin values were. All the variables entered into the regression analysis explained only 8.93% of the variability in ferritin levels, 11.8% of the variability in

Hb and 5.27% of the variability in transferrin saturation. In this study the predictive value of these variables is, therefore, very low.

Table 4.5. Summary of the association between iron status and demographic as well as dietary variables by using regression analysis.

	REGRESSION FOR DEPENDENT VARIABLES OF IRON STATUS					
	Ferritin		**Haemoglobin**		**Transferrin Saturation**	
Independent variables	$R^2 = 0.0893$		$R^2 = 0.1183$		$R^2 = 0.0527$	
	Beta	P-level	Beta	P-level	Beta	P-level
Gender	0.09	0.264	-0.13	0.117	*-0.18*	*0.041*
Age	0.07	0.448	*0.29*	*0.001*	0.08	0.367
Total dietary iron	0.01	0.961	0.13	0.213	0.02	0.883
Dietary haeme-iron	-0.11	0.292	0.01	0.945	-0.03	0.808
Dietary Vitamin A	-0.05	0.565	*0.23*	*0.005*	-0.05	0.526
Dietary Vitamin C	0.13	0.129	-0.11	0.216	0.02	0.843
Menstruation status	0.02	0.788	-0.07	0.387	0.02	0.869
Employment of the breadwinner	-0.05	0.524	-0.02	0.788	0.03	0.703
Flush toilet	0.16	0.075	-0.16	0.080	-0.01	0.931
Water supply	*-0.30*	*0.001*	0.08	0.320	0.01	0.874
Electricity supply	*-0.19*	*0.030*	-0.15	0.089	-0.10	0.313

The haematological values showed no significant differences between the changes in iron status parameters of the two groups. Possible reasons for this observation will be discussed in the following chapter.

CHAPTER 5: Discussion

5.1 Introduction

In the following section, the biochemical parameters are discussed and compared to previous literature. Possible reasons and causes for the findings of this study will be examined.

5.2 Discussion

5.2.1 Demographic data

From the demographic data it can be seen that a large part of the study group did not have water (40%) or refrigeration (67%) facilities and 55% of the subjects did not have water flush toilets at home. These factors can contribute to the prevalence of parasitic infection in a community (Fincham *et al.*, 1996). An adequate accessible water supply for drinking and for hygienic purposes is essential, as the use of sanitary facilities protects children from infections and lowers the chance of helminthic infections (Labadarios *et al.*, 1999; INACG, 2001). An important factor that might have influenced the outcome of the study is the fact that antihelminthic treatment was given to the children at baseline of the study. Parasitic infections e.g. hookworm, trichuriasis, amoebiasis and schistosomiasis cause direct blood loss, which contributes to iron deficiency (WHO, 2001). In a publication by the International Nutritional Anaemia Consultative Group (2001), it was stated that where worm infection was prevalent, anaemia is likely to also be prevalent. Jinabhai *et al.* (2001) reported in a randomised controlled study that antihelminthic treatment significantly reduced the overall prevalence of parasitic infection. This was also found by Stoltzfus *et al.* (2001). Hence, if there were children with parasitic infection, the treatment would have reduced the prevalence of the parasites, subsequently having positive effects on the iron status of the individuals.

Six girls (black tea = 4, Rooibos = 2) in the study group reported having reached menarche. Although this has an influence on the iron status of a female, the influence of this small group was non-significant in the outcome of the total study group (Table 4.5).

5.2.2 Dietary data

In Table 4.3 it can be seen that overall low intakes of many of the micronutrients were prevalent for this study group. Dietary intakes between the two groups did not differ significantly. Mean intakes of vitamins A and C decreased significantly in both groups, probably due to the seasonal availability of foods rich in these vitamins. As mean vitamins A and C and iron intakes also decreased to more

deficient intakes at the end of the intervention, one would expect that the poor diets would impair the iron status of the children, as these micro nutrients play an essential role in iron metabolism (Fishman *et al.*, 2000). In the SAVACG study (1995), children with a marginal or deficient vitamin A status were found to have a significantly higher risk of having iron deficiency anaemia. Furthermore, vitamin A is essential for the production of red blood cells and vitamin C protects mature red blood cells against oxidation and promotes iron absorption (Fishman *et al.*, 2000). It is, therefore, expected that a poor intake of these two vitamins would negatively influence biochemical parameters of iron status.

Mean vitamin B12 intake increased significantly in the black tea and Rooibos groups and riboflavin intake increased in the Rooibos group. Despite these increased intakes, the children still had deficient intakes of these micronutrients, as well as for vitamins E, B_6 and folate. These vitamins also play essential roles in the production of red blood cells. A deficient intake might impair erythropoiesis and haemolysis (Fishman *et al.*, 2000) which, in this group, could, therefore, be expected.

Though haem iron intake increased significantly in the black tea group and non-haem iron intake decreased non-significantly in both groups, total iron intake showed non-significant decreases from baseline to end. The ratio of haem to non-haem iron intakes increased in both groups (Haem : non-haem, 1:4 increased to 1:2.5 for the black tea group and ratio 1:3.66 increased to 1:3.49 for the Rooibos group). The inhibiting effect of tea is reported to only be found when tea is consumed simultaneously with foods containing non-haem iron (Derman *et al.*, 1977; Lena *et al.*, 1979; Gillooy *et al.*, 1983), as polyphenols do not have chelating effects on cooked haem iron (Galàn *et al.*, 1989). This issue, however, does only concern people who consume little or no meat products who are at risk for iron deficiency anaemia (vegetarians, infants, young children, pregnant women) (Trevisanato & Kim, 2000). The group under study consumed tea in combination with the school feeding scheme foods, which consisted of two slices of brown bread with peanut butter. This meal theoretically should contain approximately 1.1mg non-haem iron. Considering the facts discussed above, one would expect the iron to be less bio-available due to reaction with the flavonoids in the tea, resulting in a negative association with iron status over a period of time. This was, however, not the case. The fact that the total haeme iron consumption increased over the time of the study might have improved the iron status of the children. It has also been reported that when a long term diet low in non-haem iron content is consumed, partial adaptation occurs to maintain homeostatically body iron reserves, serum ferritin levels and a moderate faecal ferritin content (Merhav *et al.*, 1985; Razagni, 1991; Metha *et al.*, 1992). The latter might, therefore, also have contributed to the improvements in the relevant iron status parameters.

It is believed that when tea without milk is consumed, the tannin combines with proteins of undigested foods in the stomach. The formed tannate is digested and the tannin is liberated when the partly digested food passes into the smaller intestine where alkaline tannates are formed. With the addition of

milk to tea, the casein tannates are intact at the point of digestion, where the tannins are thought to form complexes with the non-haeme iron. Alkaline tannates would not be formed, increasing the bio-availability of non-haem iron (Farkas & Riche, 1987). Trevisanato and Kim (2000) also reported iron absorption to increase only slightly from 0.71% when tea is given without milk to 0.96% when tea with milk is given. The absorption ratio was 1.04 ($P>0.05$). The explanation given is that milk is very rich in minerals. Tea would first bind to the milk-derived ions, saturating the chelating activity so that iron from food cannot be chelated. It is clear that the addition of milk to tea does have an influence on iron absorption, but to such a small extent that it could not have carried enough weight to influence the outcome of this study.

5.2.3 Anthropometry

Weight for age in children is widely used to assess acute protein-energy malnutrition and over nutrition. A great limitation of this index, however, is that it does not take height differences into account. This indicates that children with a low weight for age are not necessarily wasted. Their low weight for age may be related to genetics or to nutritional growth failure. The latter, also known as "stuntedness", is characterised by a short stature for age but a weight appropriate for their stature. The prevalence of malnutrition in short children might, therefore, be overestimated if only weight for age is interpreted. Height for age can be used as an index for the nutritional status of a population that estimates past or chronic nutritional status. It represents a child's growth potential and identifies stunting, which can be defined as a slowing of skeletal growth and stature. Nutritional assessment based on height for age alone may result in underestimation of malnutrition, as a deficit in height takes some time to develop (Gibson, 1990).

The anthropometric results of the study group show 38% of the subjects to have normal height for age indices and 28% to have normal weight for age indices. About half of the study population (46%) had triceps skin fold measurements between the 25th and 75th percentiles, which indicates normal growth (Lewinter-Suskind *et al.*, 1993).

When measurements lie between the 10th and 25th or 75th and 90th percentiles, growth could be either normal or abnormal. Indices below the 10th percentile, however, show a risk for under nutrition, whereas values below the 5th percentile are interpreted as under nutrition and/or growth retardation (Lewinter-Suskind *et al.*, 1993). In this study group, 19% of the children appeared to be under nourished and 19% at risk for under nutrition in relation to height for age standards. Weight for age standards showed 26% under nourished children and 14% at risk for under nutrition. This correlates with a study by Monyeki *et al.* (2003) that examined primary school children living and attending schools in Potchefstroom farming areas in the North West Province. They found that the children in

their study fell below the 15th percentile when body mass indexes and sums of skin folds were interpreted, indicating a prevalence of underweight among the group. In a study performed by Schutte *et al.* (2003), which was part of the larger THUSA BANA study (Transition and Health during Urbanisation in South Africa in Children; Bana: the Setswana word for children), prevalence of under nutrition and stunted growth were also observed among these young North West Province inhabitants. A study conducted on national and provincial rural district level showed a prevalence of mild stunting ranging from 31-100% and moderate stunting from 3-25% for children 8 to 11 years old (Jinabhai *et al.*, 2003). In the neighbouring country, Mozambique, a study consisting of 2316 participants showed only 2.6% school-aged children to be stunted and 15.6% wasted (Prista *et al.*, 2003).

An inadequate diet negatively influences an individual's iron status (Bijlsma, 2001) which in turn, can positively influence dietary iron absorption (Cook, 1990). This indicates that the children could possibly have compensated for their low dietary intakes with better iron absorption.

5.2.4 Biochemical parameters

Serum ferritin is the storage protein for iron that is the first to decrease in the presence of iron deficiency. Iron stores are depleted with serum ferritin levels ≤15 µg/l (CDC, 1998; WHO, 2001). It is a sensitive and specific variable, but can be falsely elevated, as it is an acute phase reactant (Irwin *et al.*, 2001).

Serum transferrin is a plasma transport protein for iron, which can be determined as the total iron binding capacity (TIBC). TIBC can be defined as a measure of the amount of iron capable of being bound to serum protein and provides an estimate of serum transferrin (TFN) (Lee *et al.*, 1993, CDC, 1998). Transferrin saturation (TS) indicates the extent to which transferrin has vacant iron binding sites (CDC, 1998). Transferrin saturation is, however, a less reliable parameter, because of daily intra and intervariability in serum iron. A TS <16% indicates an inadequate supply for erythropoiesis (WHO, 2001). When TIBC increases and transferrin saturation decreases, serum iron will be low and iron deficiency apparent (Cook & Skikne., 1992; CDC, 1998). Iron deficiency leads to a reduction in serum iron, an elevation in transferrin and TIBC levels and hence a net reduction in transferrin saturation (WHO, 2001).

Haemoglobin and haematocrit are the most commonly used tests to screen for iron deficiency and reflect the functional iron in the body. As changes in these two parameters occur only at the late stages of iron deficiency, both tests are late indications of iron deficiency (CDC, 1998).
Serum iron concentration measurement alone provides little useful clinical information because of considerable variation from hour-to-hour and day-to-day in normal individuals (Bothwell *et al.*, 1979).

Similarly, the use of only one indicator of storage iron level or tissue iron supply (ferritin, transferrin saturation or erythrocyte protoporphyrin) may overestimate the number of cases with absent iron stores (Worwood, 1997). It is, therefore, recommended to have two or three abnormal indicators to diagnose iron deficiency or biological severity, rather than interpretation on the basis of a single indicator (NHCS, 1999).

In this study group, the mean serum iron values of the participants were on the lower edge of the normal ranges and were more or less consistent from baseline to end ($p>0.05$). The haemoglobin levels were also on the lower edge of the normal range, and mean haemoglobin of both groups increased. Serum ferritin levels were well within the normal ranges. This was also the case for the serum transferrin, TIBC and TS values of the two groups.

Earlier studies have shown Africans to have smaller red cell masses than other race groups and lower haemoglobin concentrations (Solomons, 1997), rendering haemoglobin criteria for Caucasians inappropriate for the Africans (Meyers *et al.*, 1979). Therefore, the WHO recommends cut-off values for haemoglobin 1g/dL lower to allow for this inter-racial difference (WHO, 2001). Even with this adapted criterion, there were only 4% of each study group with low haemoglobin values at baseline and only 1% of the black tea group, and no Rooibos participants at the end of the trial.

The amount of circulating red cells is termed mean corpuscular volume (MCV), and is a reliable index of haemoglobin synthesis (Cook & Skikne, 1992). MCV and mean corpuscular haemoglobin (MCH) are the two most sensitive indices of iron deficiency, measured by electronic blood counters. MCH decreases when iron reserves are depleted and iron deficiency has developed (WHO, 2001). A new and closely related parameter of the morphology of a red cell, is the red cell distribution width (RDW), which is an index of the variability of the size of the red blood cell (anisocytosis). A low MCV and an elevated RDW suggest iron deficiency (Irwin & Kirchner, 2001, CDC, 1998). Although the data showed significant increases in mean MCV, the values were still below the normal range, as indicated in Table 4. The mean MCH decreased from base to end, but were in the normal range, though on the lower edge. Low mean MCV values indicate small red cell mass as described in Africans (Solomons, 1997).

As the above mentioned variables are difficult to interpret as a whole, statistics were done to determine the number of children with low haemoglobin and ferritin blood values, as these are indicative of functional and storage iron respectively (Cook & Skikne, 1992). By combining haemoglobin and ferritin measurements, its specificity and sensitivity are greatly improved. When both variables are normal, iron deficiency is excluded and if both are low, IDA is diagnosed. In the case of a low serum ferritin along with a normal haemoglobin, iron stores may be depleted or mild

iron deficiency erythropoiesis may be diagnosed (Cook & Skikne, 1992). In the black tea group (n=72), haemoglobin levels of 13% of the children were below the cut-off ranges (Table 4.4) at baseline and 1% had haemoglobin values below cut-off range at the end. There were 11% with deficient ferritin levels at baseline and 10% with deficient levels at the end. Among the Rooibos group (n=78), 15% of the participants at baseline and 8% at the end had low haemoglobin levels. There were 12% of the children in this group who showed insufficient ferritin levels at baseline, as well as at the end of the intervention. The haemoglobin values of this study group are less abnormal than those reported by Schmidt (1995), who reported 92% of the black rural children in the North West Province to have low haemoglobin values. The number of low ferritin values in this study group, were, however, not significantly less than those found by Schmidt (1995). These findings correlate with previous cross-sectional studies (Soustre *et al.*, 1986; Van der Vijver *et al.*, 1999; Thane *et al.*, 2000; Cowin *et al.*, 2001), who found no significant associations between tea consumption and serum ferritin or haemoglobin values. Combinations of low haemoglobin and low ferritin values (Figures 4.4, 4.5, 4.6 and 4.7) showed that in the black tea group (n=72) four of the participants had low values of haemoglobin and ferritin at baseline. This number decreased to three children with low haemoglobin and low ferritin values at the end of the intervention. In the Rooibos group (n=78) there were five children with low haemoglobin and low ferritin values at baseline and four with low values of these two iron status markers at the end of the intervention. This indicates that black tea and Rooibos had the same influence on haemoglobin ($p>0.74$) and ferritin ($p>0.78$) values of the iron deficient children as well.

It is evident that the haemoglobin levels improved rather than decreased over the time of the study. This is also the case for RBC, MCV and Ht. Transferrin and TIBC increased, which would in combination with a reduced serum iron, indicate iron deficiency (WHO, 2001). In this case, however, the serum iron increased, therefore, iron deficiency cannot be diagnosed. Ferritin levels were more or less consistent during this time. In a randomised, crossover study of several months' duration, Hunt and Roughead (1999) found that serum ferritin is not responsive to short-term changes in dietary iron absorption. Years may be required for dietary changes to influence serum ferritin..

In this study black tea was compared to Rooibos regarding the effects the consumption of these drinks respectively, in addition to a non-haeme iron containing meal, would have on the iron status of primary school children. Tannins in tea are said to form unabsorbable complexes with non-haem iron in the intestine. Rooibos (*Aspalathus linearis*) contains only 4.4% tannins in dilution, where black tea has 11.5 – 17.5 % tannins (Hesseling *et al.*, 1979; Joubert, 1988). Only the study by Hesseling *et al.* (1979) is known to have investigated the effect of Rooibos on iron status. It was found that mean iron absorption after Rooibos, black tea and water consumption, was 7.25%, 1.70% and 9.34%

respectively. Rooibos, in contrast to ordinary tea ($P<0.001$), did not affect iron absorption significantly.

It must, however, be noted that the study of Hesseling *et al.* (1979) was performed in a clinical setting on participants who had been fasting before consumption of a mixture of iron sulphate, ascorbic acid and iron citrate, diluted with water, followed by either black tea, Rooibos or water consumption. Furthermore, the consumption of this drink only took place once. The radioactive tests performed after this procedure (after 4 hours and after 2 weeks) were, therefore, meant to measure the effects of this single administration. No additional variables were recognised for the two weeks time span that followed until the second radioactive tests were performed. The participants of the study by Hesseling *et al.* (1979) were adults and had mean iron status parameters (haemoglobin 15.8g/dL ; ferritin 67.7 ug/mL) far above deficient levels (Hb <13 g/dl; ferritin <12ug/L). This study consisted of children with a risk for iron deficiency that, in combination with the growth process, increases iron absorption from the diet (Cook, 1990; Anderson, 2000). It is, therefore, possible that the tea consumption might have had a less negative effect on the iron status than expected.

5.3 Summary

In contrast to intervention trials on humans (Disler *et al.*, 1975b; Morck *et al.*, 1983; Brune *et al.*, 1989; Reddy *et al.*, 1991) and clinical trials on rats (Disler *et al.*, 1975; South *et al.*, 1997) who found a strong association between tea consumption and iron absorption, this study found no significant effect of tea consumption on iron status. Other epidemiological studies investigating the effect of tea consumption on iron status have been inconclusive. Metha *et al.* (1992) found a negative correlation between tea consumption and markers of anaemia, while Merhav *et al.* (1985) found tea consumption to be positively associated with iron deficiency anaemia.

It is evident that the present study indicates a study population that is malnourished in terms of athropometrical indices (Figures 4.2 and 4.3) and nutrient intake (Table 4.3). Biochemical markers of iron status also indicate that the population could be at risk of iron depletion (Table 4.4).

During the intervention period, dietary intakes remained more or less the same between the black tea and the Rooibos groups. Significant decreases in vitamins A and C intakes did, however, occur and can be ascribed to seasonal availability of foods rich in these two micro nutrients. Serum iron status markers seemed to improve from baseline to end in both of the study groups. A possible reason is the antihelminthic treatment that the participants received when the study commenced. The effect of antihelmintic treatment has been demonstrated by previous studies, as previously discussed.

It has been illustrated that Rooibos does not inhibit iron absorption when compared to black tea (Hesseling *et al.*, 1979). If this finding were to be true and clinical studies demonstrating black tea to have an inhibiting effect on non-haem iron absorption is considered, one would expect different outcomes between the two study groups. This was not the case, which can only indicate no difference between the effect of black tea in comparison to Rooibos when considering the effects on iron status parameters.

CHAPTER 6: Conclusion and recommendations

6.1 Introduction

Iron deficiency is one of the leading micro nutrient deficiencies amongst children in developing countries (UNICEF/UNU/WHO/MI, 1994). In South Africa, 25-37% of children had been reported to have an iron intake of less than half of the recommended level. This can be narrowed down to the North West Province where more than 50% of the children aged 4-6 years had an iron intake of less than two thirds of the RDA. More than 60% of children aged 7-9 years had iron intakes lower than two thirds of the RDA (Labadarios, *et al.*, 1999). Such low intakes increase the risk for iron deficiency.

Black tea *(Camellia sinensis)* is the most commonly consumed drink in the North West Province, where children consume about 400ml daily (Kruger, 2003). Clinical studies have shown black tea to inhibit iron absorption (Disler *et al.*, 1975, South *et al.*, 1997). Soekarjo *et al.* (2001) stated that iron deficiency is associated with impaired phsychomotor development and cognitive function and may lead to a deficit in work performance. This accentuates the importance of determining whether tea consumption might compromise iron status in children who are at risk of iron deficiency. Rooibos (*Aspalathus linearis*) has been demonstrated not to inhibit iron absorption (Hesseling *et al.*, 1979). The aim of this study was, therefore, to determine the effects of regular consumption of either black tea or Rooibos on the iron status of school children with marginal nutritional status.

6.2 Conclusion

Black tea and Rooibos had similar effects on the iron status in primary school children. Several factors in this study might have contributed to this outcome. Most important are the low micro nutrient intakes (Vitamins A, C, B_6, B_{12}, E and in certain cases iron) and the possibility of previous parasitic infection in the study group as discussed earlier.

In contrast to the results found by Hesseling *et al.* (1979) where Rooibos had no significant effects on iron absorption and black tea decreased the absorption, this study found no significant differences between the effects of the two teas on the biochemical parameters of the subjects.

Due to certain limitations of the study, it is advised that the results be interpreted with caution. Determination of dietary iron absorption was not included in the study design, but could assist in more accurate interpretation of biochemical markers. The commonly used radio isotopic labelling method

(CDC, 1998; NCHS, 2003) is, however, very complex and more costly. Tea consumption of the participants when at home was uncontrolled and left to their compliance. This questions the reliability of reported intakes in this regard. More 24-hour dietary recall questionnaires should have been taken to have better representable mean dietary intakes of the group. It should also not be secluded that more sensitive markers could have been used to estimate iron status. Erythrocyte protoporphyrin (EP) in example, shows less day-to-day variation than serum ferritin and transferring do. EP is an early indicator of iron deficient erythropoiesis but is not as early and indicator of low iron stores as low serum ferritin concentration is (CDC, 1998).

Nevertheless, the outstanding outcome of this epidemiological randomized study showed that the consumption of either black tea or Rooibos, has similar effects on iron status in primary school children. Black tea does not compromise iron status when compared to Rooibos.

6.3 Recommendations

- In general, moderate tea consumption (1-2 cups daily) spread throughout the day is unlikely to have any adverse effect on iron status
- When consumed in these amounts, black tea and Rooibos have similar effects on iron status
- Black tea does not compromise iron status when compared to Rooibos
- There is no sufficient evidence to propose that black tea consumption should be limited as public health strategy to combat iron deficiency in persons with marginal iron status.

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Addendum A: 24-Hour dietary recall

24-HOUR RECALL

Subject number: _____ Interviewer: _____

School: _____ Date: ____/ ____/ 200___

Tick what the day was yesterday:

Sunday	Monday	Tuesday	Wednesday	Thursday	Friday

Would you describe the food that you ate yesterday as typical of your habitual food intake?

Yes	1	No	2

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 to the time you went to sleep. I will also ask you where you ate the food and how much you ate.

Time (approximately)	Place (Home, school, etc)	*Description of food and preparation method*	Amount	Amount in g (office use only)	Code (office use only)
From waking up to going to school, or starting day's activities					
During the morning at school or at home					

Addendum A (continued)

Time (approximately)	Place (Home, school, etc)	Description of food and preparation method	Amount	Amount in g (office use only)	Code (office use only)
Middle of the day (Lunch time)					
During the afternoon					
At night (dinner time)					
After dinner, before going to sleep					
Do you take any vitamins (tablets or syrup)?			Yes	1	No 2
Give the brand name and dose of the vitamin/tonic:					

Addendum B: Demographic questionnaire

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1 Subject number				
2 Date	D	M	Y	
3 Place and region				
Interviewer				

Home address

5 Gender	Male	1
	Female	2

6 Age				
Date of birth (for control purposes, do not code)	D	M	Y	

7 First Language	Setswana	1
	Afrikaans	2
	English	3
	Xhosa	4
	Zulu	5
	Other:	6

8 Second Language	Setswana	1
	Afrikaans	2
	English	3
	Xhosa	4
	Zulu	5
	Other:	6
9 Do you receive treatment for any chronic disease?	Yes	1
	No	2

10 If yes - what disease?		
11 Girls only: Have you started menstruating (seeing periods) yet?	Yes 1	No 2
If yes, at what age (year)?		

Addendum B (continued)

12 Do you smoke?	Yes	1
	No	2
13 If no – have you smoked regularly before?	Yes	1
	No	2
14 If yes – what do you smoke?	Cigarettes	1
	Tobacco/pipe	2
	Other:	3
If other – describe		
15 How much do you smoke? per day		
per week		
16 For how long have you been smoking (years)		
17 Calculate pack years		

18 In which grade are you this year?	Grade 1	1
	Grade 2	2
	Grade 3	3
	Grade 4	4
	Grade 5	5
	Grade 6	6
	Grade 7	7

19 Education level of parent :	Father	
	Mother	

20 Who is the breadwinner in your home?	

21 Does he/she have a job at the moment?	Yes	1
	No	2

22 If yes – what kind of job?	Doctor/nurse/teacher/professional	1
	Business/taxi/self employed formal	2
	Typist/assistant/office work	3
	Domestic worker/garden/contract	4
	Hawker/car washer/informal sector	5

23 On which days of the week does he/she work?	Irregular (piece work)	1
	Part time (1-4 days/week)	2
	Full time (5-6 days/week)	3

24 Does someone in your household receive any additional pensions?	Yes	1
	No	2

Addendum B (continued)

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

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

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

28 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		

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

30 What type of stove do you have?	None	1
	Coal/wood	2
	Gas	3
	Paraffin	4
	Electric	5

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

32 Do you watch television every week?	Yes	1
	No	2

33 Do you listen to the radio every week?	Yes	1
	No	2

Addendum C: Anthropometry data form

ANTHROPOMETRY DATA FORM

Subject no:..... Gender: M F (circle correct gender) Age:....

Anthropometric measurement

Weight (kg): .....

Height (m): .....

Mid-upper arm circumference (cm): .....

Triceps skin fold (mm): 1 ..... 2 ..... 3 ..... Mean .....