

**Dietary intake in relation to iron status in 5-12
year old primary school children and estimated
cost of a nutrient rich diet**

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ABSTRACT

Background: Poor nutrition is one of the main causes of nutritional anaemia, with half of the cases estimated to be due to iron deficiency (ID). School-age children are at high risk of these nutritional disorders due to their increased nutrient requirements, accelerated physical and intellectual development, but poor dietary intake. It is generally accepted that individuals do not consume isolated foods or nutrients and that nutrients interact with each other and influence the bioavailability and absorption. There is a need to evaluate a diet as a whole and explain how the various nutrient combinations and foods from various food groups are related to the risk of developing nutritional anaemia in school-age children. Also, it is important to compare the nutrient density and cost of the diets of children according to their anaemia and iron status and to identify foods that provide the most dietary iron per unit cost.

Aim and objectives: The aim of the study was to determine the relationship between dietary intake and iron status in three study groups of 5- to 12-year-old primary school children residing in the KwaZulu-Natal and North West provinces of South Africa. The objectives were to assess nutrient patterns and its relation to anaemia and iron status; to examine the association of dietary diversity with anaemia and iron status; and to investigate the relationship of nutrient density and cost of diet with anaemia and iron status in school children using pooled data from three study populations in South Africa.

Methods: A pooled analysis was conducted with existing baseline data of 5- to 12-year-old primary school children (n = 578) derived from three independent intervention studies conducted in two provinces in South Africa. The following data were extracted from the databases: socio-demographic information; anthropometric measurements (height and weight); biochemical data haemoglobin (Hb), plasma ferritin (PF) adjusted for inflammation, C-reactive protein (CRP); and dietary intake data (energy, and macro- and micronutrients). Nutrient patterns were determined using factor analysis; dietary diversity scores (DDS) were calculated based on data from 1-day and 3-day reference recall periods on nine food groups consumed; and the nutrient density of foods and diets was calculated using the Nutrient Rich Foods Index (NRF9.3). The nutrient density-to-price ratio of foods and total diets was estimated by attaching food prices to the dietary intake data. Descriptive statistics, factor analysis, analysis of variance, analysis of covariance, and regression analyses were used to compare the study groups and to examine the associations of dietary intake with anaemia and ID status of studied children.

Results: In the pooled group 13.8% of the children were anaemic and 27.7% were ID. More than half of the children did not meet the requirements for various nutrients, that is, vitamin A,

vitamin C, vitamin B12, folate and zinc; however, 18% of children had an iron intake below the requirements. Four nutrient patterns were identified: 'plant protein, carbohydrate, iron and B-vitamins'; 'animal protein and saturated fat'; 'vitamin A and vitamin B12'; and 'calcium and fibre'. The 'vitamin A and vitamin B12' nutrient pattern was associated with lower odds of being anaemic [OR 0.63 (0.49-0.91), $p = 0.035$]. For both reference recall periods, consumption of 'vegetables and fruits other than vitamin A-rich' and 'animal-source foods (ASF)' was associated with lower odds of being anaemic (both $p = 0.002$); and 'organ meats' with lower odds of being ID (1-day $p = 0.045$; 3-day $p < 0.001$). Consumption of 'meat and fish' was associated with lower odds of being anaemic ($p = 0.045$) and 'vegetables and fruits other than vitamin A-rich', 'legumes, nuts and seeds' and 'ASFs' with lower odds of being ID for the 3-day recall period only ($p = 0.038$, $p = 0.020$; $p = 0.003$, respectively). A DDS ≤ 4 was associated with higher odds of being anaemic (1-day $p = 0.001$; 3-day $p = 0.006$) and being ID (3-day $p < 0.001$). Diet cost did not differ according to anaemia and ID status, although the nutrient density-to-price ratio was significantly lower for anaemic versus non-anaemic children ($p = 0.001$). Children with anaemia and ID had significantly lower NRD9.3 diet scores compared to non-anaemic and non-ID children.

Conclusion: The combination of dietary vitamin A and vitamin B12 known as enhancers and facilitators of dietary iron absorption may play an important role in the aetiology of nutritional anaemia in school age children in South Africa. Dietary diversification and the importance of consuming vegetables, fruits and foods from animal sources should be considered. Selecting nutrient-dense foods in order to substitute foods with low nutrient density may be a promising way to consume a diet richer in specific nutrients, and may thus help to prevent nutritional anaemia and ID in South African school-age children without affecting the cost of the total diet.

Keywords: anaemia; iron deficiency; nutrient patterns; dietary diversity; nutrient density; diet cost; primary school children; South Africa.

LIST OF DEFINITIONS

Terms	Description
Anaemia	Anaemia is a condition in which the number of red blood cells and their oxygen-carrying capacity is insufficient to meet the body's physiologic needs (WHO, 2011a).
Anthropometry	The study and measurement of human body size and proportions (De Onis <i>et al.</i> , 2007).
C-reactive protein	A positive acute phase protein that is synthesised in the liver in response to infection, systemic inflammation or tissue damage (Thurnham <i>et al.</i> , 2010).
Dietary diversity	The number of food items or food groups consumed during the reference period (Kennedy <i>et al.</i> , 2013).
Ferritin	The main protein in which iron is stored (WHO, 2011b).
Food fortification	Addition of nutrient(s) to a food during the manufacturing process (Allen, 2006).
Iron	Iron is an essential mineral for all organisms and most commonly occurs in the forms of ferrous (Fe^{2+}) and ferric (Fe^{3+}) iron (Pantopoulos <i>et al.</i> , 2012).
Iron deficiency	Early functional iron deficiency occurs when iron transport is diminished (Lynch, 2011).
Iron status indicators	Determinants of the iron status of a population, i.e. include serum ferritin (WHO, 2011b).
Healthy diet	Eating a variety of foods that provide the nutrients people need to maintain their health, feel good, and have energy. These nutrients include protein, carbohydrates, fat, water, vitamins, and minerals (WHO, 2015).
Malnutrition	Malnutrition refers to deficiencies, excesses, or imbalances in a person's intake of energy and/or

	nutrients, which can impair physical and/or mental health (WHO, 2014).
Nutrient profiling	The science of classifying or ranking foods according to their nutritional composition in order to promote public health dietary goals (WHO, 2011c).
Unhealthy foods	Foods high in fats, sugars and/or salt (i.e. energy-dense, nutrient-poor foods) (WHO, 2015).

LIST OF ABBREVIATIONS

AGP	α 1-acid-glycoprotein
AI	Adequate intake
ALV	African leafy vegetables
AMDR	Acceptable macronutrient distribution range
BeForMi	Beverage fortified micronutrients
BMI	Body mass index
CF	Correction factor
CVD	Cardio-vascular disease
CRP	C-reactive protein
DDS	Dietary diversity score
DGLV	Dark green leafy vegetables
DRI	Dietary reference intake
ED	Energy density
ENC	Nutrients to encourage
FAO	Food and Agricultural Organization
FeFA	Ferrum (iron) and fatty acids
FSANZ	Food Standards Australia New Zealand
EAR	Estimated average requirement
EER	Estimated energy requirement
EPA	Eicosapentaenoic acid
Hb	Haemoglobin
HEI	Healthy Eating Index
ID	Iron deficiency
IDA	Iron deficient anaemia
IDE	Iron deficient erythropoiesis
KZN	KwaZulu-Natal province
LIM	Nutrients to limit
MAR	Mean adequacy ratio
NAR	Nutrient adequacy ratio
NC	Northern Cape province
NFCS-FB	National Food Consumption Survey Fortification Baseline
NFFP	National Food Fortification Program
NHANES	National Health and Nutrition Examination Survey
NP	Nutrient profiling
NRD	Nutrient rich diets

NPR	Nutrient-to-price ratio
NRF	Nutrient Rich Food nutrient profiling model
NW	North West province
NWU	North-West University
RAE	Retinol activity equivalent
RDA	Recommended dietary allowance
SAFBDG	South African Food Based Dietary Guidelines
SAFOODS	South African Food Database
SADHS	South African Demographic Health Survey
SANFCS	South African National Food Consumption Survey
SANHANES	South African Health and Nutrition Examination Survey
SANSNP	South African National School Nutrition Programme
SAMRC	South African Medical Research Council
SD	Standard deviation
SF	Serum ferritin
PF	Plasma ferritin
UL	Tolerable upper intake level
WC	Western Cape province
WHO	World Health Organization
WXYfm	Nutrient profiling model for the British Food Standards Agency
YRBS	National Youth Risk and Behaviours Survey
ZAR	South African Rand

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CHAPTER 1: INTRODUCTION

1.1 Rationale for conducting the study

The primary school age is a critical stage of the life span, and diet plays a critical role because of the importance of specific micronutrients for physical development, and for mental, emotional and social wellbeing (Best *et al.*, 2010; Kovalskys *et al.*, 2011; Srivastava *et al.*, 2012). In low- and middle-income countries, including South Africa, insufficient dietary intake is a common problem which leads to deficiencies of macro- and micronutrients (WHO, 2014). The main nutritional problems of school-age children include stunting, underweight, vitamin A and iron deficiency, and nutritional anaemia (Best *et al.*, 2010).

Iron deficiency (ID) is the most prevalent micronutrient deficiency in the world, affecting approximately 2 billion people (i.e., more than 30% of the global population), including 600 million children, and about half of these children are of primary school age (Ahmed *et al.*, 2012; WHO, 2014). Insufficient dietary iron intake and poor iron absorption are the principal factors that contribute to a depletion of the body's iron stores, causing ID which may progress to iron deficiency anaemia (IDA) (Ahmed *et al.*, 2012; WHO, 2014). Other causes of nutritional anaemia include micronutrient deficiencies other than iron (e.g. folic acid, vitamin A, and B-vitamins including vitamin B12) (Arsenault *et al.*, 2009; Balarajan *et al.*, 2011). In children of school age, ID is associated with reduced cognitive and motor development and has a negative impact on schoolwork performance (Lozoff *et al.*, 2011).. Food fortification is one of the strategies employed to increase the micronutrient intake and reduce the burden of micronutrient deficiencies (Dary & Mora, 2002).

The South African Government implemented a mandatory National Food Fortification Program (NFFP) in 2003 (Department of Health, Act 54 of 1972, 2003). Two staple foods, maize meal (used to make porridge) and wheat flour (used to make bread) are fortified with eight micronutrients (i.e., six vitamins: vitamin A, thiamine, riboflavin, niacin, pyridoxine, folic acid; and two minerals, iron and zinc). Up to now only one national survey in 2005, the National Food Consumption Survey-Fortification Baseline (NFCS-FB-1), has been conducted to evaluate the effect of the NFFP on the nutrition status of children. According to the results of the NFCS-FB-1, 28% of children 1- to 9-year-old were anaemic, and 7.8% of children had a poor iron status. Anaemia rates among school children 7- to 9-year-old were 18.6% and ID was 4.4% (Labadarios, 2007). However, the summary results of smaller independent studies conducted after the NFCS-FB-1 showed that anaemia prevalence among school children can be as high as 27% (Taljaard *et al.*, 2013a). In theory, consumption of these fortified staple foods can make a substantial contribution to nutrient intake in children (Steyn *et al.*, 2008), and subsequently

enhance their iron status and reduce the risk for anaemia. Studies conducted after the implementation of the NFFP in 2003 indicated that many school children in South Africa still do not meet their dietary requirements, including dietary iron intake (Oldewage-Theron *et al.*, 2006; Samuel *et al.*, 2010; Oldewage-Theron & Egal, 2010; Oosthuizen *et al.*, 2011). However, there are only a few studies done after 2003 that have included fortified maize meal and bread in their dietary analysis. It is therefore difficult to conclude if nutrient intakes improved after the implementation of the NFFP (Steyn *et al.*, 2016). Thus, despite the NFFP, which can make a potentially significant contribution towards essential nutrient intake, the deficiency in children may remain a problem.

In low- and middle-income countries, including South Africa, diets are limited in diversity, which may cause inadequate nutrient intake, including iron intake, which is important to maintain iron status and to prevent ID (Torheim *et al.*, 2010; Abriha *et al.*, 2014). Animal-source foods are considered the best sources of iron and provide haem and non-haem iron, while plant-based foods contain only non-haem iron. Haem iron is found in foods such as organ meat, red meat, poultry and fish, while non-haem iron is present in foods such as lentils, beans and green leafy vegetables (Hurrell & Egli, 2010; Murray-Kolbe & Beard, 2010; Pettit *et al.*, 2011). The estimated absorption of haem iron ranges from 15% to 35%, and other dietary components have a small effect on its bioavailability (Hurrell & Egli, 2010; Murray-Kolbe & Beard, 2010). Non-haem iron is absorbed at a lower rate than haem iron, ranging from 1% to 7%, but its absorption may be higher if consumed with foods that are rich in haem iron (Tetens *et al.*, 2007; Hurrell & Egli, 2010). Also, non-haem iron absorption may be influenced by various factors, such as amount of iron in the diet or the presence of enhancing or inhibiting dietary compounds consumed during the same meal (Miret *et al.*, 2003; Hurrell & Egli, 2010; Fuqua *et al.*, 2012). Thus, the bioavailability of iron from diverse diets that include foods of animal and plant origin may be much higher than that from diets that mostly consists of plant-based foods.

Diversification of diets focuses on regular intake of foods from different groups to satisfy nutritional needs. Dietary diversity across and within the various food groups is a requirement for optimal health as different food groups deliver a different variety of nutrients and may help to alleviate the multiple micronutrient deficiencies in children of school age (Labadarios *et al.*, 2011). The use of dietary diversity indicators has been proposed as useful measures of food variety and dietary diversity, and were shown to be helpful indicators of the micronutrient adequacy of women's and children's diets (Steyn *et al.*, 2006a; Arimond *et al.*, 2010; Nti, 2014). However, dietary diversity indicators do not consider the amounts for foods consumed, and neither do they consider foods with nutrients to limit, such as added sugar, saturated fat, sodium and added salt. Also, it needs to be taken into account that not all foods within a specific food group have the same nutritional value. In addition, people eat meals with a variety of nutrients

which have interactive and synergistic effects on health (Hu, 2002). Therefore, it is difficult to determine the relation of a specific food or nutrient on risk of health outcome, for instance, ID or nutritional anaemia (Hu, 2002). Nutrient pattern analyses are a complementary strategy to the traditional single nutrient approach for capturing the intrinsic complexity of diet, and the inter-relationships between its different components (Hu, 2002). There is a need to study nutrient patterns in order to evaluate the diet as a whole and elucidate the effects of how the consumption of various foods from animal sources, and intakes of plant foods, such as fruits and vegetables, are related to anaemia and ID risk amongst specific population groups.

In low- and middle-income countries, including South Africa, food procurement often depends on cost rather than health considerations (Altman *et al.*, 2009; Temple & Steyn, 2011). Though nutrient-dense foods may contribute to better health, they are generally considered to be more expensive than energy-dense foods (Temple & Steyn, 2011). The energy dense foods are often also the ones that have a longer shelf life in comparison to fresh foods such as fruits and vegetables. People may often consume larger quantities of energy-dense foods to reduce hunger; however, they lack the nutrients necessary for supporting their health (Temple & Steyn, 2011; Drewnowski, 2012). Also, consumption of high energy-dense foods which are mostly high in fats, refined carbohydrates, salt and added sugars, can lead to a limited intake of the essential macro- and micro-nutrients by replacing the intake of nutrient-rich foods which are important for children's growth and development (Steyn *et al.*, 2006b; Temple & Steyn, 2009). Therefore, for adequate growth and development of children it is essential that they consume nutrient-dense foods from a diverse diet to ensure sufficient amounts of nutrients in the diet.

Prevention and control of nutritional anaemia and ID in children is a complex issue and needs considerable research attention. Although most studies have focused on infants and pre-school children as the most vulnerable groups, ID is also prevalent among primary school children. Therefore, in order to investigate the relationship between dietary intake and anaemia and ID of school age children, a pooled analysis was conducted using the data of three independent intervention studies on primary school children in different areas of South Africa.

1.2 Aim and objectives

The aim of the study was to determine the relationship between dietary intake and iron status in three study groups of 5- to 12-year-old primary school children residing in KwaZulu-Natal and North West province.

The objectives of the thesis were:

- to determine the dietary intake of children in the three study communities;
- to determine the iron status of children in the three study communities;
- to determine the relationship between dietary intake and iron status;
- to determine nutrient density of the diet and its relationship with iron status;
- to estimate the cost of most (frequency and amount) consumed foods that contribute to iron intake.
- to estimate the cost of the diet and to compare it according to nutrient density and iron status.

1.3 Subjects and methods

The data used for the pooled analysis were derived from baseline data of three independent intervention studies on primary school children in different areas of South Africa, which has been previously reported (Baumgartner *et al.*, 2012; Taljaard *et al.*, 2013b; Van der Hoeven *et al.*, 2016). The studies were conducted between April 2009 and June 2012 at primary schools in rural and urban areas in KwaZulu-Natal and North West province. These areas were all malaria-free. At the time of data collection, all schools were participating in the National School Nutrition Program (NSNP), which provided children with a daily school meal. Parents or caregivers signed informed consent forms, and the learners gave verbal assent. Data collection included biochemical measurements, socio-demographic data, anthropometric measurements, and 24-hr dietary recalls (Baumgartner *et al.*, 2012; Taljaard *et al.*, 2013b; Van der Hoeven *et al.*, 2016). A summary of the details of the original studies is presented in Table 1-1. The relevant data from 578 participants were extracted from the original electronic data files and used after the written permission letters were obtained from the principal investigators.

1.4 Inclusion and exclusion criteria

Cases for the pooled analysis were selected from the three electronic data files using the following:

Inclusion criteria

Age range of children from 5 and 12 years; anthropometric measurements (height; weight); the iron status indicators haemoglobin (Hb) and plasma ferritin (PF); the inflammation indicator C-reactive protein (CRP); and the dietary intake components (foods consumed, macro- and micronutrients) had to be available for each participant.

Exclusion criteria

Subjects with missing data for any one of the indicators and components were excluded from the analysis.

Table 1-1: Summary of the original studies

	Study title		
	FeFA ¹	ALV ²	BeForMe ³
Study area	KZN	NW1	NW2
Data of study	2009 - 2011	2012	2011
Number of participants	352	400	540
Age, years	7 to 10	7 to 9	5 to 12
Study design	The study was a randomised double-blind placebo-controlled trial	The study was a double-blind placebo-controlled paired study	The study was an intervention, designed as a longitudinal study
Dietary intake assessment	3 x 24-hour recalls (n=102) Assessed on different days of the week	3 x 24-hour recalls (n=100) Administered at one week apart on different days of the week	3 x 24-hour recalls (n=376) Assessed on different days of the week
Ethical considerations	The study was approved by the Ethical Committee of the ETH Zürich and the NWU Potchefstroom (NWU-00033-09-A1)	The ethical approval has been obtained from the Ethics Committee of North-West University (NWU-00061-08-A1) and from the North-West Department of Education	Ethical approval was granted by the Ethics Committee of the North-West University (NWU-00065-09-A1)

ALV – African leafy vegetables; BeForMi – Beverage fortified micronutrients; FeFA – Ferrum (iron) and fatty acids; KZN – KwaZulu-Natal province; NW1 – North West province, Potchefstroom area; NW2 – North West province, Klerksdorp area.

¹Baumgartner *et al.* (2012); ²Taljaard *et al.* (2013b); ³Van der Hoeven *et al.* (2016)

1.5 Ethical approval

Ethical approval for this study was obtained from the Health Research Ethics Committee of the North-West University (NWU-00027-16-A1) (Annexure A). Ethical clearance for the individual studies were granted by the appropriate ethics committees before data collection (Baumgartner *et al.*, 2012; Taljaard *et al.*, 2013b; Van der Hoeven *et al.*, 2016).

1.6 Thesis outline

This thesis is presented in an article format and divided into six chapters. The technical aspects of this thesis (except for chapters 3 to 5) follow the guidelines specified in the manual for postgraduate studies of the North-West University (NWU, 2016).

Chapter 1 provides the background information, aim and objectives, structure of the thesis, and information about the research team.

Chapter 2 is a literature review on key components of the study to provide the necessary background.

Chapter 3 presents the first manuscript titled “*Nutrient patterns and its relation to anaemia and iron status in 5- to 12-year-old primary school children in South Africa*”. This manuscript has been submitted for publication to the *Nutrition - The International Journal of Applied and Basic Nutritional Sciences* and has been written according to the guidelines of this journal (Annexure B).

Chapter 4 presents the second manuscript titled “*The associations of dietary diversity with anaemia and iron status among 5- to 12-year-old school children in South Africa*”. This manuscript will be submitted for publication to the *Public Health Nutrition* journal and has been written according to the guidelines of the journal (Annexure C).

Chapter 5 presents the third manuscript titled “*Nutrient density but not cost of the diet is associated with anaemia and iron deficiency in school-age children in South Africa*”. This manuscript will be submitted for publication to the *The American Journal of Clinical Nutrition* and has been written according to the guidelines of the journal (Annexure D).

Chapter 6 summarises the main findings of this study. Limitations are discussed and recommendations are made.

1.7 Research team and contributors

The research team and their roles in the study are listed below.

Researcher	Role in study
Dr Tertia Van Zyl	Promoter and co-author of three manuscripts. Supervised the writing of this thesis, design and planning of the manuscripts, interpretation of results and writing of the manuscripts.
Prof Mieke Faber	Co-promoter and co-author of three manuscripts. Supervised the writing of this thesis, design and planning of the manuscripts, interpretation of results and writing of the manuscripts.
Marina Visser	PhD student and first author of three manuscripts in this thesis. Responsible for writing of this thesis, statistical

analyses, interpretation of results and writing all the manuscripts.

Prof Susanna M Hanekom

Data collection in the FeFA study and contributed to the writing of the manuscripts.

Prof Jeannine Baumgartner

Data collection in the FeFA study and contributed to the writing of the manuscripts.

Prof Marinka van der Hoeven

Data collection in the ALV study and contributed to the writing of the manuscripts.

Dr Christine Taljaard-Krugell

Data collection in the BeForMe study and contributed to the writing of the manuscripts.

Prof Cornelius M Smuts

Data collection in the FeFA study and contributed to the writing of the manuscripts.

1.8 References

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CHAPTER 2: LITERATURE REVIEW

2.1 Introduction

Good health of children during the school years contributes to their ability to attend school regularly, stay in school, and concentrate during learning (Jukes *et al.*, 2007; Mwaniki & Makokha, 2013). Diet plays a special role because of the importance of various nutrients for the physical development of children as well as their mental, emotional and social wellbeing (Best *et al.*, 2010; Kovalskys *et al.*, 2011; Srivastava *et al.*, 2012). In low- and middle-income countries, including South Africa, the failure to reach the nutrient requirements because of insufficient dietary intake is a common problem in children's nutrition (Faber, 2010; WHO, 2014). Imbalances in the consumption of macro- and micronutrients can lead to malnutrition, which can be categorised into overnutrition and undernutrition, including micronutrient deficiencies (Faber & Wenhold, 2007; Von Grebmer *et al.*, 2010). The main nutritional problems of school-age children include stunting, underweight, vitamin A deficiency, iron deficiency (ID), and nutritional anaemia (Best *et al.*, 2010).

2.2 Nutritional status

Nutritional status plays an essential role in defining the health of individuals and is influenced by dietary intake and utilisation of nutrients (Black *et al.*, 2013). Poor nutrition can have a negative impact on the health of school children and also affects children's cognitive development and performance at school (Best *et al.*, 2010; Hackman *et al.*, 2010). Undernutrition can be a result of insufficient food intake when individuals do not meet energy and nutrient needs. It can manifest in different ways, such as stunting, wasting, and underweight, depending on the cause and duration (Nelson *et al.*, 2004; Cobham *et al.*, 2012). Stunting is the result of long-term nutritional deprivation and can be used as an indicator of chronic malnutrition, and refers to a low height-for-age (Von Grebmer *et al.*, 2010). Stunting at school age is possibly due to chronic poor nutrition from the time of preschool years, though it can even be a result of poor maternal nutrition during pregnancy, and may affect mental and physical development (Von Grebmer *et al.*, 2010). Wasting in children is an indicator of acute malnutrition and a consequence of insufficient food intake or disease, and refers to a low weight-for-height. This indicator can change rapidly with changes in the availability of food or the presence of disease (Von Grebmer *et al.*, 2010). Underweight can be a measure of either chronic or acute malnutrition, but it cannot distinguish between them, and refers to a low weight-for-age (Von Grebmer *et al.*, 2010). In children undernutrition indicators are age- and gender specific, and can be measured against globally accepted reference standards (Nelson *et al.*, 2004; De Onis *et al.*, 2007). Overnutrition

can be a result of excessive consumption of an unbalanced or unhealthy diet resulting in high intake of dietary energy and macronutrients and may lead to excessive body weight (Sharma & Barasi, 2015). Nutritional status can be measured by different methods, such as anthropometric indices, adequacy of dietary intake, and biochemical indicators (Gibson, 2005).

2.3 Nutritional status of South African school children

In order to describe the nutritional status of South African children, we drew information from national surveys that investigated nutritional status in school children and from individual studies. The results of South African national surveys that have investigated nutritional status of children since 2005 are summarised in Table 2-1. The 2005 National Food Consumption Survey-Fortification baseline (NFCS-FB-1) included a nationally representative sample of 499 children 7-9 years old (Labadarios, 2007). The South African National Youth Risk and Behaviour Survey (YRBS) in 2008 collected data from nationally representative cross-sectional samples of learners 13-17 years old ($n = 9442$) (Reddy *et al.*, 2010). The 2012 South African National Health and Nutrition Examination Survey (SANHANES-1) included 2243 children 7-14 years old (Shisana *et al.*, 2013). The South African Demographic Health Survey (SADHS) 2016 included 1043 adolescence 15-19 years old (Demographic SA, 2017). Nutritional status was assessed by means of anthropometric indicators in all surveys, except NFCS-FB-1 also measured the biochemical indicators for iron, iodine, zinc, folate and vitamin A status.

2.3.1 Anthropometric status

The anthropometric indicators of children's body dimensions reflect the exposure to diet, physical activity and illness (De Onis *et al.*, 2007). The findings of South African national surveys that examined the nutritional status of school age children and adolescence from 2005 to 2016 showed that nutritional status seems to have improved in South Africa. There has been a constant decrease in the national average prevalence of stunting and underweight in children under 10 years of age, with the current prevalence being between 10.0% to 15.0% (Labadarios, 2007; Shisana *et al.*, 2013). However, undernutrition in older groups, especially among boys, still shows higher rates (Demographic SA, 2017). Yet there is an alarming increase in the prevalence of overweight and obesity among all age groups of school age, with girls found to be more overweight than boys (Labadarios, 2007; Reddy *et al.*, 2010; Shisana *et al.*, 2013; Demographic SA, 2017). Despite the prevalence of undernutrition decreasing at national level, the results of independent studies show that the prevalence of undernutrition remains high in school-age children in South Africa. Overall, the results indicated that the prevalence of underweight in children was as high as 20% (Abrahams *et al.*, 2011; Jacobs & de Ridder, 2012; Ginsburg *et al.*, 2013; Kruger, 2014). The prevalence of stunting was up to 19% (Oldewage-

Theron & Egal, 2010; Abrahams *et al.*, 2011; Kruger, 2014; Mamabolo & Alberts, 2014). With regard to overweight the prevalence was between 9% and 21% (Armstrong *et al.*, 2006; Kimani-Murage *et al.*, 2010; Abrahams *et al.*, 2011; Ginsburg *et al.*, 2013). Overall, the results showed that there is a lingering problem of undernutrition and a rapidly rising trend of overweight among children in South Africa.

Table 2-1: National surveys investigated nutritional status of South African school age children and adolescence conducted since 2005

Survey	Year	School age children and adolescence	Results
NFCS-FB-I (Labadarios <i>et al.</i> , 2007)	2005	499 children 7-9 years old	Stunted 14.3% Wasted 3.0% Underweight 9.5% Overweight 7.8%; obese 2.5%. Anaemic 18.6% Iron deficient 4.4% Vitamin A 66.3%
South African National YRBS (Reddy <i>et al.</i> , 2010)	2008	9 442 children 13-17 years old	Overweight 19.2% Obese 5.5%
SANHANES-1 (Shisana <i>et al.</i> , 2013)	2012	2 243 children 7-14 years old	7-9 years old Stunted boys 10.0%/girls 8.7% Wasted boys 2.4%/girls 1.2% Underweight boys 8.6%/girls 4.0% 10-14 years old Stunted boys 15.2%/girls 10.1% Wasted boys 5.6%/girls 2.5% Underweight girls 3.2% Overweight & obese 14.2%
SA DHS (Demographic SA, 2017)	2016	1 043 adolescence 15-19 years old	Underweight boys 14.9%/girls 5.5% Overweight boys 6.1%/girls 27.0%

NFCS-FB-1 - National Food Consumption Survey-Fortification baseline
 SANHANES-1 - South African National Health and Nutrition Examination Survey
 SA DHS - South African Demographic Health Survey
 YRBS - Youth Risk and Behaviour Survey

2.3.2 Micronutrient deficiencies

Micronutrient deficiencies (also known as ‘hidden hunger’) may occur when the diet is deficient in essential elements, that is, vitamins, minerals and trace elements, which are required in small amounts in addition to other important components for proper growth and development of children (WHO, 2014). Micronutrient deficiencies are a universal problem for children’s nutrition in developing countries (WHO, 2014), as it makes them more susceptible to diseases (Fanjiang & Kleinman, 2007; Hasan *et al.*, 2011). Micronutrient deficiencies may, for example, lead to xerophthalmia (vitamin A deficiency), beriberi (vitamin B1), pellagra (vitamin B3), scurvy (vitamin C), goiter and cretinism (iodine deficiency), and nutritional anaemia (iron deficiency, vitamin B12 and folate).

2.3.2.1 Iron

Iron is an essential micronutrient which is necessary for maintaining the normal structure and function of almost all cells of the human body. It is a component of many proteins and also of haemoglobin (Hb), needed for basic cellular function in all tissues, especially the muscles, brain and blood cells (Ganz, 2013). The iron as a part of Hb can provide transport to 98.5% of the total oxygen in the blood to body tissues (Lynch *et al.*, 2007). In the human body, the major sites of iron absorption are in the jejunum and duodenum parts of the intestine. The absorption occurs in two steps: the first step involves movement of iron from the lumen into the intestinal epithelial cells and the second step is absorption of iron from these cells into the blood (Gallaher, 2012). The liver secretes the transport protein transferrin, which binds iron in the blood and delivers to the cells in the body (Pantopoulos *et al.*, 2012). Ferritin builds up in the liver, spleen and bone marrow, which makes it easily available when iron is required by the human body (Pantopoulos *et al.*, 2012). The interaction of iron with other nutrients is important in iron absorption and metabolism. Several nutrients (i.e. vitamin A, vitamin C and vitamin B12) have been suggested to facilitate iron transport to the liver, spleen and bone marrow, and to improve iron absorption in the presence of dietary inhibitors (Fishman *et al.*, 2000). Deficiencies in these haemopoietic nutrients apart from iron may result in nutritional anaemia as well as ID (De Benoist *et al.*, 2008; WHO, 2015).

2.3.2.1.1 Iron deficiency and anaemia

Inadequate dietary iron consumption or its poor bioavailability and absorption will result in depleted iron stores and ID (Patterson & Blumfeld, 2009; Balarajan *et al.*, 2011), which is the most common cause of nutritional anaemia, and accounts for almost half of the anaemia cases worldwide (WHO, 2015). The development of ID and anaemia occurs gradually and can be observed in three stages (WHO, 2011a; WHO, 2011b). The earliest stage is iron depletion characterised by a decrease in iron concentration in the liver, spleen and bone marrow. The next stage is iron deficient erythropoiesis (IDE) which is associated with reduced serum ferritin (SF), when the iron supply is diminished but Hb remains within a normal range. The last stage namely iron deficiency anaemia (IDA), occurs when ID leads to insufficient Hb levels in the blood, thus SF is reduced and blood Hb concentration is low. The potential consequences of anaemia and ID in children of school age is related to a loss of appetite, growth retardation, reduced cognitive development and ability to concentrate which has a negative impact on schoolwork performance (Zimmermann & Hurrell, 2007).

The foremost diet-related factors contributing to ID are, for example, limited dietary diversity, low consumption from animal-source foods, and a high intake of cereals or plant-based foods

(Lynch, 2011). Food consumption is one of the most important factors in the aetiology of nutritional anaemia and ID (Ahmed *et al.*, 2012; Mwaniki & Makokha, 2013).

The amount of iron absorbed from food or a meal is determined by physiological variables such as body iron status in combination with the effect of iron enhancers and inhibitors in food (Hurrell & Egli, 2010). Iron absorption and bioavailability from a whole diet is often different from that of a single-meal (Hurrell & Egli, 2010; Murray-Kolbe & Beard, 2010). For instance, plant-source foods and animal-source foods contain different types of iron. Iron in plant-source foods presents only as non-haem, while animal-source foods provide both haem and non-haem iron and are considered as the best sources of dietary iron (Pettit *et al.*, 2011). Haem iron is highly bioavailable, on average, its absorption is 15% to 35% from diets that comprise of animal-source foods, and other dietary components have a small effect on its bioavailability (Hurrell & Egli, 2010; Murray-Kolbe & Beard, 2010). Haem iron is found in organ meat, red meat, poultry and fish, while non-haem iron found in lentils, beans and green leafy vegetables (McDermid & Lönnerdal, 2012). The non-haem iron is absorbed at a lower rate of 1% to 7% (Tetens *et al.*, 2007; Hurrell & Egli, 2010). Non-haem iron in the diet is mainly ferric iron (Fe^{3+}) which has a low bioavailability, and, it need to be reduced to ferrous (Fe^{2+}) iron before it can be absorbed (McKie *et al.*, 2001). The absorption of non-haem iron may be influenced by the amount of non-haem iron in the diet, and by the presence of enhancing or inhibiting factors consumed during the same meal (Miret *et al.*, 2003; Hurrell & Egli, 2010; Fuqua *et al.*, 2012).

Enhancers of iron absorption

The absorption of iron may increase when including animal-source foods and foods rich in vitamin A, vitamin B12 and vitamin C, which regarded as enhancers of the bioavailability of non-haem iron (Hurrell & Egli, 2010; Murray-Kolbe & Beard, 2010). The dietary reducing agents, such as vitamin C in foods converts Fe^{3+} to Fe^{2+} , and consumption of fruits and vegetables, which mostly are high in vitamin C, may improve the bioavailability of non-haem iron even in the presence of iron inhibitors (Hurrell & Egli, 2010). However, vitamin C may be unstable under the high temperature conditions, and during the food preparation and cooking this method to enhance iron absorption may not be successful (Tola & Ramaswamy, 2015; Lin *et al.*, 2016).

The haem iron in animal-source foods may enhance the absorption of non-haem iron if consumed together (Kristensen *et al.*, 2005; Hurrell *et al.*, 2006; Kennedy *et al.*, 2013). For instance, meat added to a plant-based diet may double iron absorption (Kristensen *et al.*, 2005). Vitamin A and carotenoids are important compounds that may increase the absorption of non-haem iron 2 to 3 times in plant-based diets (García-Casal, 2006). Vitamin A is found as carotenoids in plant foods, such as dark green leafy vegetables, orange-fleshed sweet potatoes,

carrots and papaya. Vitamin A intake from animal-source foods comes from organ meats (e.g. liver), butter and egg yolks, which are also valuable sources of vitamin B12 (Schonfeldt *et al.*, 2013).

Inhibitors of iron absorption

The main inhibitors of non-haem iron absorption include phytate, polyphenols and oxalates, which are mainly found in plant-based diets. These substances bind non-haem iron in the digestive tract and limit its absorption by the body (Samman *et al.*, 2001; Fuqua *et al.*, 2012). The inhibiting effect of polyphenols on iron absorption has been shown with cereals and legumes consumption, such as white and wholegrain bread, and nuts and seeds (Cade *et al.*, 2005), and with a black tea (Mennen *et al.*, 2007; Pynaert *et al.*, 2009). Nonetheless, the study by Asakura *et al.* (2009) did not find a relation between consumption of beans and pulses and iron absorption. Calcium may also have negative effects on iron absorption and may affect the absorption of iron from both animal and plant sources, compared to other inhibitors that affect only iron from plant sources (Hurrell & Egli, 2010; Lönnerdal, 2010). However, consumption of mixed meals with a variety of foods and including enhancers of iron absorption showed a low or a moderate effect of calcium as inhibitor (Roughead *et al.*, 2005; Walczyk *et al.*, 2014). In addition, animal proteins - such as casein milk protein and egg protein albumin - have been shown to inhibit iron absorption (Camara-Martos & Amaro-Lopez, 2002; Armah *et al.*, 2013). Proteins from soya beans also decrease iron absorption (Hallberg & Hulthen, 2000).

2.3.2.1.2 Iron status indicators

In order to assess the iron status of a population the World Health Organization (WHO) considered the most effective indicators that can be used, such as SF and Hb. Serum ferritin is used to reflect the iron stores, and Hb is used for the diagnosis of anaemia and when used in combination with SF to indicate IDA (WHO 2011a; WHO, 2011b). It is important to note that SF concentrations may increase independently of iron status, in response to conditions, such as acute or chronic inflammation; therefore a person with a sufficient SF concentration could actually be iron deficient (WHO, 2011b). It is therefore essential to measure inflammatory markers simultaneously. Inflammatory indicators, such as C-reactive protein (CRP) and α 1-acid-glycoprotein (AGP), can assist in the interpretation of SF values (WHO, 2011b; Nel *et al.*, 2015). There are some methods to adjust for the effect of inflammation, such as excluding subjects with inflammation, adjustment of SF cut-off points, or applying a correction factor to SF (Thurnham *et al.*, 2010).

In primary school children 5- to 12-year-old, the suggested SF cut-off value is < 15 µg/L and can be applied for the diagnosis of ID, the Hb cut-off value is < 115 g/L and can be used to define the presence of anaemia, and for the diagnosis of IDA the combination of Hb level of < 115 g/L and SF < 15 µg/l can be used (WHO 2011a; WHO, 2011b).

2.3.2.1.3 Iron status and anaemia of South African school children

The most recent national data with regard to the anaemia and iron status of South African school children were collected in 2005 by the NFCS-FB-1. It was found that 18.6% of children 7-9 years old were anaemic and 4.4% of children had a poor iron status (SF was not adjusted for the inflammation) (Labadarios, 2007). In addition to the results from the NFCS-FB-1, the information from small local studies on the iron status of primary school children in South Africa is available. An independent study in the North West province, conducted in 2005, found that the prevalence of anaemia (Hb < 120 g/L) was 13% to 15% and prevalence of iron deficiency (SF < 12 µg/L) was 12% in primary school children (Breet *et al.*, 2005). A review by Taljaard *et al.* identified independent studies in South Africa that involved primary school children 7-9 years old that investigated ID and anaemia prevalence and were conducted after NFCS-FB-1, 2005 (Taljaard *et al.*, 2013a). The pre-screening data of the intervention studies showed that anaemia prevalence was 11.5% in KwaZulu-Natal (KZN), 5.4% in the Northern Cape (NC), and 6.9% in the North West (NW) province. The ID prevalence was 7.3% in KZN, 3.3% in the NC and 14.8% in NW [data were not available for the Western Cape (WC)]. The results are summarised in Table 2-2. In their conclusion, Taljaard *et al.* (2013a) suggested that since the NFCS-FB-1, overall the prevalence of anaemia in school age children did improve in some provinces of South Africa. However, more recent studies conducted among primary school children from KZN and Gauteng provinces found that the prevalence of anaemia was 23.4% and 38.7%, respectively (Gwetu *et al.*, 2016; Hlatswayo *et al.*, 2016).

Thus, these studies showed that there are wide variations of anaemia and ID prevalence between some provinces. Even though the independent studies are not representative of the national population, these studies do provide valuable data on the prevalence of anaemia and ID in primary school children.

2.3.2.1 Vitamin A

Vitamin A is an essential nutrient required for a child's growth, maintaining immune function, cellular differentiation and vision. Vitamin A plays an important part in erythropoiesis and has been shown to improve Hb concentration and increase the mobilisation of iron stores, increasing iron absorption (Fishman *et al.*, 2000; Gallaher, 2012).

Table 2-2: Prevalence of anaemia and ID among school children 7- to 9-year-old reported in the NFCS-BF-1 (2005) and in four independent studies

NFCS-FB-1, 2005, by province				Independent studies			
Province	n	Anaemia (%)	ID (%)	Province where study area was located (year of study)	n	Anaemia (%)	ID (%)
KwaZulu-Natal	97	14.4	2.2	KwaZulu-Natal (2009)	321	11.5	7.3
Northern Cape	9	22.2	0	Northern Cape (2009)	200	5.4	3.3
North West	37	27.0	2.9	North West (2010)	407	6.9	14.8
Western Cape	32	18.8	4.2	Western Cape (2006)	--	--	--

^a Adopted and modified from Taljaard *et al.*, 2013a;

Anaemia based on haemoglobin < 115 g/L;

Iron deficiency based on serum ferritin < 12 µg/L.

There is a paucity of data on the prevalence of vitamin A deficiency in South African school children as most of studies focused on children younger than 5 years. The latest national data with regard to the vitamin A deficiency of school children 7- to 9-year-old were collected in 2005 by the NFCS-FB-1. The results showed a high (66.6%) prevalence of vitamin A deficiency [cut-off of serum retinol < 0.7 µmol/L (20 µg/dL)] (Labadarios, 2007). Independent studies reported in North West province found that only 4.7% of the studied children 6- to 12-year-old were deficient (Taljaard *et al.*, 2013b). Another study from the same province observed vitamin A deficiency between 2.5% and 7.0% in primary school children (Van der Hoeven *et al.*, 2016). Thus, the prevalence of vitamin A deficiency observed in independent studies showed lower figures compared to the national prevalence.

2.3.2.2 Vitamin B12

Vitamin B12 is an essential nutrient for the human body and is involved in the synthesis of various proteins and DNA, cell growth and cell division. Vitamin B12 deficiency can lead to the damage of erythrocytes during the developmental stage, resulting in fewer and larger erythrocytes that are incapable of carrying oxygen and finally, megaloblastic anaemia may develop (De Benoist *et al.*, 2008; Gallaher, 2012). Limited data on the prevalence of vitamin B12 deficiency in school children in South Africa is available to date. One study found that 13.6% of 1-year-old had vitamin B12 intakes below the RDA, but no 3-year old children were found to be deficient (Mamobolo & Alberts, 2014). For the diagnosis of megaloblastic anaemia, appropriate folate as well as vitamin B12 biochemical values need to be determined (Wick *et al.*, 2013).

2.3.3 Dietary intake

An adequate dietary intake is one of the factors enhancing the function of the vital body systems of children (Srivastava *et al.*, 2012). Poor dietary intake both in terms of variety of foods and

nutrient-dense foods, is often a major cause of micronutrient malnutrition (Thompson, 2007). Dietary intake data are used to assess the nutritional adequacy of diets and to provide information about nutrients, energy, food and eating habits (Collins *et al.*, 2010).

An adequate intake of dietary iron is important to maintain sufficient iron stores and to prevent ID (Zielińska-Dawidziak, 2015). People who are mostly consuming plant-based foods are at greater risk of developing ID due to the low bioavailability of iron in these foods (Hurrell & Egli, 2010). Animal-source foods are good sources of bioavailable iron, vitamin A and vitamin B12, and low consumption of these foods predisposes children to deficiencies in these micronutrients. Literature shows that in children of school age vitamin A deficiency is one of the main predictors of nutritional anaemia (Thurlow *et al.*, 2005; Jamil *et al.*, 2008). A study by Oliveira *et al.* (2016) also showed that insufficient dietary intake of vitamin A and vitamin B12 in children was one of the factors most strongly associated with anaemia. An adequate supply of both vitamin A and vitamin B12 is needed for the production of red blood cells, and facilitating the absorption of non-haem iron from plant-based diets (Fishman *et al.*, 2000; García-Casal, 2006). Irregular consumption of animal-source foods (i.e. less than once per week), including organ meats, was shown to be significantly associated with anaemia (Obse *et al.*, 2013). However, the diets of the majority of children from low- and middle-income countries lack variety and consist mostly of plant-based foods such as, for example, cereals that contain dietary iron inhibitors which may affect iron absorption (Fishman *et al.*, 2000; Jamil *et al.*, 2008).

2.3.3.1 Dietary intake of South African school age children

In South Africa the national data on dietary intake of school-age children are limited, and only the National Food Consumption Survey (NFCS), which included children 6- to 9-year-old, was conducted in 1999 (Labadarios *et al.*, 2005). A recent review conducted by Steyn *et al.* (2016), analysed the results of smaller studies (children 6- to 15-year-old) conducted between 2000 and 2015. The studies were included that analysed the dietary intake of vitamin A and vitamin B6, zinc, folate (Oldewage-Theron *et al.*, 2006; Petersen *et al.*, 2006; Samuel *et al.*, 2010; Oosthuizen *et al.*, 2011), and iron (Oldewage-Theron *et al.*, 2006; MacKeon *et al.*, 2007; Oldewage-Theron & Egal, 2010; Oldewage-Theron & Kruger, 2011). Overall, the findings of the Steyn *et al.* (2016) review showed that the mean intakes of nutrients, such as vitamin A and vitamin B6, zinc and folate, across the studies were below the requirements. The results on dietary iron intake were reported as being above the recommended levels and the mean iron varied from 5.3 mg to 10.6 mg, where the recommendation in this age range is 5.9 mg for boys and 5.7 mg for girls, which suggest that there is no risk of ID in school age children. Nonetheless, Steyn *et al.* (2016) emphasised that certain limitations must be considered in

studies reviewed, such as small sample sizes in some studies, differences in methodologies in assessing dietary intakes and the reference standards for the interpretation of the adequacy intake of the nutrients (Steyn *et al.*, 2016). The other limitation is that the mean nutrient intakes were compared with the nutrient requirement values (Oldewage-Theron *et al.*, 2006; Oosthuizen *et al.*, 2011; Steyn *et al.*, 2016). It should, however, be noted that the mean nutrient intakes cannot be directly compared to the nutrient requirements values to judge the adequacy of nutrient intake. The nutrient requirement values can be used as a cut-off point to estimate the proportions of participants whose nutrient intake is below the cut-off for the specific nutrients (Otten *et al.*, 2006).

In terms of foods intake, it is important to consume a variety of foods to ensure that nutrient requirements are met (Kabahenda *et al.*, 2014; Korkalo *et al.*, 2017). It is not possible to achieve nutritional requirements by consuming essential nutrients from only one or two food groups. Vegetables and fruits, dairy products and animal-source foods in addition to starchy foods and cereals, need to be consumed regularly (Clausen *et al.*, 2005; Labadarios *et al.*, 2011). Staple food in most parts of the world is predominantly cereal-based, such as maize meal and bread in South Africa, with low intake of animal-source foods and fruits and vegetables, including those rich in vitamin A (Labadarios *et al.*, 2011; Faber *et al.*, 2013). The results of the NFCS in 1999 highlighted that children consumed a predominantly cereal based diet with limited amounts of animal protein. Maize meal porridge was the most frequently and regularly consumed food item with an average daily intake of 559 g for 6- to 9-year-olds and from 690 g to 879 g for children older than nine (Nel & Steyn, 2002).

2.4 Public health strategies aimed at improving nutritional status

Public health nutrition strategies aimed at improving nutritional status of targeted populations are needed, thus preventing many chronic disorders and improving quality of life, which may directly benefit overall health care budgets, employee productivity, and economies. In supply-driven approaches, the decisions of governments are used to modify food content, for example, the fortification of staple foods. In demand-driven approaches, the population is educated and encouraged to make a better food choice in order to reduce the burden of malnutrition. These approaches may include food-based dietary guidelines, dietary diversification, and increasing consumption of nutrient-dense or nutrient-rich foods

2.4.1 Food fortification

Worldwide the food fortification approach is presented as the most important way to increase micronutrient intake at population level and reduce the concerns of micronutrient deficiencies

(Dary & Mora, 2002). Food fortification is characterised by increasing nutrient content in foods to a higher level than they naturally occur (Allen *et al.*, 2006). A number of systematic reviews were conducted in recent years to analyse the impact of multi-nutrient fortification of staple foods on the health of women and children. Overall, it was found that multi-micronutrient food fortification may improve the micronutrient status and has a significant impact on increasing serum micronutrient concentrations and reduce risk of ID and anaemia prevalence in school age children (Serdula, 2010; Best *et al.*, 2011; Gera *et al.*, 2012; Das *et al.*, 2013). However, the micronutrient status may only improve gradually (Thompson, 2007). In order to improve iron status, the iron compounds in fortified foods have to be bioavailable (WHO, 2016). The use of electrolytic iron powder as food fortificant, among others, i.e., ferrous sulphate and ferrous fumarate, has been recommended by the WHO as a bioavailable component with a long shelf life (WHO, 2016).

2.4.1.1 The South African National Food Fortification Programme

The South African National Food Fortification Program (NFFP) was legislated in October 2003 to improve the average micronutrient intake of South Africans (Department of Health, South Africa, 2003). The identified staple foods maize meal and wheat flour were set as the most appropriate vehicles for fortification. These staple foods are fortified to provide a person of 10 years or older with vitamin A (31%), thiamine (25%), niacin (25%), pyridoxine (25%), folate (50%), riboflavin (17% from maize and 20% from wheat), iron (25% from unsifted maize meal and 50% from maize meal), and zinc (20%) of the recommended dietary allowance per 200 g of raw foods. In order to assess the theoretically potential effect of the NFFP on micronutrient intake, Steyn *et al.* (2008) recalculated documented micronutrient intakes of children derived from the South African National Food Consumption Survey (SANFCS) 1999, by replacing the values of unfortified bread and maize meal with values of the fortified equivalent. The authors concluded that the consumption of the staple foods fortified as part of the NFFP can make a potentially significant contribution towards nutrient intake in children, including dietary iron intake (Steyn *et al.*, 2008).

Studies conducted in the past decade suggested that the low bioavailability of electrolytic iron, as a fortificant used in maize meal and wheat flour in South Africa, does not seem to be effective in reducing the prevalence of anaemia or ID in children (Assuncao *et al.*, 2007; Van Stuijvenberg *et al.*, 2008; Paganini *et al.*, 2016). Furthermore, chemical analysis of uncooked fortified food items, sampled in different areas in South Africa, suggested that the amount of fortificants, including vitamin A and iron, are below the recommended fortification premix values (Yusufali *et al.*, 2012; Van Jaarsveld *et al.*, 2015). This raises concerns over bioavailability and

quantity of fortificants used in the NFFP, which may limit the potential of preventing anaemia and ID. These results are in line with studies conducted earlier in other countries with national fortification programmes (Andango *et al.*, 2007; Assuncao *et al.*, 2007). From the current literature, it is uncertain whether electrolytic iron is effective in order to prevent anaemia and ID, and food fortification should be viewed as a complementary strategy for improving the nutrient status of children (Assuncao *et al.*, 2007; Van Stuijvenberg *et al.*, 2008; Paganini *et al.*, 2016).

2.5 Quality of diets

While fortified foods contain increased amounts of selected micronutrients, they are not a substitute for a good quality diet that supplies adequate nutrient intake. The inclusion of nutrient-dense foods from a variety of food groups is important to improve diet quality, enhance the nutrient intakes according to requirements, and construct a healthy balanced diet (Tetens *et al.*, 2007; Drewnowski, 2010). Poor diet quality can be described as a diet that contain foods high in energy density and low in nutrient density (i.e. sweets and salted snacks, and cold drinks), and can lead to limited intake of the necessary macro- and micro-nutrients for children's growth and development (Steyn *et al.*, 2006; Temple & Steyn, 2009).

The quality of a diet can be estimated by evaluating the nutrient adequacy and compliance with nutrient requirements, assessing the variety of food intake, and nutrient or food patterns, in order to assess the combination of foods or nutrients consumed by participants.

2.5.1 Nutrient adequacy

Evaluating a diet in terms of nutrient adequacy may determine risk of inadequate nutrient intake, whether low intake or high intake. The Nutrient Adequacy Ratio (NAR) is an index of adequacy, which compares a participant's daily intake of selected nutrients with the specific requirements, and the Mean Adequacy Ratio (MAR) calculates the average for the NAR values for each participant (Gibson, 2005). The NAR can be calculated using specific requirements established for gender and life-stage group, that is, the dietary reference intake by the Food and Nutrition Board of the Institute of Medicine, United States (Otten *et al.*, 2006). Nutritional requirements start to differ between genders at the beginning of the teenage years. Dietary reference intake (DRI) presents a set of four reference values: Estimated Average Requirements (EAR); Recommended Dietary Allowances (RDA); Adequate Intakes (AI); and Tolerable Upper Intake Levels (UL) (Otten *et al.*, 2006). The EAR cut-off point method measures the prevalence of inadequate nutrient intakes of population groups with usual intakes below the EAR. The EAR is a daily nutrient intake value that is estimated to meet the requirement of 50% of healthy individuals in the specific group or population. The AI is the recommended intake value based

on observed or experimentally determined estimates of nutrient intake by a group of healthy individuals that are assumed to be adequate. The AIs are used when the EAR cannot be determined (Otten *et al.*, 2006). The RDA is the average daily intake values required to meet the nutrient needs of 97% to 98% healthy individuals in a specific age and gender group.

2.5.2 Dietary and nutrient patterns

It is generally accepted that people do not consume isolated foods or nutrients, but consume foods or meals consisting of one or more than one different food item with a variety of nutrients (Hu, 2002). Nutrients may interact with each other, and influence their bioavailability and absorption. For instance, as was mentioned previously, non-haem iron absorption is influenced by other nutrients that may enhance and inhibit absorption when consumed in the same meal. Thus, the combinations of foods and beverages, rather than individual foods and nutrients, in meals and diets most likely have interactive and synergistic effects on the health outcome (Hu, 2002; Newby & Tucker, 2004). Therefore, nutrient or food pattern analysis, or a combination of both, has been suggested as an advanced method for studying the relationship between diet and health outcomes.

Although results from dietary (food) pattern analyses may be interpreted to make health recommendations more easily, the nutrient pattern approach has an additional benefit (Jacobs & Tapsell, 2007). Nutrients in foods are universal, and their function in the human body does not change. Nutrients are directly involved in the metabolism and aetiology of health disorders (Moskal *et al.*, 2014). Also, contrary to food patterns, nutrient patterns may be a more comparable way across populations to study their association with health disorders (Moskal *et al.*, 2014).

Thus, nutrient pattern analyses are a complementary strategy to the traditional single nutrient approach for capturing the intrinsic complexity of diet, the inter-relationships between its different components (Hu, 2002). There is a need to study nutrient patterns in order to evaluate the diet as a whole and elucidate the effects of how the consumption of various foods from various sources are related to anaemia and ID risk amongst specific population groups.

2.5.3 Dietary diversity

The consumption of a greater variety of foods is considered beneficial for the improvement of the nutritional quality of a diet and, compared to a monotonous diet, thus alleviates multiple micronutrient deficiencies (Oldewage-Theron & Kruger, 2011; Labadarios *et al.*, 2011). Dietary diversity across and within the various food groups is needed for optimal health as different

foods deliver a variety of different nutrients and may help to alleviate multiple micronutrient deficiencies in children (Labadarios *et al.*, 2011). The use of dietary diversity indicators has been proposed as useful measures of food variety and dietary diversity, and were shown to be helpful indicators of the micronutrient adequacy of women's and children's diets (Steyn *et al.*, 2006a; Arimond *et al.*, 2010; Nti, 2014). Calculating dietary diversity scores (DDS) is one reliable and quick way of assessing dietary diversity at the population level (Clausen *et al.*, 2005; Kennedy *et al.*, 2013). Dietary diversity scores (DDS) are most widely used to assess the dietary variety in low- and middle-income countries and often used to identify micronutrient adequacy (Clausen *et al.*, 2005; Labadarios *et al.*, 2011). It shows a positive correlation with higher micronutrient intake, such as vitamin A, vitamin C, iron and zinc in adult and children study groups (Arimond *et al.*, 2010; Nti, 2014; Gashu *et al.*, 2016). A study on South African children also showed that the adherence to dietary diversity may be a good indicator of the nutrient adequacy (Steyn *et al.*, 2006a). While most studies evaluate the DDS using the cut-off value of 4 as an indicator of poor dietary diversity (Steyn *et al.*, 2006a; Labadarios *et al.*, 2011; Oldewage-Theron & Kruger, 2011; Drimie *et al.*, 2013), no established cut-off points are available in literature (Kennedy *et al.*, 2013).

Different methods of determining DDS for various population groups can be used, such as using different combinations of food groups in calculating DDS. For example, studies conducted on school children and adolescence from various countries used indices based on 7-14 groups' combination (Azadbakht & Esmailzadeh, 2011; Vakili *et al.*, 2013; Gewa *et al.*, 2014; Abizari *et al.*, 2017). The suggested Food and Agricultural Organization (FAO) guidelines recommend that foods be grouped into nine food groups to determine DDS at individual level (Kennedy *et al.*, 2013). These food groups are: starchy staples; dark green leafy vegetables; vitamin A-rich fruits and vegetables; other fruits and vegetables; organ meat; meat and fish; eggs; legumes and nuts and seeds; and milk and milk products. The fruits and vegetables are divided into groups according to their nutrient composition similarities, such as vitamin A-rich fruits and vegetables, and dark green leafy vegetables, or animal-source foods, such as organ meats, which are good sources of bioavailable iron (Pennington & Fisher, 2010; Kennedy *et al.*, 2013). For instance, DDS can be useful to obtain information on the consumption of specific foods or food groups (such as iron-rich foods), or consumption of fortified foods, for example, those fortified with iron or vitamin A. The type of food groups selected to calculate DDS may affect the value of the score (Ruel *et al.*, 2004). However, it needs to be taken into account that not all foods within a specific food group have the same nutritional value and may still contain some of the nutrients to limit, such as added sugar, saturated fat, and added salt or sodium.

2.5.4 Nutrient density

The nutrient density of individual foods can be assessed using a technique known as the nutrient profiling (NP), which can also be applied to meals and total diets (Francou *et al.*, 2015). Various NP models are used as a quantitative tool for classifying foods as “more” or “less” healthy based on their specific nutrient components, or grouping foods based on their overall nutrient composition (Rayner *et al.*, 2005; Drewnowski & Fulgoni, 2014). Numerous NP models have been widely used for various purposes, such as providing scientifically-based nutrition information to the public (Cooper *et al.*, 2016), or guiding the regulation of foods and beverages marketing and advertising to children (Rayner *et al.*, 2013). For example, the nutrient profiling model WXYfm, developed in the United Kingdom, to regulate food advertising aimed at children (Rayner *et al.*, 2005); or Food Standards Australia and New Zealand (FSANZ) profiling model was used to regulate health claims on foods in Australia and New Zealand (FSANZ, 2007). The NP models can be based on the nutrients that are deficient in a specific study population, and on nutrients for which dietary intake should be limited. For example, nutrients consumed in inadequate amounts generally include protein, fibre and a selection of vitamins and minerals; however, nutrients consumed in excess include saturated fat, added or total sugars and sodium (Darmon *et al.*, 2009). Using NP models may be helpful in identifying healthier food and diet options.

The Nutrient Rich Food (NRF) index model has been suggested as a complete accepted metric, that score and classify individual foods on a continuum scale, considering overall nutritional composition of foods (Fulgoni *et al.*, 2009; Drewnowski, 2010). The NRF approach is applicable to individual food items and a diet as a whole (Drewnowski, 2010). The dietary references used in this model must be based on age and gender appropriate values (Drewnowski & Fulgoni, 2014). The choice of nutrients for the NRF index model is based on the selection of nutrients that are of public health concern within a specific study population, and also includes nutrients that are consumed in excess and need to be limited (Fulgoni *et al.*, 2009). The NRF model is a validated metric that has been validated against the United States Healthy Eating Index (HEI-2005) score, using data from the United States National Health and Nutrition Examination Survey (NHANES) 1999-2002 (Fulgoni *et al.*, 2009). All foods were scored using the NRFn.3 range of algorithms, and the results showed that the most optimal model was NRF9.3, which include nine beneficial nutrients and three nutrients to limit. The NRF profiling techniques have also been tested in several other countries, including China (Zhou *et al.*, 2014), France (Francou *et al.*, 2015), the Netherlands (Streppel *et al.*, 2014; Sluik *et al.*, 2015), and Australia (O'Sullivan *et al.*, 2015).

2.6 Diet quality and anaemia and ID

The indices of diet quality may be used to explore the relationships between diet quality and health outcomes (Wirt & Collins, 2009; Kimokoti & Millen, 2011; Gil *et al.*, 2015). The principal component analysis or factor analysis scores can be used to investigate the relationship between dietary intake and iron status (Hu, 2002; Newby & Tucker, 2004) by considering that multiple foods and nutrients impact on iron absorption. However, limited evidence exists on the relationship of nutrient patterns with anaemia and ID. Most of the studies that have explored the association between nutrient patterns and health outcomes, focused on risks of various types of cancer (Deneo-Pellegrini *et al.*, 2013; Edefonti *et al.*, 2015), metabolic syndrome (Bian *et al.*, 2013), obesity (Pisa *et al.*, 2015), or behavioural disorders (Zhou *et al.*, 2016). However, a few studies have explored the relationship of food patterns with anaemia and ID, and these studies focused on adults (Shi *et al.*, 2006; Broderstad *et al.*, 2011; Beck *et al.*, 2013). For example, Beck *et al.* (2013) used factor analysis and logistic regression to demonstrate that following a 'meat and vegetables' dietary pattern was associated with a 41% reduced risk of suboptimal iron status, based on ferritin concentrations in an adult women. Also, following a 'milk and yoghurt' pattern was associated with a 50% increased risk of suboptimal iron status in this population. Overall results demonstrated that food patterns characterised by a low intake of meat and vegetables are associated with an increased risk of suboptimal iron status or anaemia.

Assessing the variety of food groups consumed can be an important approach to evaluate dietary intake in relation to anaemia and ID and has been demonstrated in the literature. The studies showed that a higher dietary diversity is associated with a decreased risk of nutritional anaemia in different age groups (Acham *et al.*, 2013; Saaka & Abdul Ralf, 2015; Gwetu *et al.*, 2016). Increased number of food groups significantly increased SF and serum retinol in school age children (Acham *et al.*, 2013; Gwetu *et al.*, 2016). A study on pre-school children indicated that a high consumption of cereals and legumes, but a low consumption of fruit and vegetables, animal-source foods and vitamin A-rich foods, are predictors of low Hb concentration and poor iron status (Gashu *et al.*, 2016). However, overall, results showed that, despite poor diet quality being characterised as consumption of foods from mostly two or three food groups, the prevalence of anaemia was low (Gashu *et al.*, 2016). Other studies showed contrary results, that is, dietary diversity was not associated with anaemia (Ali *et al.*, 2014; Saaka *et al.*, 2017). Nevertheless, in one study involving pregnant women dietary diversity had a positive effect on Hb (Saaka & Abdul Ralf, 2015). Thus, varying results have been reported. While most of the studies showed a positive association of dietary diversity and haematological status, the relationship between dietary diversity and anaemia remained inconclusive.

Review of the available literature indicates that studies exploring the relationship of nutrient density of foods and diet with anaemia and ID are rare. The closest example that can be used is association between the nutrient density of foods and cardio-vascular disease (CVD) (Vlismas *et al.*, 2010; Streppel *et al.*, 2014), anthropometry (Streppel *et al.*, 2012), diabetes (Nansel *et al.*, 2012), and all-cause mortality (Streppel *et al.*, 2014). Overall, the higher nutrient density of diets was inversely associated with the development of health disorders. However, in one study a higher nutrient density of diets was positively related to a higher body mass index (BMI) and body weight (Streppel *et al.*, 2012), and was not associated with hemotological indicators of diabetes (Nansel *et al.*, 2012). Thus, results that have been reported showed that the relationship between nutrient diversity of diet and health outcome remained questionable.

2.7 The cost of nutrient-dense foods and diets

During 2008 and 2009 studies were conducted in South Africa to estimate the cost of healthier foods (lower energy density, higher nutrient density) by comparing them with the cost of less healthy foods (high energy density, low nutrient density) according to prices collected at supermarkets (Temple *et al.*, 2011; Temple & Steyn, 2011). Although, healthier food choices are usually available in stores, people with a low income may select the less healthy foods because of lower cost. An analysis of healthier food selections showed that the healthier option estimates for 33 foods (out of 42 analysed), were more costly per 100 g compared to less healthy foods (Temple *et al.*, 2011). The estimated comparison of the cost of a typical South African diet with a healthy diet showed that the latter one would cost up to 70% more and theoretically, the majority of the South African population cannot afford a healthy diet (Temple *et al.*, 2011; Temple & Steyn, 2011). The studies conducted in the United States confirmed that the prices of foods increase with a higher nutrient density (Bernstein *et al.*, 2010; Drewnowski, 2010a; Rehm *et al.*, 2011; Aggarwal *et al.*, 2012; Drewnowski & Rehm, 2013). Studies in other countries also reported that healthier diets may cost more than less healthy ones (Darmon *et al.*, 2005; Andrieu & Darmon, 2006; Maillot *et al.*, 2007; Monsivais & Drewnowski, 2007; Monsivais *et al.*, 2011). For example, the diets of people who eat more fruits and vegetables are more expensive in comparison to energy dense and nutrient poor diets (Maillot *et al.*, 2007; Drewnowski, 2010a).

However, using NP models in combination with food prices may help to calculate the nutrient-to-price ratio and to identify the most nutrient-dense foods and diets per unit cost. For example, Darmon *et al.* (2005) found that some fruits and vegetables had a high nutrient-to-price ratio, i.e. fresh oranges or carrots, and also canned or frozen vegetables, such as tomatoes or green peas. Also, Maillot *et al.* (2007) showed that not all vegetables are equally expensive, and that

there is always variability in nutrient density and cost within each food group, making eating healthier at low cost possible. It was also found that milk and yogurt, eggs and legumes had the highest nutrient-to-price ratio, followed by meat, poultry, and fish that also were available at reasonable prices (Drewnowski, 2010; Drewnowski, 2011).

Several studies have found that not all healthy diets are more expensive than less healthy ones. For example, Drewnowski and Eichelsdoerfer (2009) suggested that diets (such as Mediterranean style diet) that are rich in vegetables, fruits, beans, whole grains, olive oil and fish and low in energy density may cost less than high energy-dense diets. The authors showed that some vegetables, beans and other legumes, grains, nuts, and some dairy products were among the lower-cost foods. Thus, it should be possible to plan a nutrient rich diet using the lower-cost selections of foods in every food category. Also, Temple and Steyn (2011) suggest that careful choice of foods, including more oats, lentils, dry beans, canned fish and beans, may help to reduce the cost of the healthier diet. Another study found that by reducing expenditures on red and processed meats, the diet may cost less if buying more nuts, soya, beans, and whole grains (Bernstein *et al.*, 2010). Moreover, some meals, for instance for school children, can be made more nutritious by including more whole grains, beans, and potatoes, which are rich in potassium, iron and fibre, but cost less (Drewnowski, 2010; Drewnowski & Rehm, 2013). Also, it has been shown in German children and adolescents study that there is no positive correlation between diet quality and cost (Alexy *et al.*, 2014).

Thus, overall results of abovementioned studies suggest that food costs may not be an obstacle to consume a better diet quality. The nutrient density of a whole diet can be increased without additional cost by choosing affordable nutrient-dense foods (Marty *et al.*, 2015). Also, it can be achieved by reducing the intake of certain foods with a low nutrient density, and thus compensate the higher costs of foods with higher nutrient density (Dubois *et al.*, 2017).

2.8 The cost of diets in relation to health outcomes

Studies that determined associations of nutrient density of diets and cost of these diets with anaemia and ID as health outcomes are rare. The closest example of such relationship is a study conducted on Japanese university students that found an inverse association between cost of dietary energy and BMI (Murakami *et al.*, 2007). Similar results were received in study on Spanish graduates showing a positive association between low dietary energy cost and weight gain (Lopez *et al.*, 2009). However, in a study on Greek adults no correlation was observed between food cost and the incidence of CVD (Vlismas *et al.*, 2010). A more recent study on cost analyses and health outcome has been suggested that the risk of becoming

obese is linked to a lower cost diet (Drewnowski, 2012). However, the relation of cost nutrient-dense diet and nutritional anaemia still remains a questionable.

2.9 Conclusion

It is evident from the findings of the reviewed studies that the main nutritional problems among South African school-age children include stunting, overweight, vitamin A deficiency, anaemia and ID. Consumption of foods from plant-based diets and a low consumption of foods from animal sources place children at risk of nutritional anaemia. Fortification of staple foods with micronutrients of concern can make a significant contribution towards nutrient intake in children, and preventing nutritional anaemia at population level. However, there is a dearth of data available on nutrient intake of school age children in South Africa. Current knowledge on the association between dietary intake and nutritional anaemia is focused mostly on single nutrients; however, it is relevant to assess the combined effect of nutrients, in order to evaluate a diet as a whole. This may help to explain the effects of how the consumption of various animal source foods, and intakes of plant- and cereal-source foods, are related to anaemia and ID risk amongst specific population groups, such as children of school age. However, foods are eaten in combination with one another as meals, thus the need to study the nutrient patterns. Yet, the information on nutrient patterns and their relationship with anaemia and ID is lacking. Additionally, although dietary diversity is an accepted indicator of diet quality, it still remains questionable how it affects the anaemia and iron status of school-age children. Thus, there is the need to evaluate the dietary diversity in relation to anaemia and ID as a health outcome. During the last decade, the cost of healthy eating has become a topic of interest. Although studies conducted in various countries reported that healthier diets cost more than less healthy ones, there are some studies that assumed that diet quality can be increased without additional cost. Thus, studies are needed to examine the overall nutrient density of foods and diets and their cost which may help to identify foods that provide the best nutrient content per unit cost.

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CHAPTER 3: MANUSCRIPT 1:

Nutrient patterns and its relation to anaemia and iron status in 5- to 12-year-old primary school children in South Africa

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8 Abstract

9 *Objective:* To assess nutrient patterns and its relation to anaemia and iron status of school
10 children using pooled data from three study populations in South Africa.

11 *Methods:* Data of 5- to 12-year-old primary school children (n=578) from three independent
12 studies conducted in two provinces in South Africa were pooled. Data used in the analysis were
13 dietary intake, haemoglobin and plasma ferritin concentrations. Nutrient patterns were
14 determined using factor analysis. Logistic regression analysis was performed to determine
15 relationships of nutrient patterns with anaemia and iron deficiency.

16 *Results:* In the pooled group, 13.8% of the children were anaemic and 27.7% were iron deficient
17 (ID). More than half of the children did not meet the Estimated Average Requirement for various
18 nutrients, including vitamin A, vitamin C, vitamin B12, folate and zinc; however, only 17.7% of
19 children had an iron intake below the requirements. Median intakes for vitamin A and vitamin C
20 were lower for anaemic versus non-anaemic children (p=0.03 and p=0.02, respectively) and for
21 ID versus non-ID children (p=0.03 and p=0.046, respectively). Four nutrient patterns were
22 identified: 'plant protein, carbohydrate, iron and B-vitamins'; 'animal protein and saturated fat';
23 'vitamin A and vitamin B12'; and 'calcium and fibre'. The 'vitamin A and vitamin B12' nutrient
24 pattern was associated with lower odds of being anaemic [OR 0.63 (0.49-0.91), p=0.035].

25 *Conclusion:* Our results highlight the potential role of the combination of dietary vitamin A and
26 vitamin B12 in the aetiology of nutritional anaemia in school-age children in South Africa.
27 Nutrient pattern analysis may improve the understanding of the synergistic role of nutrients
28 related to anaemia and may assist in planning intervention strategies.

29 *Keywords:* anaemia; iron deficiency; nutrient patterns; primary school children; South Africa

30 Introduction

31 Global estimates show that anaemia affects 600 million children and about half of these cases
32 are children of primary school age (WHO, 2015). The most common cause of anaemia is iron
33 deficiency (ID) (WHO, 2015). Other causes include micronutrient deficiencies other than iron
34 (e.g. folic acid, vitamin A, and B-vitamins including vitamin B12), poor health, and acute and
35 chronic infections (Arsenault *et al.*, 2009; Balarajan *et al.*, 2011). In children of school age,
36 anaemia is associated with reduced cognitive and motor development and has a negative
37 impact on schoolwork performance (Lozoff *et al.*, 2006).

38 The only national data available for South African children 6- to 9-years-old are based on the
39 National Food Consumption Survey (NFCS-FB-1) of 2005, which indicated an anaemia
40 prevalence of almost 19.0% (Labadarios, 2008). The results of smaller independent studies
41 conducted after the NFCS-FB-1 were summarized by Taljaard *et al.* and show that anaemia
42 remains a common problem in school children, with a prevalence from 6.9% to 27% (Taljaard *et al.*
43 *et al.*, 2013a).

44 In low- and middle-income countries, including South Africa, the diets of the majority of children
45 lack variety, which may result in insufficient intake of various nutrients important for growth and
46 proper functioning of the body (Ochola & Masibo, 2014). An adequate intake of dietary iron is
47 important to maintain sufficient iron stores and to prevent iron deficiency anaemia (IDA), an
48 advanced stage of ID, which is characterized by low haemoglobin production (Lozoff *et al.*,
49 2006). Individuals who mostly consume plant-based diets are at greater risk of developing ID
50 due to the low bioavailability of iron in plant foods (Heath & Fairweather-Tait, 2002; Hurrell &
51 Egli, 2010). A South African study in a rural disadvantaged community showed that less than
52 15% of children consumed foods from animal sources on the day of recall (Gwetu *et al.*, 2016),
53 which is a good source of various nutrients, placing these children at risk of nutritional anaemia
54 (Schonfeldt *et al.*, 2013).

55 Fortification of staple foods with micronutrients of concern is one of the effective strategies to
56 prevent nutritional anaemia at population level, which has been introduced in some low- and
57 middle-income countries, including South Africa (Nutrition Service of the World Food Program,
58 2006). The South African Department of Health in 2003 legislated mandatory fortification of
59 frequently consumed staple foods – that is, bread (made with wheat flour) and maize meal –
60 with six vitamins: vitamin A, thiamine, riboflavin, niacin, pyridoxine, folic acid, and two minerals,
61 iron and zinc (South Africa. Department of Health, 2003). Theoretically, these fortified staple
62 foods can make a significant contribution towards nutrient intake in children (Steyn *et al.*, 2008).
63 However, there are no national data available on nutrient intake of school-age children in South
64 Africa. A summary of dietary studies in 6- to 12-year-old primary school children done between

65 2000 and 2015 showed that only a few studies included the fortified values for the fortified
66 maize meal and bread in their dietary analysis, and it was therefore difficult to determine if
67 nutrient intakes improved after mandatory fortification of these staple foods (Steyn *et al.*, 2016).

68 Current knowledge on the association between dietary intake and nutritional anaemia is focused
69 mostly on single nutrients. Diets, however, consist of a combination of nutrients that have an
70 interactive and synergistic effect on health, and thus it is relevant to assess the combined effect
71 of nutrients (Moskal *et al.*, 2014). Factor analysis is one of the modern reduction techniques
72 whereby the number of variables (nutrients) are reduced to determine the whole nutrient pattern
73 (Hu, 2002; Moskal *et al.*, 2014).

74 Information on nutrient patterns and their relationship with anaemia and ID in school-age
75 children in South Africa is lacking. Therefore, in this study, we conducted a pooled analysis of
76 existing data with the aim to assess the relationship of nutrient patterns with anaemia and ID by
77 using the factor analysis approach.

78 **Subjects and methods**

79 The data used for this pooled analysis were derived from the baseline point of three
80 independent intervention studies with school children in different sites in South Africa, which
81 have been previously reported (Baumgartner *et al.*, 2012; Taljaard *et al.*, 2013; Van der Hoeven
82 *et al.*, 2016). The studies were conducted between April 2009 and June 2012 at primary schools
83 in rural and urban areas in the KwaZulu-Natal (KZN) and North-West (NW) provinces. These
84 areas are all malaria-free. All children received deworming medication at the baseline point. At
85 the time of data collection, all schools were participating in the National School Nutrition
86 Programme (NSNP), which provides children with a daily school meal. Parents or caregivers
87 signed informed consent forms, and the learners gave verbal assent. Data collection included
88 biochemical measurements, socio-demographic data, anthropometric measurements, and 24-hr
89 dietary recalls.

90 For the purpose of the present study, only the relevant data were extracted from the original
91 electronic data files and used electronically after written permission letters were obtained from the
92 principal investigators. The following data were extracted from the databases: socio-
93 demographic information; anthropometric measurements (height and weight); biochemical data
94 haemoglobin (Hb), plasma ferritin and C-reactive protein (CRP); and dietary intake data
95 (energy, and macro- and micronutrients). Cases for the pooled analyses were selected from the
96 three original databases, using the following inclusion criteria: children 5- to 12-years-old;
97 availability of three biochemical values, namely Hb, plasma ferritin and CRP; and complete
98 dietary intake data. Cases with missing data for any of these variables were excluded.

99 *Dietary intake assessment*

100 Dietary data were collected by means of the 24-hr dietary recall method. Three 24-hr recalls per
101 child were completed on non-consecutive days for all children in one of the studies (Taljaard *et al.*,
102 2013) but only on a sub-sample of children in the other two studies (Baumgartner *et al.*,
103 2012; Van der Hoeven *et al.*, 2016). Both the child and the parent or caregiver responsible for
104 the food preparation were present during the 24-hr recall interviews, which were conducted by
105 trained fieldworkers. Food portion sizes were estimated using plastic food models, household
106 utensils, food packaging materials and “dish-up and measure”. Where the amount of food
107 consumed was reported in household measures or volume, it was converted to grams using the
108 South African Medical Research Council (SAMRC) Food Quantities Manual (Langenhoven *et al.*,
109 1991). The South African Food Database (SAFOODS) was used to code the foods
110 consumed by the children. The SAMRC FoodFinder3 software program was used to convert
111 food intake to energy, macro- and micronutrients. FoodFinder3 did not include the fortified
112 values for maize meal and bread and these values were added to the database of FoodFinder3,
113 based on the values in the SAFOODS database (Wolmarans *et al.*, 2010). The average daily
114 energy and nutrient intake of the three 24-hr recalls was calculated for each child. The
115 percentage of children with an average daily intake below the Estimated Energy Requirements
116 (EER) was calculated and, for micronutrients, the Estimated Average Requirements (EAR) was
117 calculated (Otten *et al.*, 2006; Institute of Medicine, 2011).

118 *Anthropometric measurements*

119 Weight and height of the children were measured according to the 2007 WHO recommended
120 procedures, as described in previously published papers (Baumgartner *et al.*, 2012; Taljaard *et al.*,
121 2013; Van der Hoeven *et al.*, 2016). The 2007 WHO references values were used to
122 calculate age- and sex-specific indicators of nutritional status, such as stunting, underweight
123 and overweight. Stunting was defined as a height-for-age z-score (HAZ) < -2 SD, underweight
124 as weight-for-age z-score (WAZ) < -2 SD, and overweight as BMI-for-age z-score (BAZ) > +1
125 SD (De Onis *et al.*, 2007).

126 *Biochemical measurements*

127 To determine biochemical values, blood samples were collected from children in the original
128 studies. For the purpose of the present study, the Hb, plasma ferritin and CRP concentrations
129 were used. The Hb concentrations were measured on whole blood by using the direct
130 cyanmethemoglobin method (Bio Rad Laboratories (PTY) Ltd) by using Drabkin’s solution and a
131 standard miniphotometer in the Baumgartner *et al.* (2012) study, while in the other two studies a
132 haematology analyser (Coulter® Ac·T™ 5diff CP; Beckman Coulter) was used (Taljaard *et al.*,
133 2013; Van der Hoeven *et al.*, 2016). Plasma ferritin and CRP were measured using an

134 automated chemiluminescent immunoassay system (CLIA, IMMULITE) in the Baumgartner *et*
135 *al.* (2012) study, while in the other two studies a ferritin ELISA kit (Ramco Laboratories Inc.) was
136 used for plasma ferritin and an immunoturbidimetric method (Technicon RA-1000 auto analyser)
137 for CRP (Taljaard *et al.*, 2013; Van der Hoeven *et al.*, 2016).

138 For the pooled data analysis, the plasma ferritin values of children with a CRP concentration ≥ 5
139 mg/L were adjusted for inflammation by multiplying plasma ferritin values with a correction factor
140 (CF) of 0.65 (Thurnham *et al.*, 2010).

141 Selected cut-off points of iron status markers were used to classify children as ID, anaemic, or
142 IDA. Anaemia was defined as Hb < 115 g/L; ID based on plasma ferritin < 15 μ g/L; and IDA was
143 defined as a combination of Hb < 115 g/L and plasma ferritin < 15 μ g/L (WHO, 2007; WHO,
144 2011a; WHO, 2011b).

145 *Ethical approval for the present study*

146 Ethical approval for the pooled data analysis was obtained from the Health Research and Ethics
147 Committee of the North-West University (NWU-00027-16-A1). The appropriate ethics
148 committees granted ethical permission for the three original studies (Baumgartner *et al.*, 2012;
149 Taljaard *et al.*, 2013; Van der Hoeven *et al.*, 2016).

150 *Determination of nutrient patterns*

151 Exploratory factor analysis was conducted as described by Field (2013) in order to develop a
152 number of nutrient patterns (called factors) that explained most of the variances in the observed
153 nutrients (variables). The factor analysis was applied with the correlation matrix in order to
154 standardise data. The reliability of the factor analysis was verified using the Kaiser-Meyer-Olkin
155 (KMO) test. Factors were rotated by orthogonal Varimax rotation method to provide a simpler
156 structure and to improve interpretation. Factors were retained and interpreted for further
157 analysis based on their natural interpretation, 'eigenvalues' of > 1.00 and scree-plot construction.
158 In order to define the extent to which each of the input nutrient variables contributes to the
159 meaning of each of the factors, the nutrients (at least three variables) with factor loadings
160 greater than or equal to 0.53 on a given factor were retained for nutritional interpretation. Factor
161 scores for each of the nutrient patterns were computed for each individual child and indicate the
162 degree to which each child's nutrient intake conforms to the identified patterns. For each factor,
163 children were grouped into three categories according to tertiles of factor scores, and the
164 distribution of children across the tertiles of each pattern according to their anaemia and ID
165 status was determined.

166 *Statistical analyses*

167 The Statistical Software Package SPSS v.25 (Inc., Chicago, IL, USA) was used for all statistical
168 analyses. The Kolmogorov-Smirnoff test was used to assess the normality of the data
169 distribution. The Levene's test was used to test the homogeneity of variances. For normally
170 distributed data, the results are expressed as either mean and standard deviation (\pm SD) or
171 mean and 95% confidence interval (CI). The dietary data were log-transformed and after log
172 transformation, most dietary data were still not normally distributed. Dietary data are therefore
173 reported as medians (25th; 75th percentile). The results for categorical data are presented as
174 frequencies and percentages. Differences between two groups were tested using the
175 independent t-test for normally distributed data and the Mann-Whitney U test for non-normally
176 distributed data. Differences between categorical data were determined using the Pearson chi-
177 square test with z-test with adjusted p-values (Bonferroni method). The analysis of variances
178 (ANOVA) was used for continuous variables to compare across more than two groups. If
179 significant differences were detected, the Bonferroni test was used to identify which groups
180 differed. The analysis of covariance (ANCOVA) by means of the general linear model univariate
181 procedure was used, taking into account covariates such as energy intake, age, gender, and
182 study site. The Bonferroni test was used to identify which groups differed if significant
183 differences were detected. Binary logistic regression models were computed to identify the
184 association of nutrient patterns with anaemia and ID. We calculated two models for each
185 nutrient pattern. Each model 1 referred to the crude analysis and in each model 2, we included
186 the same set of potential confounding factors, such as dietary energy intake, study site, age and
187 gender. Odds ratio (OR) and 95% CI for the regression parameters are reported. Statistical
188 significance was set at $p < 0.05$ and trend towards statistical significance at $p < 0.1$.

189 **Results**190 *Characteristics and anaemia and iron status of children in the pooled group and by study sites*

191 A total of 578 cases were pooled for analysis, consisting of study site 1 (KZN) $n=102$, study site
192 2 (NW1) $n=100$, and study site 3 (NW2) $n=376$. The characteristics of children as well as the
193 biochemical indicators of anaemia and iron status for each of the study sites and the pooled
194 group are presented in Table 1. The gender distribution was 51.0% boys and 49.0% girls, 5- to
195 12-years-old. The age differed significantly across the three study sites ($p < 0.001$).

196 In the pooled group ($n=578$), 13.8% of the children were anaemic, 27.7% were ID, 19.8%
197 presented with elevated CRP levels, and 27.2% were stunted. Differences across study sites
198 were observed: in terms of anaemia and ID prevalence, children in KZN had significantly higher
199 rates of anaemia (26.5%) compared to the other two study sites (14.0% and 10.4%,
200 respectively; $p < 0.001$). Children in KZN had an ID prevalence of 42.2%, which was significantly

201 higher compared to the other two study sites (18.8% and 26.3%, respectively; $p < 0.001$). Raised
202 CRP levels were more prevalent in children in NW2 (22.8%) than in the other two study sites
203 (19.6% and 8.0%, respectively, $p < 0.01$).

204 The highest prevalence of stunting (34.4%) was found in children in NW2, the prevalence of
205 underweight in NW1 was 24.0%, and the highest prevalence of overweight (12.6%) was found
206 in children in KZN.

207 *The energy and nutrient intake of children in the pooled group and by study sites*

208 More than 80% of children did not meet the Estimated Energy Requirements (EER) (Table 2).
209 Though the intake of protein was within the adequate range (between 10% and 30% of total
210 energy intake), the mean percent of energy from protein was just above the minimum range of
211 10% in the pooled group and in all study sites. Overall, more than 50% of children did not meet
212 the EAR for the variety of nutrients, including vitamin A, vitamin C, vitamin B12, folate and zinc;
213 although, only 17.7% of children had an iron intake below EAR.

214 *The energy and nutrient intake of children in the pooled group stratified by anaemia and ID* 215 *status*

216 Within the pooled group, children were stratified by anaemia and iron status (Supplement Table
217 S1). Results showed that energy intake did not differ significantly between the compared sub-
218 groups. Median intakes for vitamin A and vitamin C were lower for anaemic than non-anaemic
219 children ($p = 0.03$ and $p = 0.02$, respectively) and for ID versus non-ID children ($p = 0.03$ and
220 $p = 0.046$, respectively).

221 *The nutrient patterns identified by factor analysis in the pooled group and by study sites*

222 The exploratory factor analysis procedure was tested statistically and found KMO > 0.8 (data not
223 shown), which confirmed that the multivariate reduction technique is applicable to our study
224 sample. The factor analyses were conducted for the three study sites separately as well as for
225 the pooled group. As similar nutrient patterns were observed in all study sites (data not shown),
226 the final results are presented for the pooled group (Table 3). Estimates from a factor analysis
227 performed on 23 nutrients (equal to the number of nutrient variables) were reduced to 4 nutrient
228 patterns (factors) based on the convergence of the scree plot and Kaiser's criterion. The four
229 retained factors explained 60.2% of the total variance after Varimax rotation. Factor loadings,
230 which are the equivalents to the correlations between the nutrients and nutrient patterns, and
231 the names assigned to each nutrient pattern are presented in Table 3. The factor loading values
232 did not change when the variables (nutrients) that were not prevalent in the nutrient patterns
233 and did not show correlations with nutrient patterns were not included in the analysis (Field,
234 2013). The first nutrient pattern (factor 1) is mainly representative of plant protein, carbohydrate,

235 B-vitamins, and iron (27.0% of the total variance explained); the second nutrient pattern (factor
236 2) represents animal protein, saturated fat, and zinc (12.0% of the total variance explained); the
237 third nutrient pattern (factor 3) is mainly representative of vitamins A and B12 (11.1% of the total
238 variance explained); and the fourth nutrient pattern (factor 4) mainly represents fibre and calcium
239 (10.1% of the total variance explained). Factors were named according to the nutrients with
240 higher loadings (>0.53 , as dominant nutrients) that cluster around the same pattern (Field,
241 2013). Factor 1 was named 'plant protein, carbohydrate, iron and B-vitamins' nutrient pattern;
242 factor 2 - 'animal protein and saturated fat'; factor 3 - 'vitamin A and vitamin B12'; and factor 4 -
243 'calcium and fibre' nutrient pattern. The comparisons of the nutrient intakes of children across
244 the tertiles for each nutrient pattern score showed that intake of dominant nutrients in each
245 nutrient pattern was significantly lower in the first tertiles (T1) compared to the T2 and T3 (all
246 $p<0.001$), adjusted for energy intake, age, gender, and study site (data not shown).

247 We compared the distribution of anaemic or ID children across the scores tertiles for each
248 nutrient pattern (Table 4). A significantly higher proportion of anaemic children (41.3%) fell
249 within T1 and a lower proportion (26.2%) in T3 of the 'vitamin A and vitamin B12' pattern
250 compared to non-anaemic children (30.3% and 34.3%, respectively; $p=0.012$). Within the other
251 nutrients patterns, we found no significant difference in the proportion of anaemic or ID children.
252 However, there was a trend for a higher proportion of anaemic children within T3 of the 'plant
253 protein, carbohydrate, iron and B-vitamins' and 'calcium and fiber' patterns. Furthermore, there
254 was a trend for a lower proportion of ID children within T1 of the 'calcium and fiber' pattern.
255 Comparisons of the continuous factor scores between sub-groups of children according to their
256 anaemia and ID status showed similar results (data not shown).

257 The results of the logistic regression analysis (Table 5) present the OR of anaemia and ID and
258 corresponding 95% CIs in the pooled group of children ($n=578$) according to continuous factor
259 scores of nutrient patterns. The 'vitamin A and vitamin B12' nutrient pattern scores were
260 associated with lower odds of being anaemic [OR 0.63 (0.49-0.91), $p=0.035$] and tended to be
261 associated with lower odds of being ID. The 'plant protein, carbohydrate, iron and B-vitamins'
262 nutrient pattern scores tended to be associated with higher odds of being anaemic or ID. No
263 significant association was observed between other nutrient pattern scores and the odds of
264 being anaemic or ID.

265 **Discussion**

266 In order to determine the various nutrient combinations that could be associated with anaemia
267 and ID, we identified four nutrient patterns explaining 60.2% of the total variance in nutrient
268 intakes. Results showed that the 'vitamin A and vitamin B12' nutrient pattern was inversely
269 associated with anaemia and tended to be inversely associated with ID. The 'plant protein,

270 carbohydrate, iron and B-vitamins' nutrient pattern tended to be positively associated with
271 anaemia and ID.

272 Almost half of the children in the pooled group had an inadequate intake of vitamin A and
273 vitamin B12. The study site KZN had the highest rates of inadequate intake of these vitamins
274 (91.2% and 75.3%, respectively) and also the highest rates of anaemia and ID. Moreover,
275 comparisons of the sub-groups of study children according to their anaemia and iron status
276 showed that anaemic and ID children had significantly lower dietary intake of vitamin A. These
277 results are in agreement with the nutrient pattern analysis, as significantly higher proportion of
278 anaemic children fell within the lowest tertile of the 'vitamin A and vitamin B12' nutrient pattern
279 compared to children without anaemia. Literature shows that in children of school age, vitamin A
280 deficiency was one of the main predictors of anaemia (Thurlow *et al.*, 2005; Jamil *et al.*, 2008).
281 Another study by Oliveira *et al.* (2016) also showed that insufficient dietary intake of vitamin A
282 and vitamin B12 in children was one of the factors most strongly associated with anaemia. An
283 adequate supply of both vitamin A and vitamin B12 is needed for the production of red blood
284 cells and facilitates the absorption of non-haem iron from plant-based diets (Fishman *et al.*,
285 2000; García-Casal, 2006). The 'vitamin A and vitamin B12' nutrient pattern probably reflects
286 the combination of these nutrients within a specific food matrix, particularly organ meat, such as
287 liver. In appropriate amounts, these foods are valuable sources of many essential
288 micronutrients, including vitamin A and vitamin B12 (Schonfeldt *et al.*, 2013). Irregular
289 consumption of foods like organ meats (i.e. less than once per week) was significantly
290 associated with anaemia (Obse *et al.*, 2013).

291 In the present study, the scores of the nutrient pattern 'plant protein, carbohydrate, iron and B-
292 vitamins' tended to be associated with higher odds for anaemia and ID. This pattern is
293 characterized by several of the micronutrients (riboflavin, niacin, pyridoxine, folic acid, iron) that
294 are used in the mandatory fortification of wheat flour (bread) and maize meal (South Africa,
295 Department of Health, 2003). The high loadings for plant protein and carbohydrates further point
296 to consumption of these two fortified staple foods in this nutrient pattern. Potentially,
297 consumption of the fortified staple foods can make a significant contribution towards daily iron
298 intake, since on average, children 5- to 12-years-old need to consume 4 to 6 mg of iron every
299 day (Steyn *et al.*, 2008). The average daily portion size of fortified bread consumed by the
300 children was 99 g (equal to 3 slices), which provides 3.4 g of iron. In addition, the average daily
301 amount of fortified maize meal was 119 g (eaten as either a soft, stiff or crumbly porridge),
302 which provides 3.5 g of iron (data not shown). In the pooled group, only 17.7% of the children
303 had an iron intake below the EAR, yet 13.8% were anaemic and 27.7% were ID. These results
304 suggest that an adequate dietary iron intake does not necessarily equate to adequate iron
305 status. It should be noted that the study site KZN had the highest percentage of children below

306 the EAR for iron intake (23.4%), and also the highest prevalence of anaemia (26.5%) and ID
307 (42.2%), compared to the other two study sites. Also, children in KZN had the highest intake of
308 carbohydrates as proportion of TE intake, which further points to higher consumption of the two
309 fortified staple foods. Electrolytic iron, which is used as fortificant in maize meal and wheat flour,
310 does not seem to be effective in reducing the prevalence of anaemia or ID in children (Van
311 Stuijvenberg *et al.*, 2008; Hurrell *et al.*, 2010; Paganini *et al.*, 2016). Furthermore, chemical
312 analyses of uncooked fortified food items, sampled in different areas in South Africa, suggest
313 that the levels of fortificants, including vitamin A and iron, are below the recommended
314 fortification premix values (Yusufali *et al.*, 2012; Van Jaarsveld *et al.*, 2015). This raises
315 concerns of bioavailability and quantity of fortificants used in the NFFP, which may limit the
316 potential of preventing anaemia and ID.

317 The high loading of plant protein in the 'plant protein, carbohydrate, iron and B-vitamins' nutrient
318 pattern suggests the presence of inhibitors of iron absorption, such as phytate and polyphenols,
319 which are present in plant foods. Absorption of non-haem iron from plant foods or fortificants
320 can be enhanced by vitamin C in the meal (Péneau *et al.*, 2008). A high proportion of children
321 in our study had an intake of vitamin C below the EAR, and our results show that anaemic
322 children had significantly lower vitamin C intake compared to non-anaemic children. Studies
323 have further shown that the absorption of non-haem iron from plant foods will be enhance by
324 the addition of a little amount of animal protein in the same meal (Hurrell *et al.*, 2005; Ready &
325 Cook, 2006). Studies evaluating the relationship of nutrient patterns with anaemia and iron
326 status are scarce in comparison to food patterns. As far as we are aware, few studies have
327 explored the relationship of food patterns with anaemia and ID, and these studies focused on
328 adults (Shi *et al.*, 2006; Broderstad *et al.*, 2011; Beck *et al.*, 2013). Overall, these studies
329 suggest that food patterns characterized by a low intake of meat and vegetables are associated
330 with an increased risk of suboptimal iron status or anaemia. In the most recent study, Beck *et al.*
331 observed a low odds of suboptimal iron status based on ferritin concentrations in young women,
332 who followed a 'meat and vegetable' pattern (Beck *et al.*, 2013). These associations observed
333 between food patterns and anaemia and/or iron status can, however, not be directly compared
334 to our results on nutrient patterns.

335 Some strengths and limitations of this study should be considered. The identification of nutrient
336 patterns, rather than individual nutrients, as was done in this study, may be a better way of
337 exploring the cumulative and synergistic effects of various nutrients in the aetiology of nutritional
338 anaemia. However, the present study was cross-sectional and unable to identify causal
339 relationships between exposure (diet) and outcome (anaemia and iron status). Also, the dietary
340 intake data may be affected by misreporting of food intakes, which is known to be a common
341 challenge in dietary assessment, and is described in the literature (Archer *et al.*, 2013; Mitka,

342 2013). There are also limitations with regard to the food composition database used to convert
343 food intake data to nutrient intakes. The nutrient values in any food composition database
344 reflect averages and therefore give an approximate indication of the nutrient content of a food
345 (Wolmarans *et al.*, 2013). Also, the nutrient content of fortified bread and maize meal varies
346 (Yusufali *et al.*, 2012; Van Jaarsveld *et al.*, 2015) and may differ from the values in the food
347 composition database.

348 **Conclusion**

349 This study showed that the 'vitamin A and vitamin B12' nutrient pattern was associated with a
350 lower odd of anaemia in 5- to 12-year-old school children from three study sites in South Africa.
351 These results highlight the potential role of inadequate intake of both vitamins A and B12 in the
352 aetiology of nutritional anaemia. The 'vitamin A and vitamin B12' nutrient pattern probably
353 reflects consumption of animal-source foods that are rich in vitamin A and vitamin B12,
354 particularly liver. Nutrient pattern analysis is a novel and potentially powerful tool for exploring
355 the relationship between nutrient intake and anaemia status and can possibly contribute
356 towards planning intervention strategies.

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364 **Author contributions**

365 T.V.Z. and M.F. conceptualized this study, M.V. analysed the data and wrote the draft article.
366 S.M.H., J.B., M.v.d.H., C.T-K. and C.M.S. were responsible for data collection in the original
367 studies and contributed to the writing of the paper. T.V.Z. and M.F. contributed to the writing of
368 the paper. All authors read and approved the final manuscript.

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Table 1: The characteristics of 5- to 12-year-old school children and biochemical indicators of anaemia and iron status in the pooled group and at each study site

	Pooled group n=578	KZN n=102	NW1 n=100	NW2 n=376	P value
Age, years ¹	8.7±1.3	10.0±1.0 ^a	9.0±1.9 ^b	8.2±0.9 ^c	<0.001
Gender Boys, % (n)	51.0 (296)	47.0 (48)	54.0 (54)	52.0 (194)	0.593
Girls, % (n)	49.0 (282)	53.0 (54)	46.0 (46)	48.0 (182)	
Haemoglobin (Hb, g/L) ²	125 (124; 126)	120 (118; 122) ^a	127 (124; 129) ^b	126 (125; 127) ^b	0.001
Plasma ferritin (µg/L) ²	23.7 (14.4; 36.9)	17.6 (9.0; 29.0) ^a	23.1 (16.4; 33.2) ^b	21.9 (13.6; 34.4) ^b	0.006
Anaemia, % (n)	13.8 (80)	26.5 (27) ^a	14.0 (14) ^b	10.4 (39) ^b	0.001
Iron deficiency, % (n)	27.7 (160)	42.2 (43) ^a	18.8 (18) ^b	26.3 (99) ^b	0.001
Iron deficiency anaemia, % (n)	6.7 (39)	16.7 (17) ^a	4.0 (4) ^b	4.8 (18) ^b	0.001
Inflammation, % (n)	19.8 (114)	19.6 (20) ^a	8.0 (8) ^b	22.8 (86) ^c	0.01
Stunted, % (n)	27.2 (154)	12.7 (12) ^a	13.0 (13) ^a	34.4 (129) ^b	0.05
Underweight, % (n)	15.0 (87)	3.9 (4) ^a	24.0 (24) ^b	15.8 (59) ^c	0.01
Overweight, % (n)	6.1 (35)	12.6 (12) ^a	4.0 (4) ^b	5.0 (19) ^b	0.001

KZN, KwaZulu-Natal, NW, North West province; HAZ, height-for-age z-score; WAZ, weight-for-age z-score; BAZ, BMI-for-age z-score; SD, standard deviation;

¹reported as mean ± SD; ² mean, 95%CI;

Anaemia defined as Hb <115 g/L; Iron deficiency as plasma ferritin <15 µg/L; Iron deficiency anaemia as Hb < 115 g/L + plasma ferritin <15 µg/L; Inflammation as C-reactive protein (CRP) ≥5 mg/L; Stunting, underweight and overweight was defined as HAZ <-2SD, WAZ <-2SD and BAZ >+1SD, respectively;

Plasma ferritin values of children with a C-reactive protein (CRP) concentration ≥5 mg/L were adjusted for inflammation by multiplying plasma ferritin values with a correction factor (CF) of 0.65 (Thurnham *et al.*, 2010).

¹ANOVA used for continuous variables were compared across groups; Pearson Chi-Square for categorical data, a,b,c- the values with different letters in superscript differed significantly, multiple comparisons: Bonferroni test.

Table 2: Dietary intake of 5- to 12-year-old school children in the pooled group and at each study site

	Pooled group n=578	KZN n=102	NW1 n=100	NW2 n=376	P value²
Total energy (TE), kJ ¹	6413 (5144; 7507)	6940 (5776; 7988) ^a	5753 (4548; 7066) ^b	6455 (5175; 7577) ^c	0.030
Total energy < EER, %	82.7	93.1 ^a	88.2 ^b	78.4 ^c	0.048
Total protein, g	46.9 (36.8; 57.7)	42.1 (30.6; 52.9) ^a	49.0 (37.6; 67.4) ^b	47.5 (39.2; 58.2) ^c	<0.001
Total protein, %TE	13.1	10.3 ^a	14.5 ^b	12.5 ^c	0.031
Total protein < EAR, %	8.1	27.4	5.0	3.7	<0.001
Plant protein, g	26.9 (21.8; 32.3)	24.9 (21.4; 31.1) ^a	25.0 (19.0; 30.9) ^a	27.9 (23.1; 33.4) ^b	0.006
Animal protein, g	18.4 (11.1; 26.8)	12.3 (5.0; 23.5) ^a	23.3 (13.4; 35.3) ^b	18.4 (12.3; 25.8) ^c	<0.001
Total fat, g	37.6 (27.8; 50.0)	42.0 (38.3; 54.5) ^a	33.2 (22.5; 42.3) ^b	37.7 (27.8; 49.5) ^c	0.011
Total fat, % TE	22.3	23.0	21.9	22.1	0.054
Carbohydrate, g	243.7 (199.6; 289.5)	272.4 (210.7; 323.0) ^a	215.2 (169.6; 258.9) ^b	248.4 (202.8; 289.1) ^c	0.014
Carbohydrate, % TE	64.6	66.7 ^a	63.6 ^b	65.4 ^a	0.048
Total fiber, g	18.0 (14.1; 23.2)	15.2 (11.5; 18.8) ^a	17.7 (12.4; 24.3) ^b	18.5 (15.1; 23.6) ^c	0.001
Calcium, mg	217.0 (133.7; 309.7)	171.7 (124.9; 255.6) ^a	297.8 (120.0; 318.9) ^b	216.9 (139.0; 300.0) ^c	0.001
Calcium < EAR, %	97.5	100.0	97.0	98.0	0.230
Iron, mg	12.7 (10.2; 15.7)	10.2 (7.3; 11.6) ^a	12.4 (9.1; 16.1) ^b	13.5 (11.3; 16.8) ^c	0.030
Iron < EAR, %	17.7	23.4 ^a	19.0 ^b	12.8 ^c	0.023
Zinc, mg	9.6 (7.2; 12.1)	7.3 (5.5; 9.3) ^a	7.7 (5.9; 11.1) ^a	10.5 (8.4; 12.9) ^b	0.010
Zinc < EAR, %	18.2	51.9 ^a	25.0 ^b	7.2 ^c	<0.001
Vitamin A, µg, RE	461.8 (332.9; 718.5)	334.5 (210.1; 432.5) ^a	499.8 (341.0; 803.9) ^b	496.8 (367.8; 862.6) ^b	<0.001
Vitamin A < EAR, %	47.8	91.2 ^a	46.0 ^b	36.4 ^c	<0.001
Vitamin C, mg	18.1 (9.7; 38.3)	19.6 (8.2; 26.5) ^a	18.6 (5.4; 28.9) ^a	15.3 (9.5; 29.2) ^b	0.042
Vitamin C < EAR, %	70.8	64.1	69.0	72.9	0.032
Vitamin D, µg	1.4 (0.6; 2.8)	1.5 (0.7; 2.9)	1.4 (0.3; 4.5)	1.5 (0.6; 2.6)	0.650
Vitamin D < EAR, %	99.3	100.0	98.0	100.0	0.733
Vitamin E, mg	7.3 (4.2; 12.5)	10.7 (10.2; 19.2) ^a	7.0 (2.2; 8.2) ^b	6.4 (4.1; 10.8) ^c	<0.001
Vitamin E < EAR, %	56.1	25.3 ^a	80.0 ^b	57.9 ^c	<0.001
Riboflavin, mg	1.31 (0.77; 1.92)	0.91 (0.50; 1.53) ^a	1.00 (0.60; 1.42) ^a	1.51 (1.00; 2.10) ^b	0.030
Riboflavin < EAR, %	7.8	8.8 ^a	9.0 ^a	6.5 ^b	0.042
Niacin, mg	14.5 (10.9; 19.7)	13.1 (8.4; 16.9) ^a	14.0 (10.7; 19.0) ^b	15.1 (11.5; 20.9) ^b	0.037
Niacin < EAR, %	2.7	2.9	2.0	2.8	0.600
Vitamin B6, mg	2.7 (1.9; 3.7)	2.3 (1.8; 3.3) ^a	1.7 (0.9; 2.6) ^b	3.0 (2.2; 3.9) ^c	0.010
Vitamin B6 < EAR, %	6.6	6.8 ^a	12.0 ^b	5.1 ^c	0.010
Folate, µg	345.7 (233.2; 458.5)	253.5 (186.9; 359.6) ^a	193.3 (118.5; 276.6) ^b	395.3 (310.5; 520.9) ^c	<0.001
Folate < EAR, %	27.9	55.1 ^a	66.0 ^b	10.4 ^c	<0.001
Vitamin B12, mcg	1.6 (0.7; 3.4)	0.9 (0.4; 1.8) ^a	1.8 (0.4; 4.8) ^b	1.8 (0.8; 3.5) ^b	0.030
Vitamin B12 < EAR, %	46.9	75.3 ^a	42.0 ^b	40.4 ^b	0.045

EER (Estimated Energy Requirements); EAR (Estimated Average Requirements); KZN, KwaZulu-Natal, NW, North West provinces;

¹reported as median (25th; 75th percentile); all such values.

²Mann-Whitney U test used for non-parametric variables; Pearson Chi-Square for categorical data.

a,b,c- the values with different letters in superscript differed significantly, multiple comparisons: Bonferroni test.

Table 3: Extracted nutrient patterns and factor loadings identified by factor analysis in the pooled group of 5- to 12-year-old school children (n=578)

Nutrients and variance explained	Factors (nutrient patterns)*			
	Factor 1 'Plant protein, carbohydrate, iron and B-vitamins'	Factor 2 'Animal protein and saturated fat'	Factor 3 'Vitamin A and Vitamin B12'	Factor 4 'Calcium and fibre'
Plant protein	0.814	0.394	-0.116	0.392
Animal protein	0.190	0.873	0.398	0.123
Saturated fat	0.180	0.683	-0.147	0.162
Carbohydrate	0.656	0.340	-0.190	0.385
Total fiber	0.670	0.325	0.197	0.632
Iron	0.688	0.492	0.230	0.339
Zinc	0.385	0.536	0.226	0.283
Vitamin A	0.361	-0.190	0.763	0.298
Riboflavin	0.407	0.285	0.403	0.491
Niacin	0.693	0.267	0.247	0.281
Vitamin B6	0.811	0.321	0.137	-0.142
Folate	0.779	-0.136	0.276	0.383
Vit_B12	0.305	0.249	0.836	-0.247
Vitamin D	0.116	0.337	0.259	-0.107
Calcium	0.183	0.266	0.139	0.671
Percent of variances				
Total 60.23%	27.00	12.01	11.13	10.09

*Estimates from a factor analysis performed on 23 nutrients after rotation. The loadings are measure the significance of the corresponding nutrient to the factor. The leading nutrients were defined as loadings >0.53 for each factor and shown in bold; nutrients with loadings <0.1 were suppressed (Field, 2013).

Table 4: Comparisons of proportions of 5- to 12-year-old school children in sub-groups of anaemia and iron deficiency status across the tertiles scores of four nutrient patterns

Tertiles of nutrient patterns	Anaemic n=80	Non-anaemic n=498	P value	ID n=160	Non- ID n=418	P value
'Plant protein, carbohydrate, iron and B-vitamins' nutrient pattern						
T1	30.3 ^a	34.9 ^a	0.064	30.5	31.6	0.361
T2	28.7 ^a	34.1 ^a		32.5	33.7	
T3	41.0 ^a	30.9 ^b		37.0	34.7	
'Animal protein and saturated fat' nutrient pattern						
T1	33.5	32.5	0.345	32.5	33.5	0.222
T2	31.5	33.5		33.1	33.7	
T3	35.0	33.9		34.4	32.8	
'Vitamin A and Vitamin B12' nutrient pattern						
T1	41.3 ^a	30.3 ^b	0.012	34.5	33.2	0.304
T2	32.5 ^a	35.3 ^a		33.4	31.6	
T3	26.2 ^a	34.3 ^b		32.1	35.1	
'Calcium and fibre' nutrient pattern						
T1	25.3 ^a	28.9 ^a	0.068	26.1 ^a	32.0 ^b	0.076
T2	33.2 ^a	34.9 ^a		35.6 ^a	31.6 ^a	
T3	41.5 ^a	36.1 ^b		38.3 ^a	36.4 ^a	

T-tertile; Values are expressed as percentage of the sub-groups for categorical variables; Chi-square test used for categorical variables;

Superscript letters in a row that are the same denote a subset of sub-groups that did not differ significantly from each other, superscript letters in a row that differ denote a subset of sub-groups that differ significantly from each other at the 0.05 significance level; z-test with adjusted p-values (Bonferroni method)

Table 5: Odds ratios (95% CI) of anaemia and ID in the pooled group of 5- to 12-year-old school children (n=578) according to continuous factor scores of nutrient patterns

	Factor 1 'Plant protein, carbohydrate, iron and B-vitamins'	p-value	Factor 3 'Vitamin A and vitamin B12	p-value
Anaemia				
Model 1	1.10 (0.90-1.34)	0.140	0.91 (0.81-1.18)	0.192
Model 2	1.37 (1.09-1.89)	0.051	0.63 (0.49-0.91)	0.035
Iron deficiency				
Model 1	1.08 (0.82-1.39)	0.174	1.01 (0.83-1.14)	0.151
Model 2	1.14 (1.10-1.32)	0.070	0.96 (0.95-1.31)	0.050

Model 1 - crude; Model 2 - adjusted for energy intake, study site, age and gender

Supplement Table S1 Nutrient intake of 5- to 12-year-old school children stratified by ID and anaemia status in the pooled group

Nutrients	Anaemic n=80	Non-anaemic n=498	P value	ID n=160	Non- ID n=418	P value
Energy, kJ	6709 (4855; 7566)	6755 (4913; 7852)	0.23	6623 (3873; 6978)	6596 (4976; 7865)	0.60
Total protein, g	47.7 (28.0; 57.4)	49.3 (36.8; 57.7)	0.39	46.8 (36.3; 57.8)	47.8 (38.0; 57.3)	0.25
Plant protein, g	27.4 (21.3; 33.5)	27.8 (22.2; 32.3)	0.71	27.1 (21.5; 31.8)	27.2 (21.8; 32.4)	0.76
Animal protein, g	19.3 (9.0; 28.5)	18.4 (11.5; 26.7)	0.50	19.1 (11.9; 26.5)	18.2 (10.9; 26.8)	0.18
Total fat, g	41.2 (29.6; 53.8)	38.3 (27.8; 49.0)	0.44	40.4 (31.1; 51.7)	38.1 (27.6; 47.3)	0.31
Saturated fat, g	9.0 (6.7; 14.2)	9.6 (6.6; 13.2)	0.26	9.8 (6.8; 13.2)	9.5 (6.6; 13.4)	0.25
Carbohydrate, g	251.5 (219.3; 288.6)	248.6 (224.3; 279.3)	0.20	248.4 (175.8; 320.1)	249.0 (192.3; 318.1)	0.16
Total fiber, g	17.3 (17.7; 23.8)	18.2 (14.3; 22.9)	0.14	17.3 (13.0; 22.9)	18.2 (14.4; 23.5)	0.65
Calcium, mg	215.7 (141.4; 315.0)	221.3 (133.0; 308.4)	0.52	213.3 (127.6; 296.0)	222.9 (138.9; 325.0)	0.10
Iron, mg	9.1 (6.7; 9.7)	10.9 (10.1; 15.8)	0.08	11.3 (9.4; 15.4)	12.9 (10.6; 15.8)	0.33
Zinc, mg	9.4 (6.3; 12.1)	9.6 (7.2; 12.1)	0.61	9.5 (6.7; 12.0)	9.7 (7.3; 12.1)	0.62
Vitamin A, µg, RE	446.8 (323.3; 642.5)	463.2 (333.1; 730.7)	0.03	431.7 (305.1; 596.2)	479.6 (341.3; 768.1)	0.03
Vitamin C, mg	17.3 (9.3; 26.2)	21.4 (15.7; 34.8)	0.02	17.5 (9.5; 24.2)	21.6 (10.6; 34.6)	0.046
Vitamin D, µg	1.5 (0.6; 3.0)	1.4 (0.6; 2.9)	0.70	1.4 (0.6; 2.8)	1.5 (0.6; 2.7)	0.14
Vitamin E, mg	9.1 (4.6; 15.1)	6.9 (4.0; 11.9)	0.03	8.3 (4.3; 13.9)	6.8 (4.0; 11.8)	0.05
Thiamin, mg	1.4 (1.0; 2.0)	1.5 (1.2; 2.0)	0.64	1.4 (1.2; 1.9)	1.5 (1.2; 2.0)	0.79
Riboflavin, mg	1.2 (0.7; 1.8)	1.3 (0.8; 1.9)	0.13	1.3 (0.8; 1.9)	1.3 (0.7; 1.8)	0.84
Niacin, mg	14.6 (11.1; 19.7)	14.5 (10.8; 19.7)	0.53	13.9 (10.7; 18.0)	14.8 (11.0; 20.1)	0.27
Vitamin B6, mg	3.4 (1.9; 3.8)	2.8 (1.8; 3.7)	0.12	2.4 (1.9; 3.8)	2.8 (1.9; 3.7)	0.38
Folate, µg	339.3 (190.0; 455.5)	344.9 (235.1; 458.3)	0.30	341.4 (225.8; 459.1)	346.6 (235.9; 456.3)	0.25
Vitamin B12, µg	1.4 (0.4; 3.6)	1.6 (0.7; 1.9)	0.22	1.4 (0.7; 3.2)	1.7 (0.8; 3.5)	0.42

Results are reported as median (25th; 75th percentile); all such values; T-test used to compare parametric variables, Mann-Whitney U test used to compare non-parametric variables.

CHAPTER 4: MANUSCRIPT 2:**The associations of dietary diversity with anaemia and iron status among 5- to 12-year-old school children in South Africa**

This manuscript will be submitted for publication to the *Public Health Nutrition* journal and has been written according to the guidelines of the journal (Annexure C).

Title: The associations of dietary diversity with anaemia and iron status among 5- to 12-year-old school children in South Africa

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Shortened version of the title: Dietary diversity, anaemia and iron status in children

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Conflict of interest: none.

Authorship:

T.V.Z. and M.F. conceptualized this study;

M.V. analysed the data and wrote the draft article;

S.M.H., J.B., M.v.d.H., C.T-K. and C.M.S. were responsible for data collection in the original studies and contributed to the writing of the paper;

T.V.Z. and M.F. contributed to the writing of the paper.

All authors read and approved the final manuscript.

Ethical standards:

All original intervention studies were conducted according to the guidelines laid down in the Declaration of Helsinki and all the procedures involving human participants were permitted by the appropriate ethics committees before data collection. Additional approval from the Health Research Ethics Committee was obtained to perform the present study (NWU-00027-16-A1).

Abstract

Objective: To examine the association of dietary diversity with anaemia and iron status among primary school-age children in South Africa.

Design: A pooled analysis was conducted with existing data derived from three independent intervention studies. Two different dietary diversity scores (DDS) were calculated based on data from 1-day and 3-day dietary recall periods on nine food groups consumed. Logistic regression analysis was performed to examine the association of dietary diversity with anaemia and iron status.

Setting: KwaZulu-Natal and North West province, South Africa.

Subjects: Children ($n = 578$) 5- to 12-year-old.

Results: For both recall periods, consumption of 'vegetables and fruits other than vitamin A-rich' and 'animal-source foods (ASF)' was associated with lower odds of being anaemic (both $p = 0.002$); and 'organ meats' with lower odds of being iron deficient (ID) (1-day $p = 0.045$; 3-day $p < 0.001$). Consumption of 'meat and fish' was associated with lower odds of being anaemic ($p = 0.045$) and 'vegetables and fruits other than vitamin A-rich', 'legumes, nuts and seeds' and 'ASFs' with lower odds of being ID for the 3-day recall period only ($p = 0.038$, $p = 0.020$; $p = 0.003$, respectively). A $DDS \leq 4$ was associated with higher odds of being anaemic (1-day $p = 0.001$; 3-day $p = 0.006$) and being ID (3-day $p < 0.001$).

Conclusion: In order to improve anaemia and iron status among primary school-age children, dietary diversification, with emphasis on consumption of vegetables, fruits and ASF (including organ meats), should be considered.

Key words: dietary diversity; anaemia; iron deficiency; school-age children; South Africa

Introduction

According to the World Health Organization, the global prevalence of anaemia is about 30% (approximately 2 billion people, including 600 million children of whom about half are of primary school age) ⁽¹⁾. Nutritional anemia is a common condition that results from a lack of certain vitamins and minerals, including iron deficiency (ID) ⁽¹⁾. The potential consequences of nutritional anemia in children are related to a loss of appetite, growth retardation, reduced

cognitive development and ability to concentrate which has a negative impact on schoolwork performance ⁽²⁾.

Food consumption is one of the most important factors in the etiology of nutritional anemia and ID in children of school age ^(3, 4). The intake of a greater variety of foods across and within the various food groups is needed to meet nutrient requirements, and may help to alleviate multiple micronutrient deficiencies and reduce the risk of nutritional anaemia ⁽⁵⁻⁷⁾.

In low- and middle-income countries, including South Africa, the diets of the majority of children lack variety, and mainly consist of plant-based foods that are predominantly from cereals, roots and tubers groups with limited consumption of animal-source foods (ASF) and fruits and vegetables ⁽⁷⁻¹⁰⁾. This may lead to a risk of various health disorders, including nutritional anaemia ⁽¹¹⁻¹³⁾. A South African study showed that less than 15% of school age children consumed ASF on the day of recall ⁽¹⁴⁾, placing these children at risk of nutritional anaemia ⁽¹⁵⁾. Consumption of ASF plays an important role in reducing the risk of developing anaemia and contributes to dietary intake of nutrients such as highly bioavailable iron, vitamin B12 and vitamin A ⁽¹⁵⁾. Moreover, individuals who consume mostly cereal-based diets are at greater risk of developing iron deficiency (ID) due to the high content of iron inhibitors in their diets ^(16, 17). Increased intake of ASF, and fruits and vegetables rich in vitamin C and carotenoids as precursors of vitamin A, may increase the absorption of iron from cereal-based diets ⁽¹⁸⁾.

Studies have shown that low dietary diversity is associated with metabolic syndrome, obesity and cardiovascular disorders ⁽¹⁹⁻²¹⁾. A more diverse diet has been shown to be positively associated with micronutrient adequacy of the diet ^(22, 23). An association between higher dietary diversity and lower risk of nutritional anaemia has been reported in preschool ⁽¹³⁾ and school children ^(14, 24). However, in pregnant women, varying results have been reported ^(25, 26).

A dietary diversity score (DDS) can be calculated as proxy indicator of the micronutrient adequacy of a diet. The cut-off point to define low dietary diversity and the reference recall period on which the DDS is based, differ between studies. In several dietary diversity studies, a 1-day period was chosen as it is easier to administer ^(22, 23). However, a 1-day reference recall period for the DDS calculation did not show an association with anaemia in pregnant women ⁽²⁶⁾. Furthermore, a study on school children found no association between dietary diversity and anaemia using a 1-day reference recall period, but when a 7-day was used, low dietary diversity was significantly associated with anaemia risk ⁽¹⁴⁾. Thus, considering the limited number of conclusive results, there is a need to study the diversity of diets as a whole and the consumption of specific food groups is related to anaemia and ID.

The aim of this article is to describe the association of dietary diversity based on two different reference recall periods with anaemia and ID among primary school-age children in South Africa.

Methods

A pooled analysis was conducted with existing data derived from the baseline collection point of three independent intervention studies on school children at different sites in South Africa, which have been previously reported ⁽²⁷⁻²⁹⁾. The studies were conducted between April 2009 and June 2012 at primary schools in the KwaZulu-Natal and North West provinces. These areas were malaria-free. All children received deworming medication at the baseline point. At the time of data collection, all schools were participating in the National School Nutrition Programme (NSNP) of South Africa, which provided children with a daily meal. Parents or caregivers signed informed consent forms and the learners gave verbal assent. Data collection included socio-demographic data, biochemical measurements and 24-hour dietary recalls. Cases for the pooled analysis were selected from the three original databases, using the following inclusion criteria: 5- to 12-year-old school children; availability of three biochemical values, namely haemoglobin (Hb), plasma ferritin (PF) and C-reactive protein (CRP); and complete dietary intake data. Cases with missing data for any of these variables were excluded.

Biochemical measurements

In the original studies, blood samples taken from the children were used to determine biochemical values. For the pooled analysis, the Hb, PF and CRP concentrations were used. The Hb concentrations were measured on whole blood through the direct cyanmethemoglobin method (Bio Rad Laboratories [PTY] Ltd) by using Drabkin's solution and a standard miniphotometer ⁽²⁷⁾, while in the other two studies, a haematology analyser (Coulter® Ac-T™ 5diff CP; Beckman Coulter) was used ^(28, 29). The PF and CRP were measured using an automated chemiluminescent immunoassay system (CLIA, IMMULITE) ⁽²⁷⁾, while, in the other two studies, a ferritin ELISA kit (Ramco Laboratories Inc.) was used for PF and an immunoturbidimetric method (Technicon RA-1000 auto analyser) for CRP ^(28, 29). The PF values of children with a CRP concentration ≥ 5 mg/L were adjusted for inflammation by multiplying PF values with a correction factor (CF) of 0.65 ⁽³⁰⁾. Anaemia was defined as Hb < 115 g/L and ID as PF < 15 μ g/L ^(31, 32).

Dietary diversity assessment

In all three intervention studies, dietary data were collected for three non-consecutive days by means of the 24-hour dietary recall method ⁽²⁷⁻²⁹⁾. The children and their parents or

caregivers were interviewed by trained fieldworkers and were asked to recall all food and beverage items and amounts consumed the previous day. For each child, foods reported during the 24-hour recalls were categorised into the following groups: 1) starchy staples (combination of cereals, white roots/tubers); 2) dark green leafy vegetables; 3) other vitamin A-rich fruits and vegetables (including vitamin A-rich vegetables, tubers and fruits); 4) other fruits and vegetables; 5) organ meat; 6) meat and fish; 7) eggs; 8) legumes, nuts and seeds; and 9) milk and dairy products⁽³³⁾. A score of “1” was assigned to each food group if at least one food item within the specific food group was consumed during the 24-hour recall reference period. A score of “0” was assigned if the child did not consume any food item from a given food group. The DDS for each child was calculated as the sum of the scores, with a maximum possible score of “9”. For the purpose of the study, low dietary diversity was defined as $DDS \leq 4$ ⁽¹³⁾. In addition, an ASF score (ASFS) was calculated based on five animal-source foods, namely: 1) organ meat; 2) red meat (beef mince, beef and pork sausages, processed cold meat); 3) white meat (chicken meat); 4) fish; and 5) eggs. A score of “1” was assigned to each of these groups if at least one food item within the specific food group was consumed during the 24-hour recall reference period. The ASFS for each child was calculated as the sum of the scores given, with a maximum possible score of “5”⁽³³⁾. Two different DDS were calculated: one for a 1-day recall period (1-day DDS) and one for a 3-day (3-day DDS) recall period. The 1-day DDS was calculated as the number of different food groups consumed during the first 24-hour recall period. The 3-day DDS was calculated as the number of different food groups consumed during the three 24-hour recall days.

Statistical analyses

The SPSS v. 25 (Inc., Chicago, IL, USA) was used for all statistical analyses. Data are presented as the mean and SD for continuous variables or as percentages for categorical variables. The t-test was used to compare the mean (SD) values between two sub-groups, and the paired sample t-test was used to compare the mean DDS and ASFS between the two reference recall periods. Pearson’s Chi-square test was used to compare the proportions of children consuming specific food groups, including the ASF group, and the percentage of children below the cut-off values for the DDS and ASFS. Logistic regression analysis was done with the binary outcome (anaemic versus non-anaemic, and ID versus non-ID) as the dependent variables, and DDS and food groups as independent variables. The results are presented as odds ratio (OR) and 95% confidence intervals (CI). The potential confounders (age, gender and study site) were included in the model. Statistical significance was set at $p < 0.05$ and a trend as $p < 0.1$.

Results

The mean (SD) age of the children ($n = 578$) was 8.7 (1.3) years, the gender distribution was 51.0% boys and 49.0% girls, and 13.8% of the children were anaemic and 27.7% were ID. Table 1 presents the results of the proportions of children who consumed specific food groups, DDS and ASFS as calculated from two reference recall periods. All children consumed foods from the 'starchy staples' group for both recall periods, but a significantly higher proportion of children consumed foods from certain groups, in particular 'vegetables and fruits' (other than vitamin A-rich), 'organ meat', 'eggs', and ASF groups, according to the 3-day recall period compared to the 1-day recall period (all $p < 0.05$). The mean DDS and mean ASFS were significantly higher in the 3-day recall period compared to the 1-day recall period, and the proportion of children with $DDS \leq 4$ was significantly lower for the 3-day period compared to the 1-day period.

Children were stratified according to anaemia and iron status (Table 2); the proportion of children who consumed different food groups and DDS and ASFS from the 1-day and 3-day 24-hour recalls was compared between these sub-groups. Results showed that a significantly lower proportion of anaemic (versus non-anaemic) children consumed foods from the 'vegetables and fruits (other than vitamin A-rich)', 'organ meat' and the 'ASF' groups in both reference periods. The proportion of anaemic (versus non-anaemic) children consumed foods from 'meat, fish, and seafood' and 'eggs' groups, and also proportion of ID (versus non-ID) children consumed 'legumes, nuts and seeds', was significantly lower in the 3-day period only.

Food groups consumed by the lowest proportion of children were 'vitamin A-rich vegetables and fruits' and 'organ meat'. The proportion of children with $DDS \leq 4$ was significantly higher for the anaemic sub-group (versus non-anaemic) for both recall periods. The means of the DDS were significantly lower in the anaemic and ID sub-groups (versus non-anaemic and non-ID, respectively), according to both reference recall periods, but the mean ASFS was significantly lower in the 3-day period only.

Binary logistic regression models were developed using anaemia and ID as dependent variables, and food groups and DDS categories, calculated from both reference periods, as independent variables (Table 3). Consumption of 'vegetables and fruits other than vitamin A-rich' and 'ASF' was associated with lower odds of being anaemic, and consumption of 'organ meats' was associated with lower odds of being ID in both reference recall periods (all $p < 0.05$). Consumption of 'meat and fish' was associated with lower odds of being anaemic, and 'vegetables and fruits other than vitamin A-rich', 'legumes, nuts and seeds' and 'ASF' with

lower odds of being ID for the 3-days recall period only (all $p < 0.05$). The $DDS \leq 4$ was associated with higher odds of being anaemic for both reference recall periods (1-day, $p = 0.001$; and 3-days, $p = 0.006$) and higher odds of being ID for the 3-days recall period ($p < 0.001$).

Table 1: Proportions of 5- to 12-year-old school children who consumed foods from specific food groups, according to the 1-day and 3-day 24-hour recall periods

Food groups and scores	1-day 24-hr n = 578 %	3-day 24-hr n = 578 %	p-value
Starchy staples (cereals/white roots)	100.0	100.0	1.000
Dark green leafy vegetables	26.1	27.5	0.631
Vitamin A-rich vegetables and fruits	13.2	13.8	0.709
Vegetables and fruits other than vitamin A-rich	66.2	68.2	0.048
Meat, fish, and seafood	73.0	73.4	0.823
Organ meat	9.5	10.6	0.022
Eggs	20.9	30.7	0.010
Legumes, nuts and seeds	51.9	58.1	0.042
Milk and dairy products	30.3	31.8	0.358
ASF	78.1	82.5	0.035
DDS ≤ 4	43.3	37.5	0.012
DDS			
Mean	4.69	4.84	<0.001
SD	1.2	1.1	
ASFS			
Mean	2.42	2.91	<0.001
SD	1.4	1.2	

DDS, dietary diversity score; ASF, animal-source foods; ASFS, animal-source foods score; SD, standard deviation. Continuous data reported as mean and SD, categorical data presented as percentages.

Paired t-test used to compare the means; Chi-square test used to compare the proportions. Statistical significance was set at $p < 0.05$ and a trend as $p < 0.1$.

Table 2: Proportions of 5- to 12-year-old school children who consumed specific food groups over the two reference periods according to anaemia and iron status

Food groups and scores	Sub-groups of children (n = 578)					
	Anemic (n = 80) %	Non-anemic (n = 498) %	p-value	ID (n = 160) %	Non – ID (n = 418) %	p-value
Starchy staples (cereals/white roots)						
1-day 24-hr	100.0	100.0	1.000	100.0	100.0	1.000
3-day 24-hr	100.0	100.0	1.000	100.0	100.0	1.000
Dark green leafy vegetables						
1-day 24-hr	20.0	27.4	0.217	26.9	25.8	0.830
3-day 24-hr	29.9	27.4	0.190	31.7	25.8	0.063
Vitamin A-rich vegetables and fruits						
1-day 24-hr	5.0	14.5	0.019	11.9	13.6	0.681
3-day 24-hr	11.3	14.3	0.304	13.8	13.9	1.000
Vegetables and fruits other than vitamin A-rich						
1-day 24-hr	33.8	71.5	0.001	66.9	66.0	0.833
3-day 24-hr	30.0	74.3	0.001	59.4	71.5	0.007
Meat, fish, and seafood						
1-day 24-hr	65.0	74.3	0.057	75.6	72.0	0.408
3-day 24-hr	60.0	75.5	0.006	73.1	73.4	1.000
Organ meat						
1-day 24-hr	0.0	11.2	--	5.0	11.5	0.018
3-day 24-hr	0.0	12.2	--	8.1	11.5	0.016
Eggs						
1-day 24-hr	13.8	22.1	0.058	20.0	21.3	0.418
3-day 24-hr	20.0	32.3	0.027	31.9	30.1	0.689
Legumes, nuts and seeds						
1-day 24-hr	42.5	53.4	0.072	48.9	53.9	0.050
3-day 24-hr	52.5	59.4	0.103	51.9	60.6	0.032
Milk and dairy products						
1-day 24-hr	25.0	31.1	0.209	34.4	28.7	0.190
3-day 24-hr	25.0	32.9	0.196	30.6	32.3	0.715
ASF						
1-day 24-hr	68.5	79.7	0.005	73.3	81.6	0.018
3-day 24-hr	73.8	83.9	0.001	74.4	85.6	0.002
DDS ≤ 4						
1-day 24-hr	65.0	39.8	0.001	46.9	41.9	0.060
3-day 24-hr	58.8	34.1	0.001	40.6	36.6	0.051
DDS (SD)						
1-day 24-hr	3.5 (1.2)	4.7 (1.2)	0.003	4.2 (1.2)	4.9 (1.1)	0.035
3-day 24-hr	3.8 (1.2)	4.9 (1.1)	0.001	4.2 (1.1)	4.8 (1.1)	0.035
ASFS (SD)						
1-day 24-hr	1.4 (0.9)	2.1 (1.1)	0.062	1.7 (1.0)	2.4 (1.2)	0.070
3-day 24-hr	1.8 (1.0)	3.5 (1.2)	0.004	1.8 (1.1)	3.4 (1.0)	0.001

DDS, dietary diversity score; ASF, animal-source foods; ASFS, score; SD, standard deviation.

Continuous data reported as mean (SD), categorical data presented as percentages.

T-test used to compare the mean (SD) values; Chi-square test used to compare the proportions.

Statistical significance was set at $p < 0.05$ and a trend as $p < 0.1$.

Table 3: Odds ratios (95% CI) for having anaemia and ID for 5 to 12 years old children according to the food groups consumed and DDS cut-off values

Food groups consumed	1-day recall period			3-day recall period		
	OR	95% CI	p-value	OR	95% CI	p-value
Anaemia						
“Vegetables and fruits other than vitamin A-rich”	0.67	0.40-0.71	0.002	0.38	0.23-0.62	0.002
“Meat, fish, and seafood”				0.78	0.57-0.96	0.045
ASF	0.66	0.52-0.85	0.002	0.67	0.51-0.87	0.003
DDS ≤ 4	1.71	1.30-2.21	0.001	1.56	1.45-2.67	0.006
DDS > 4	1.0			1.0		
ID						
“Vegetables and fruits other than vitamin A-rich”				0.80	0.65-0.82	0.038
“Organ meat “	0.86	0.69-1.02	0.045	0.54	0.42-0.80	<0.001
“Legumes, nuts and seeds”				0.73	0.67-0.93	0.020
ASF				0.78	0.46-0.92	0.003
DDS ≤ 4	1.30	0.91-1.84	0.060	2.79	1.77-4.48	<0.001
DDS > 4	1.0			1.0		

ID, iron deficiency; CI, confidence interval; DDS, dietary diversity score; ASF, animal-source foods; OD, Odds ratio adjusted for age, gender and study site; DDS > 4 is the reference. Statistical significance was set at $p < 0.05$ and a trend as $p < 0.1$.

Discussion

The association of dietary diversity with nutritional anaemia and ID were investigated using a pooled sample of primary school-age children in South Africa. Results showed that children who consumed foods from ‘vegetables and fruits other than vitamin A-rich’ group and from various animal food sources during the reference recall period were less likely to be anaemic and ID compared to non-consumers. Children who consumed a diet with low variety (DDS ≤ 4) were more likely to be anaemic and ID.

Comparisons between anaemic versus non-anaemic and ID versus non-ID children showed that significantly fewer anaemic and ID children consumed ‘vegetables and fruits other than vitamin A-rich’; however, for ID children, this was only the case for the 3-day reference recall period. Available evidence indicates that fruit and vegetable intake is associated with a reduced risk of many nutrition-related diseases in South Africa⁽³⁴⁾. Low consumption of fruits and vegetables has been shown to be a predictor of poor iron status, which may lead to nutritional anaemia⁽¹¹⁾. A study of Brazilian children aged 4 to 10 years showed that children who did not consume fruits and vegetables had a higher prevalence of anaemia compared to consumers⁽³⁵⁾. Similarly, results of a recent study conducted on adult women showed that non-consumers of fruits and vegetables had a higher chance of suffering from anaemia⁽³⁶⁾. The inverse relationship between the consumption of fruits and vegetables, other than vitamin A-rich, to anaemia and ID can, at least partly, be explained by the presence of

nutrients that facilitate absorption of iron from the diet. The high vitamin C content in most fruits and vegetables may play an important role in enhancing the bioavailability of iron from plant-based foods, thus preventing nutritional anaemia ⁽³⁷⁾. These results confirm our previous findings ⁽³⁸⁾, which showed that vitamin C intake was lower in anaemic versus non-anaemic and ID versus non-ID children. The South African population generally has a low intake of fruits and vegetables ⁽⁷⁾. Low consumption has also been reported for school children ⁽⁸⁾. To thrive, a more frequent and regular intake of a variety of fruits and vegetables should be promoted. The South African Food-Based Dietary Guidelines (SAFBDG) recommends eating “plenty of fruit and vegetables every day” as well as various types and colours ⁽³⁹⁾ in order to increase nutrient intake. Though, we did not find any association of fruits and vegetables rich in vitamin A with anaemia and ID, probably the number of children who reported intake of vitamin A rich fruits and vegetables during the recall period was low (<14%). However, these fruits and vegetables are rich in carotenoids as precursors of vitamin A and consumption may increase the absorption of iron from cereal-based diets ⁽¹⁸⁾. Strategies to increase intake of fruits and vegetables should thus be explored, as is recommended by the Centres for Disease Control and Prevention (2011) ⁽³⁴⁾. For example, local production in home gardens should be promoted and subsidies, and can be motivated the selling of these foods at schools. Also, the importance of fruit and vegetables consumption may be included in the school curriculum.

Consumption of foods from various animal food sources were also a factor which showed significant association with anaemia in primary school children. More than 70% of the children in our study ate foods from the ‘ASF’ group, regardless of the reference recall period. However, significantly fewer anaemic versus non-anaemic and ID versus non-ID children consumed from the ‘ASF’ group. Animal-source foods, particularly organ meat (e.g. liver, kidney), followed by red and white meat, and fish, are rich in highly bioavailable nutrients such as iron, vitamins A and B12, which are important and effective for iron mobilisation and Hb synthesis and play an important role in managing nutritional anaemia ⁽⁴¹⁻⁴³⁾. The children in this study had a low dietary intake of those nutrients ⁽³⁸⁾, which can be obtained mostly from ASF. Specially, organ meat can potentially make a significant contribution to high bioavailable iron intake. None of the anaemic children in our study consumed organ meat during the 3-day recall period. A portion of 50 to 60 g of cooked beef or chicken liver can provide approximately 5 to 11 mg of highly bioavailable haem iron (compared to children’s age specific EAR for iron of 4 to 6 mg) ⁽⁴⁴⁾. A number of studies in different age groups, including school-age children, have shown a positive association between consumption of various ASF and haematological concentrations. For instance, including small amounts of ASFs (at least 50 g of red meat per day) in one’s diet can

improve Hb concentration and iron status ⁽⁴⁵⁻⁴⁸⁾ and lower risk of anaemia and ID ⁽⁴⁹⁾. Other studies have shown that the consumption of 60 g minced meat per day improved the cognitive performance of and led to higher levels of physical activity in Kenyan school children ^(50, 51). Additionally, foods from ASF groups are effective enhancers of non-haem iron absorption when consumed in the same meal with plant-based foods and may increase iron absorption by two- to three-fold ⁽¹⁷⁾. The SAFBDG recommends that foods from the ASF group can be eaten daily, but to maintain a healthy balance diet, a variety of foods from other food groups is recommended ⁽³⁹⁾.

Significantly fewer ID than non-ID children in our study consumed legumes during the 3-days period, and the logistic regression analysis results showed an inverse association between consumption from the 'legumes' group and ID in the 3-day reference recall period. Adding foods from the legumes group (i.e. dry beans, split peas, lentils and soya) to the diet, increases the nutrient content of meals, and the SAFBDG recommends that such foods are eaten regularly ⁽⁵²⁾. A study showed that adding legumes can slightly improve the iron content of plant-based diets, although the bioavailability of non-haem iron from these foods is low ⁽⁴⁴⁾. Legumes are generally rich in iron absorption inhibitors (i.e. phytate), which may affect the absorption of iron derived from both animal and plant sources ⁽¹⁷⁾. Other studies, however, could not find an association between the intake of legumes and ID ^(47, 53). Diets containing a variety of fruits and vegetables, and the presence of vitamin C, for example, could play an important role in enhancing iron absorption from the total diet ⁽³⁷⁾. In meals containing legumes, non-haem iron bioavailability may be enhanced by adding sufficient amounts of foods rich in vitamin C, which has been shown to reverse the inhibitory effects of phytate ⁽⁵⁴⁾.

In terms of overall dietary variety, approximately 40% of children in this study consumed foods from four or less food groups. Also, children who consumed a diet of low variety (DDS \leq 4) were more likely to be anaemic and ID. This is similar to other studies that showed a positive association between dietary diversity and haematological concentration in different age groups ^(13, 24, 25) and that a lower dietary diversity was associated with anaemia ⁽¹⁴⁾.

Our results showed that results on consumption of specific food groups may depend on the reference recall period. In dietary assessment studies, associations between less frequently consumed food groups and anaemia and ID as outcome might be missed if only a 1-day reference recall period is used. For instance, no association was found between intake of foods from the 'meat and fish group' and anaemia, and foods from the 'vegetables and fruits other than vitamin A-rich', 'ASF' and 'legumes' groups and ID in the 1-day reference recall period, but associations were found when the 3-day reference recall period was used.

However, for frequently consumed foods there was an association regardless of the reference recall period. When using the DDS to describe dietary variety, there was a statistically significant difference in the mean DDS between the two reference recall periods, although the difference in the score values was relatively small.

This study contributes to the knowledge about dietary diversity and aetiology of nutritional anaemia in South African primary school-age children. Considering the fact that South Africa has a National Food Fortification Programme in place, which also aims to address nutritional anaemia and ID, and that all children in this study consumed fortified starchy staples, which are expected to be fortified, our finding strengthens the point that a diverse diet should be promoted despite or in addition to fortification efforts. However, the limitation of the study is the cross-sectional design: the results reflect associations but cannot establish causality.

Conclusion

In terms of overall dietary diversity, approximately 40% of the children in this study consumed a low diverse diet from four or less food groups. Low dietary diversity was associated with higher chance of being anaemic among the children studied. Our results emphasise the importance of the multiple-day dietary recall to get information on foods that are not frequently consumed by the study population (i.e. 'meat, fish, and seafood'). The promotion of dietary diversification, with a focus on greater consumption of vegetables, fruits, and ASF, including organ meats, may have a positive effect on improving anaemia and iron status in children of primary school age.

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CHAPTER 5: MANUSCRIPT 3:**Nutrient density but not cost of the diet is associated with anaemia and iron deficiency in school-age children in South Africa**

This manuscript will be submitted for publication to the *The American Journal of Clinical Nutrition* and has been written according to the guidelines of the journal (Annexure D).

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Short running head: Nutrient density, cost of diet and anemia and ID in children

Abbreviations used: AI, adequate intake; CF, correction factor; CRP, C-reactive protein; DGLV, dark green leafy vegetables; EAR, estimated average requirements; ED, energy density; ENC, nutrients to encourage; Hb, haemoglobin; HEI, Healthy Eating Index; ID, iron deficiency; KZN, KwaZulu-Natal; LIM, nutrients to limit; NFCS-FB, National Food Consumption Survey; NRD, Nutrient Rich Diet; NPR, nutrient-to-price ratio; NRF, Nutrient Rich Foods Index; NSNP, National School Nutrition Programme; NW, North West; PF, plasma ferritin; ZAR, South African Rand.

ABSTRACT

Background: School-age children are at high risk of nutritional anaemia and iron deficiency (ID) due to their increased nutrient requirements, accelerated physical and intellectual development, but poor dietary intake.

Objective: The aim of the study was to investigate the relationship of nutrient density and cost of foods and diet with anaemia and ID in children.

Design: Dietary intake data of 5- to 12-year-olds ($n = 578$) were obtained from three independent intervention studies at different sites in South Africa. Nutrient density was calculated using the Nutrient Rich Foods Index (NRF9.3). The nutrient density-to-price ratio of foods and total diets was estimated by attaching food prices to dietary intakes from three 24-hour recalls. A series of descriptive and correlation analyses examined associations of dietary nutrient density and diet cost with anaemia and ID status.

Results: Foods mostly consumed by children were from starchy staples, vegetables other than vitamin A-rich and legumes groups, with the mean NRF9.3 scores per 100 g ranging from 35.9 to 56.3. The least consumed foods were cheese, organ meat, fish and vitamin A-rich vegetables and fruits, but these food groups were most nutrient dense with the NRF9.3 scores ranging from 112.6 to 184.7. Diet cost did not differ according to anaemia and ID status, although the nutrient density-to-price ratio was significantly lower for anaemic versus non-anaemic children ($P = 0.001$). Children with anaemia and ID had significantly lower NRF9.3 scores compared to non-anaemic and non-ID children ($P < 0.001$ and $P = 0.039$, respectively).

Conclusion: Careful selection of nutrient-dense foods as substitution for foods with a lower nutrient-density could make it possible to consume a diet richer in specific nutrients and may help to prevent anaemia and ID in South African school-age children without affecting the cost of the total diet.

Key words: nutrient density; anaemia; iron deficiency; school-age children; South Africa

Introduction

Anaemia is the most common health problem worldwide affecting about a third of the population, including almost 300 million children of primary school age (1). National data for primary school children in South Africa is scarce. The National Food Consumption Survey (NFCS-FB-1) of 2005 indicated that 18.6% of 7- to 9-year-old children were anaemic (2). However the results of smaller studies conducted after the NFCS-FB-1 were summarized by Taljaard *et al.*, and reported anaemia prevalence of up to 27.0% (3). Some of the adverse effects of anaemia in children are reduced oxygen delivery to the organs and weakened functioning of the brain and muscles, which has a negative impact on schoolwork performance (4, 5).

Nutritional deficiency anaemia mainly results from a lack of iron, which accounts for up to half of the cases, however, at the same time, it can be caused by various other micronutrient deficiencies as well (i.e. vitamin A, vitamin B12, folate and zinc) (6). An individual's diet can consist of a variety of foods and meals with complex combinations of nutrients. It has been suggested that nutrient based profiling techniques be used to evaluate the quality of individual foods and diets (7). A key element of these methods is the scoring of foods and diets according to their nutrient density in relation to recommended dietary intake references (8), which may help identifying more nutrient-dense or nutrient-rich foods and diets. There is evidence that eating a healthy nutrient-rich diet can reduce risk of cardiovascular and chronic diseases and all-cause mortality (9-11). However, one study showed that nutrient-rich diets were not associated with weight management (12). Moreover, the relationship between nutrient density and anaemia has been rarely explored, especially in school children.

During the last decade, the cost of healthy eating has become a growing concern. Studies conducted in various countries reported that healthier diets cost more than less healthy ones (13-17). A study in South Africa concluded that healthier food choices can be more expensive than some of the commonly consumed foods and that most of the South African population cannot afford a healthy diet (18). Analysing the overall nutrient density of foods and their cost may help to identify foods that provide the best nutrient content per unit cost (13, 19). Some studies suggest that nutritious and healthier school meals can be provided without increasing expenses by choosing lower-cost substitutes from the specific food group (17, 20). For instance, a study conducted in the United States analysed the nutrient composition and food prices of the local foods and found that grains, dry beans and eggs were the cheapest sources of iron (20). However, animal-source foods are better sources of bioavailable iron, which play an important role in preventing anaemia (21). Products such as

eggs, poultry, organ meats and canned sardines, among others, were identified as animal-source foods with a good nutrients-to-cost ratio (20, 22). In this regard, the nutrient density-based models may be used in combination with food prices to identify those food sources that provide more targeted nutrients at the lowest cost (20).

In this study, a pooled analysis of existing data was conducted with the aim to investigate the relationship of nutrient density and cost of diet with anaemia and ID in children. The Nutrient Rich Foods (NRF) index model, which has been previously validated against the US Department of Agriculture Healthy Eating Index (HEI-2005) (23), was used.

SUBJECTS AND METHODS

Study design

The data used for this pooled analysis were derived from the baseline data collected in three independent intervention studies (24-26) that were conducted in school children at different sites in South Africa. The studies were conducted between April 2009 and June 2012 at primary schools in rural and urban areas in KwaZulu-Natal (KZN) and North West (NW) provinces. These areas were all malaria-free. Prior to data collection, parents or caregivers signed informed consent forms and the learners gave verbal assent. All children received deworming medication at the baseline point. At the time of data collection, all schools were participating in the National School Nutrition Programme (NSNP), which provided children with a daily school meal. The following data were extracted from the datasets: socio-demographic information; biochemical data, namely haemoglobin (Hb), plasma ferritin (PF) and C-reactive protein (CRP); and dietary intake data (energy, and macro- and micronutrients). Cases for the pooled analysis were selected using the following inclusion criteria: participating children were 5- to 12-year-old; the availability of three biochemical values, namely haemoglobin (Hb), plasma ferritin (PF) and C-reactive protein (CRP); and complete dietary data. Cases with missing data for any of these variables were excluded.

Biochemical measurements

To determine biochemical values, blood samples were collected from children in the original studies. The Hb concentrations were measured on whole blood, using the direct cyanmethemoglobin method (Bio Rad Laboratories [PTY] Ltd) by using Drabkin's solution and a standard miniphotometer (24), while, in the other two studies, a haematology analyser (Coulter® Ac-T™ 5diff CP; Beckman Coulter) was used (25, 26). The PF and CRP were measured using an automated chemiluminescent immunoassay system (CLIA, IMMULITE) (24), while, in the other two studies, a ferritin ELISA kit (Ramco Laboratories Inc.) was used

for PF and an immunoturbidimetric method (Technicon RA-1000 auto analyser) for CRP (25, 26).

For the pooled data analysis, the PF values of children with a CRP concentration ≥ 5 mg/L were adjusted for inflammation by multiplying plasma ferritin values with a correction factor (CF) of 0.65 (27). Selected cut-off points of iron status markers were used to classify children as iron deficient, anaemic, or iron deficient anaemic. Anaemia was defined as Hb < 115 g/L; ID based on PF < 15 μ g/L; and IDA was defined as a combination of Hb < 115 g/L and plasma ferritin < 15 μ g/L (28, 29).

Dietary intake assessment

Dietary data were collected for three days by means of the 24-hour dietary recall method. The 24-hour recalls were collected for each child on non-consecutive days of the week in all three intervention studies (24-26). The children and parents or caregivers were interviewed by trained fieldworkers and were asked to recall all food and beverages as well as amounts consumed in the previous 24 hours. Food portion sizes were estimated using plastic food models, household utensils and food packaging materials. Where the amount of food consumed was reported in household measures, it was converted into weight using the SAMRC Food Quantities Manual (30). The SAFOODS was used to code the foods consumed by the children. The SAMRC FoodFinder3 software program was used to convert food intake to energy intake, macro- and micronutrients. FoodFinder3 did not include the fortified values for maize meal and bread, and these values were added to the database of FoodFinder3 based on the values in the Condensed Food Composition Tables for South Africa (31).

Estimation of the nutrient density of foods and food groups by calculating the NRF scores

To identify the nutrient-rich foods, the nutrient density of the foods and beverages was calculated using the NRF9.3 index (23). The NRF index assigns scores to foods and beverages based on their content of nutrients to encourage (ENC) and nutrients to limit (LIM). Higher scores indicate more nutrient-dense foods. The choice of ENC nutrients used in the NRF9.3 algorithms was guided by the results of our prior analyses on the daily nutrient intake of the children (32). The choice of nutrients to limit (LIM) was guided by the recommendations of Fulgoni *et al.* (2009) and also supported by global dietary recommendations (23). Finally, the nine ENC nutrients were protein, fiber, vitamin A, vitamin C, vitamin D, vitamin B12, calcium, iron and zinc, and the three LIM nutrients were saturated fat, added sugar and sodium. The nutrient values per 100 g edible portion were used from

the 2010 version of the Condensed Food Composition Tables for South Africa (31). The Dietary Reference Intake (DRI) values, such as estimated average requirements (EAR) for ENC nutrients and adequate intake (AI) for LIM nutrients (**Supplement Table S1**), were used to calculate the percentage of daily intake of each nutrient according to age group and gender (33, 34). The algorithms for calculating the NRF9.3 scores for each food item are tabulated in **Table 1**. The NRF9.3 scores were calculated in relation to the energy density (ED) of each individual food per 100 g of food (8). The final NRF9.3 score is the arithmetic difference between the ENC score and the LIM score for individual foods (100 g).

Table 1: The NRF9.3 algorithms for nine nutrients to encourage and three nutrients to limit¹

Score	Algorithm	Description
ENC_{<i>i</i>} (100 g)²	$(\text{Nutrient}_i / \text{EAR}_i) \times 100$	Nutrient _{<i>i</i>} - content of nutrient to encourage in 100 g food; EAR _{<i>i</i>} - for nutrient _{<i>i</i>}
ENC₁₋₉ (100 g)	$\sum_{1-9} (\text{ENC}_i / \text{ED}_i) \times 100$	<i>n</i> = 1-9, the number of nutrients to encourage; ED, kJ/100 g
LIM_{<i>i</i>} (100 g)	$(\text{Nutrient}_i / \text{AI}_i) \times 100$	Nutrient _{<i>i</i>} - content of nutrient to limit in 100 g food; AI _{<i>i</i>} - for nutrient _{<i>i</i>}
LIM₁₋₃ (100 g)	$\sum_{1-3} (\text{LIM}_i / \text{ED}_i) \times 100$	<i>n</i> = 1-3, the number of nutrients to limit; ED, kJ/100 g
NRF9.3	$\sum \text{ENC}_9 - \sum \text{LIM}_3$	Difference between sums

¹ adopted and modified from Drewnowski, 2010 (8); AI, adequate intake; EAR, estimated average requirements; ED, energy density; ENC, nutrients to encourage; LIM, nutrients to limit; NRF, Nutrient Rich Foods Index

² including ENC_{iron}

Foods and beverages consumed over the 3-day recall period were grouped into 18 food groups, guided by food grouping used in the Condensed Food Composition Tables for South Africa (31), as well as grouping used to calculate dietary diversity scores (35). Foods from organ meat, flesh meat (red and white) and fish were grouped into three separate groups as these foods contain bioavailable haem iron, which is a nutrient of interest (35). The NRF9.3 score for each food group was calculated as the mean of all NRF9.3 index scores of each individual food within each specific group. The groups were: 1 – cereals; 2 – dark green leafy vegetables (DGLV), including imifino, indigenous DGLV; 3 – vitamin A-rich fruits and vegetables; 4 – other vegetables; 5 – other fruits; 6 – organ meat; 7 – red meat (beef mince, beef and pork sausages, processed cold meat); 8 – white meat (chicken meat); 9 – fish; 10 – eggs; 11 – legumes, nuts and seeds; 12 – milk and milk products; 13 – cheese; 14 – sweets and jam; 15 – salted snacks; 16 – added sugar (sugar added to food by participants during preparation); 17 – sugar-sweetened soft drinks; and 18 – fats and oils. Fruit juices were not consumed during the recall period. Foods and beverages with no nutrient density – for

example, diet sodas, unsweetened coffee, or tea and water – were excluded from the calculation.

Estimation of the nutrient density to cost ratio of consumed foods

The retail prices of the 47 food items consumed by children were collected from local supermarkets (Shoprite, Pick 'n Pay, and Checkers) - licensed stores with constant prices per kg of food in all provinces of South Africa. Although information on the foods consumed by the children was collected from 2009 to 2012, the retail prices were collected in 2017 in South African Rand (1 ZAR = 0.7 US dollars). There was no need to correct for inflation, since we used the comparative costs of foods in this study (36). The average price of every food item was then adjusted for preparation and waste that occur during food processing and cooking prior to consumption (37). Also, the prices of mixed dishes were calculated using standard recipes obtained from the Condensed Food Composition Tables for South Africa (31). For example, the price of beef stew with vegetables can be calculated as beef and each of the vegetables and other components in this dish, using the proportions of the relevant ingredients as is recommended in the Condensed Food Composition Tables for South Africa. Finally, all estimated prices in ZAR per 100 g weight of edible portion were then assigned to each food in our dataset.

Nutrient cost was calculated as a ratio of the nutrient density provided by each food item (calculated as NRF9.3 index per adjusted 100 g of food) to food prices (ZAR per 100 g) and to yield a nutrient-to-price ratio (NPR) per 100 g of food. Finally, the mean NRF9.3 scores and NPR of all food groups were compared. The iron nutrient-to-price ratio (NPR_{iron}) was determined as ratio of ENC_{iron} to cost per edible 100 g of food by using the formulas provided in **Table 1**.

Cost of the nutrient-dense and nutrient-poor diets

To quantify the nutrient density of the diets, the Nutrient Rich Diet (NRD9.3) model was used in which the principle of the NRF9.3 model was applied (**Supplement 2**). Higher NRD9.3 index scores indicate higher nutrient density of the diet. Children with higher NRD9.3 index scores were thus considered to have a healthier dietary intake than those with a lower NRD9.3 index score.

For each child, diet cost (ZAR/day) was calculated by multiplying the cost of consumed foods per weight of each food eaten (g/day) and then summing these values (8, 15). The NPR of diets was calculated as a ratio of the nutrient density of the total diet (NRD9.3) to the diet cost.

Statistical analyses

The Statistical Software Package SPSS v.25 (Inc., Chicago, IL, USA) was used for all statistical analyses. Continuous data were tested for normality using the Shapiro-Wilk test and are reported as the mean and standard deviation (SD) for normally distributed data, or as the median with the 25th, 75th percentiles for data not normally distributed. Categorical variables are reported as frequencies and percentages. The biochemical variables and nutrient density scores were compared between quartiles of the NRD9.3 scores by using analyses of variance (ANOVA). The analysis of covariance (ANCOVA) by means of the general linear model univariate procedure was used taking into account covariates such as energy intake, age, gender, and study site. The Bonferroni test was used to identify which groups differed if significant differences were detected. Pearson's chi-square test was used to determine the association between categorical data. The relationship between the nutrient density scores and the anaemia and iron status indicators was determined using the partial correlations, adjusted for gender, age, and study site. Statistical significance was set at $P < 0.05$.

Ethical approval for the present study

Ethical approval was obtained from the Human Research and Ethics Committee of the North-West University (NWU-00027-16-A1). The appropriate ethics committees granted ethical permission for the three original studies (24-26).

RESULTS

The mean (SD) age of the 578 children included in this study was 8.7 (1.3) years; 13.8% of the children were anaemic and 27.7% were ID. Information on the 18 food groups consumed by the children over the 3-day period, the mean portion size, price per 100 g and iron-rich foods as well as the mean index scores and nutrient-to-price ratio are provided in **Table 2**. The mean NRF9.3 scores across the food groups varied from -99.9 to 184.7 and differed significantly between food groups ($P = 0.001$). Food groups consumed by more than half of the children were starchy staples (100.0%), vegetables other than vitamin A-rich (63.9%), and legumes (58.1%). The mean NRF9.3 scores for these foods ranged from 35.9 to 56.3. The food groups that were consumed by 25% to 50% of the children were white meat (47.6%), red meat (32.8%), milk (31.8%), eggs (30.7%) and DGLV (27.5%), and the mean NRF9.3 scores for these groups ranged from 37.3 to 112.9. The food groups consumed by the least children were cheese (6.3%), organ meat (12.2%), vitamin A-rich vegetables and fruits (13.8%), fruits other than vitamin A-rich (15.2%) and fish (15.5%), and these foods were the most nutrient-rich with NRF9.3 scores ranging from 112.6 to 184.7. Chicken liver

had the highest NRF9.3 score of 256. The vitamin A-rich vegetables and fruits, eggs, fish, DGLV and organ meat groups provided more nutrients per unit cost (NPR). However, in the organ meat group, chicken liver had the highest NRF9.3 score. **Table 2** also shows that DGLV, organ meat, fish and eggs are the most iron-rich foods ($P < 0.001$), and organ meat and DGLV provided most dietary iron per unit cost ($P = 0.004$). Foods from sugar (85.7%), fats and oils (67.2%), salty snacks (39.5%), sugar-sweetened soft drinks (37.5%), and sweets and jam (30.6%) were the least nutrient-dense, with mean NRF9.3 scores are ranging from -99.9 to -12.3.

The biochemical values and prevalence of anaemia and iron status as well as nutrient density indices and cost of the diet across the quartiles of NRD9.3 index are presented in **Table 3**. In terms of biochemical values, anaemia and ID prevalence, significant differences across NRD9.3 score quartiles were observed. A higher NRD9.3 score (quartile 4) was associated with higher Hb and PF concentrations ($P < 0.001$ and $P = 0.009$, respectively). The prevalence of anaemia and ID differed significantly across the NRD9.3 quartiles. The proportions of children with anaemia and ID were significantly lower in the higher NRD9.3 quartiles compared to those in the lower NRD9.3 quartiles ($P < 0.001$ and $P = 0.012$, respectively). Significant differences in nutrient density and cost of the diets across NRD9.3 score quartiles were observed. The density of nine nutrients to encourage (ENC9) was significantly lower in quartile 1 of the NRD9.3 score ($P < 0.001$). However, the density of three nutrients to limit (LIM3) did not differ across quartiles. A comparison of the cost of the diet and NRD-to-price ratio across quartiles showed higher values in the higher quartiles ($P = 0.013$ and $P < 0.001$, respectively).

The nutrient density and cost of the diets were also compared when children were stratified by anaemia and ID status (**Table 4**). Children with anaemia and ID had significantly lower NRD9.3 scores compared to non-anaemic and non-ID children (all $P < 0.05$). Diet costs did not differ between the sub-groups, though the nutrient density-to-price ratio was significantly lower for anaemic versus non-anaemic children ($P = 0.001$).

Table 5 presents the partial correlation coefficient for the associations of the nutrient density scores, cost and nutrient-to-price ratio of the diets with Hb and PF. The nutrient density of the diets score (NRD9.3), nutrients to encourage score (ENC9) and nutrient density-to-price ratio (NRD-to-price ratio) showed a significant positive moderate to weak correlation (all correlation coefficients $r < 0.4$) with Hb and PF (all $P < 0.05$) after adjustment for covariates.

Table 2: Frequency of the consumption, the nutrient density and nutrient-to-price ratio of specific food groups consumed by 5- to 12-year-old school children over the 3-day period

Food groups, g or ml	Frequency, %	Portion size, g/day	Price per 100 g (edible), ZAR [†]	NRF9.3 100 g	NPR 100 g	ENC _{Iron} 100 g	NPR _{Iron} 100 g
Starchy staples (cereals/white roots)	100.0 ^d	239 (145; 265) ^a	1.4 (0.5) ^c	31.9 (9.1)	23.1 (9.9) ^b	9.5 (8.7)	6.1 (3.2) ^b
- bread ¹	86.9	99 (60; 120)	1.3 (0.3)	44.1 (3.9)	35.6 (3.8)	13.1 (1.8)	10.1 (1.5)
- maize meal ^{1,2}	97.9	275 (195; 450)	0.6 (0.0)	39.8 (3.3)	54.6 (9.6)	9.7 (1.2)	15.6 (1.1)
- potatoes	39.9	105 (55; 125)	1.3 (0.3)	41.3 (1.6)	32.0 (1.1)	2.6 (0.2)	2.2 (0.3)
- rice	43.8	125 (75; 180)	0.7 (0.1)	14.8 (2.5)	22.7 (2.4)	1.5 (0.2)	3.1 (0.3)
- other	18.4	139 (83; 155)	1.9 (0.4)	21.8 (8.2)	11.1 (6.2)	2.9 (4.1)	1.3 (0.2)
DGLV³	27.5 ^b	71 (55; 126) ^c	2.4 (1.3) ^b	112.9 (9.7) ^a	45.9 (6.3) ^a	27.7 (4.1) ^a	11.9 (2.2) ^a
Vitamin A-rich vegetables and fruits⁴	13.8 ^a	46 (35; 80) ^d	2.1 (1.2) ^b	126.9 (69.1) ^a	63.4 (12.5) ^a	4.2 (0.4) ^e	3.3 (0.6) ^b
Other than vitamin A-rich vegetables	63.9 ^c	65 (33; 110) ^c	1.7 (1.0)	40.1 (10.6) ^c	26.3 (6.1) ^b	5.1 (1.9) ^d	2.9 (0.9) ^b
Other than vitamin A-rich fruits	15.2 ^a	103 (70; 155) ^b	1.8 (0.8)	44.3 (33.4) ^c	23.1 (5.8) ^b	1.1 (0.2)	0.6 (0.2)
Organ meat	12.2 ^a	82 (38; 105) ^c	6.2 (1.8) ^a	184.7 (42.6) ^a	30.8 (9.3) ^b	39.2 (9.6) ^a	7.9 (4.1) ^a
- chicken liver	10.2	65 (35; 79)	4.0 (1.1)	256.4 (19.8)	65.7 (11.2)	38.5 (6.3)	10.4 (3.8)
Red meat	32.8 ^b	84 (55; 145) ^b	7.1 (1.9) ^a	85.4 (42.3) ^b	12.4 (6.3)	11.8 (6.0) ^b	1.8 (1.2) ^c
White meat	47.6 ^b	42 (35; 92) ^d	5.4 (1.4) ^a	91.4 (25.6) ^b	16.3 (3.4)	6.6 (2.8)	1.4 (0.5) ^c
Fish	15.5 ^a	92 (48; 120) ^b	3.7 (1.1) ^b	182.4 (44.9) ^a	49.3 (8.4) ^a	15.3 (9.2) ^c	4.6 (2.0) ^b
Eggs	30.7 ^b	78 (50; 120) ^c	3.8 (0.9) ^b	165.9 (10.8) ^a	43.2 (1.0) ^a	13.8 (0.2) ^c	3.4 (0.3) ^b
Legumes, nuts and seeds⁵	58.1 ^c	205 (45; 240) ^a	1.6 (0.2) ^c	56.3 (10.1) ^c	31.7 (6.8) ^b	7.3 (1.9) ^d	4.1 (1.8) ^b
Milk and milk products	31.8 ^b	149 (64; 250) ^b	1.4 (0.4) ^c	37.3 (3.5) ^c	27.7 (6.6) ^b	0.8 (0.1)	0.6 (0.1)
Cheese	6.3 ^a	15 (0) ^e	7.9 (1.9) ^a	112.6 (12.5) ^a	14.6 (2.1) ^c	5.4 (0.9) ^e	0.7 (0.3)
Sweets and jam	30.6 ^b	23 (15; 40)	7.2 (3.3) ^a	-78.4 (44.8)	-12.1 (8.3)	--	--
Salty snacks, NikNaks/crisps	39.5 ^b	37 (25; 50) ^d	10.1 (1.9) ^a	-68.4 (20.4)	-7.2 (3.1)	--	--
Added sugar	85.7 ^d	23 (15; 35) ^e	1.3 (0.3) ^c	-99.9 (0)	-76.8 (0) ^a	--	--

Food groups, g or ml	Frequency, %	Portion size, g/day	Price per 100 g (edible), ZAR [†]	NRF ^{9.3} 100 g	NPR 100 g	ENC _{Iron} 100 g	NPR _{Iron} 100 g
Sugar-sweetened soft drinks	37.5 ^b	260 (150; 300) ^a	1.2 (0.1) ^c	-12.3 (0.8)	-10.3 (1.3)	--	--
Fats and oils	67.2 ^c	15 (10; 30) ^e	1.8 (0.2) ^c	-21.9 (4.1)	-10.7 (3.7)	--	--
One-way ANOVA by food groups⁶	<i>P</i> = 0.024	<i>P</i> = 0.001	<i>P</i> < 0.001	<i>P</i> = 0.001	<i>P</i> < 0.001	<i>P</i> < 0.001	<i>P</i> = 0.004

Data reported as percentages, means (SD), and median (25th; 75th percentiles);

ENC, nutrients to encourage; DGLV – dark green leafy vegetables; LIM, nutrients to limit; NRF; NPR, nutrient-to-price ratio; NRF, Nutrient Rich Foods; ZAR, South African Rand;

[†] 1 ZAR = 0.07 US dollars;

¹ fortified;

² eaten as a stiff (contains 27% maize meal) or soft (contains 12% maize meal) porridge;

³ DGLV - the price for imifino was assumed the same as for spinach;

⁴ no fruits were eaten;

⁵ including beans from samp and beans mix (1:1);

⁶ the different superscripts indicate a significant difference between food groups

Table 3: Anaemia and iron status, and nutrient density and cost of the diets in 5- to 12-year-old school children according to the quartiles of the nutrient-rich diet score (NRD)

Characteristic of children	Q1 n=144	Q2 n=145	Q3 n=145	Q4 n=144	P-value
Age, years	9.1 (1.5) ^a	8.7 (1.4) ^b	8.5 (1.1) ^c	8.3 (1.1) ^d	0.003
Hb, g/L	12.2 (1.1) ^a	12.3 (1.3) ^a	12.7 (0.9) ^b	12.9 (0.9) ^b	0.009
PF, µg/L	21.7 (15.4) ^a	25.7 (16.3) ^b	27.6 (19.6) ^c	28.4 (19.9) ^d	<0.001
Anaemic, %	25.0 ^a	17.2 ^b	8.3 ^c	4.9 ^d	<0.001
ID, %	38.2 ^a	25.5 ^b	23.8 ^c	24.3 ^c	0.012
NRD9.3 100 g ¹	39.3 (10.3) ^a	62.1 (14.2) ^b	75.8 (13.3) ^c	109.7 (23.2) ^d	<0.001
ENC9 100 g ¹	53.7 (11.7) ^a	75.8 (7.3) ^b	88.6 (5.7) ^c	123.3 (33.5) ^d	<0.001
LIM3 100 g	14.5 (7.7)	13.7 (5.8)	13.5 (6.7)	13.6 (6.2)	0.134
Diet cost, ZAR/day ^{†2}	36.3 (7.5) ^a	37.8 (8.7) ^b	38.1 (8.3) ^c	41.3 (6.7) ^d	0.013
NRD-to-price ratio	1.1 (0.4) ^a	1.4 (0.5) ^b	2.1 (0.8) ^c	2.8 (1.3) ^d	<0.001

Data reported as means (SD), categorical data reported as percentages;

ENC – nutrients to encourage; Hb – haemoglobin; ID – iron deficiency; LIM – nutrients to limit; NRD – nutrient-rich diets; PF – plasma ferritin; SD – standard deviation; Q – quartiles; ZAR South African Rand;

[†] 1 ZAR = 0.07 US dollars;

The values of the NRD9.3 scores across quartiles – Q1 (< 53.5), Q2 (53.5-63.8), Q3 (68.8-84.3), Q4 (> 84.3);

Anaemia – Hb < 115 g/L; ID - PF < 15 µg/L;

^{1,2} ANCOVA test used: ¹ adjusted for age, gender and study site, ² adjusted for energy intakes. Chi-square test used to compare column's proportions. Multiple comparisons: Bonferroni method; different superscript letters in a row denotes values significantly differ from each other across quartiles at $P < 0.05$ level.

Table 4: Nutrient density and cost of the diets in 5- to 12-year-old school children according to anaemia and iron status

Nutrient density and cost of diets	Anaemic n=80	Non-anaemic n=498	P-value	ID n=160	Non-ID n=418	P-value
NRD9.3 100 g ¹	58.8 (27.3)	73.8 (30.0)	<0.001	67.5 (36.1)	72.6 (27.5)	0.039
ENC9 100 g ¹	71.7 (21.7)	87.5 (30.3)	<0.001	83.8 (37.4)	86.2 (28.3)	0.040
LIM3 100 g ¹	13.1 (5.7)	13.6 (6.3)	0.455	13.6 (6.8)	13.6 (6.0)	0.976
Diet cost, ZAR/day ^{†2}	34.8 (7.3)	36.1 (6.9)	0.063	36.9 (5.8)	37.6 (8.1)	0.110
NRD-to-price-ratio	1.69 (0.7)	2.06 (0.8)	0.001	1.83 (1.0)	1.98 (0.8)	0.050

Data reported as means (SD),

ENC – nutrients to encourage; ID – iron deficiency; LIM – nutrients to limit; NRD – nutrient rich diets; SD – standard deviation; Q – quartiles; ZAR – South African Rand;

[†] 1 ZAR = 0.07 US dollars;

ANCOVA test used, adjusted for ¹ age, gender and study site, and ² energy intakes.

Table 5: Partial correlation coefficient of haemoglobin and plasma ferritin with the nutrient densities scores, cost and nutrient-to-price ratio of diets of 5- to 12-year-old school children

	NRD9.3 100 g	ENC9 100 g	LIM3 100 g	Diet cost, ZAR [†]	NRD-to-price ratio
Haemoglobin, g/L	$r = 0.346$ $P < 0.001$	$r = 0.239$ $P < 0.001$	$r = -0.016$ $P = 0.815$	$r = 0.010$ $P = 0.695$	$r = 0.223$ $P < 0.001$
Plasma ferritin, µg/L	$r = 0.121$ $P = 0.013$	$r = 0.110$ $P = 0.019$	$r = -0.039$ $P = 0.351$	$r = 0.045$ $P = 0.220$	$r = 0.196$ $P = 0.023$

^a Adjusted for age, gender, and study site;

NRD – nutrient-rich diet; NR – nutrient rich; LIM – nutrients to limit; ZAR – South African Rand; [†] 1 ZAR = 0.07 US dollars.

Discussion

The aim of this study was to determine the relationship of nutrient density and cost of diet with anaemia and ID status in 5- to 12-year-old school children. The NRF9.3 index scores were used to classify the foods and diets based on their nutrient composition and cost (7, 8). Foods that were less frequently consumed had the highest nutrient density. The nutrient density of the diet was positively associated with Hb and PF concentrations. Anaemic and ID children consumed diets of lower nutritional density, with no significant difference in diet cost compared to non-anaemic and non-ID children.

There was a wide variability in nutrient density and cost between food groups. The food groups that were least consumed - that is, organ meat, fish (mostly canned sardines), eggs, vegetables and fruits, such as vitamin A-rich, and other fruits, and DGLV, were most nutrient-dense, with NRF9.3 scores ranging from 112.6 to 184.7, with the highest score assigned to the organ meat group. The high scores indicate that these foods have a higher amount of ENC nutrients per 100 g compared to LIM nutrients. Organ meat is an important source of high quality protein and provide a variety of essential nutrients required in the human diet (i.e. vitamin A [found in liver], B-vitamins, including vitamin B12, zinc, and iron), although it also contains some amount of saturated fat (21, 38). Combining nutrient density and cost helped in identifying foods that provide more nutrients while having a better price. Organ meat, fish and eggs, and various vegetables and fruits, including vitamin A-rich and DGLV had the highest density of nutrients to encourage per unit cost. These results are in line with findings of other studies which showed that organ meats and canned sardines, DGLV and non-leafy vegetables, and deep-yellow vegetables, including sweet potatoes, had the highest nutrient density per unit cost (20, 22). Our results show that chicken liver is one of the cheapest animal-source foods that can supply haem iron, which is more readily absorbed in the human body than iron from plant sources. Among the vegetable-source foods, the DGLV such as spinach, kale and indigenous leafy vegetables, were the cheapest source of iron. Boiled DGLVs are often eaten with maize meal porridge, particularly in rural areas (39). Indigenous leafy vegetables are usually not available in the food supermarkets, but can be found in the wild, bought from some informal vendors or grown in home gardens. In order to obtain prices for these foods, we assumed the same price as for spinach.

The results of our study also showed that diets with a higher mean nutrient density had a higher diet cost and higher ratio of nutrient density per unit cost. The estimated mean diet cost per day was ZAR 38.5, thus, assuming ZAR 1,160 per month for a child's diet. However, as diet quality increased, so did the mean cost of diets: the higher quartile of the NRD scores showed a mean diet cost per day of ZAR 41.3 (6.7), thus the estimated monthly

cost on a child's diet will increase up to ZAR 1,230. According to the National Agricultural Marketing Council, the estimated cost of a typical food basket for the general South African population in March 2018 was approximately ZAR 860 (40). According to the 2018 South African Monthly Food Price Barometer, the estimated cost of a basic diet was ZAR 546 for 3- to-9-year-old children and ZAR 589 for 10- to-13-year-old children (41). Although these figures may not be directly comparable to our results, it is clear that a nutrient-dense diet may be difficult to afford for the majority of the South African population. Temple et al (42) compared the cost of healthier foods (nutrient dense) with the cost of less healthy options, using prices collected at supermarkets in South Africa, and found that the healthier options were more costly per 100g than the less healthy options. They further estimated that a healthier version of a typical South African diet would cost up to 70% more than a less healthy version and concluded that theoretically the majority of the South African population cannot afford a healthy diet. It therefore follows that although healthier food choices are usually available in stores, people with a low income may select relatively less healthy foods because of the lower cost.

Overall, as is indicated in the literature, a healthy nutrient-dense diet characterised by meat, fish, vegetables, and fruit consumption, as well as reduced intake of animal fats and sweets, account for a higher cost. A positive relation between diet quality and cost across different age groups was confirmed in several studies (14, 16, 43, 44). For instance, a study on women in the United Kingdom showed that a healthier diet costs almost twice as much as a less healthy diet (42). Not all healthier diets are however associated with higher cost, such as for example a healthy Mediterranean-style diet can have a lower cost, depending on the foods included (19).

Furthermore, our results showed that the nutrient density and nutrient-to-price ratio were significantly lower for anaemic versus non-anaemic sub-groups, even though the diet cost of the sub-groups did not differ. This finding raises the question of whether replacing nutrient-poor foods with healthier options would allow anaemic children to consume diets with a higher nutrient density without affecting the overall cost of the diet. In our study, several food groups (i.e. sweets and salted snacks, and sugar-sweetened soft drinks) had a negative NRF9.3 score, indicating that the amounts of LIM nutrients were higher than ENC nutrients. The costs per 100 g edible portion of these foods are in the same range, or even higher, than most nutrient-dense foods such as vitamin A-rich fruit, vegetables, and chicken liver. Therefore, if foods are carefully selected and intake of certain foods with a low nutrient density is reduced, it is quite possible to consume a diet that has a higher nutrient density with no extra cost (45, 46). Thus, we can assume that in our sample of anaemic children, an

increase of diet nutrient density would be possible without increasing the diet cost, which is also reported in other studies (47, 48). Overall, the nutrient density analyses and added cost variable may be used as a guide to choose the more nutrient-dense foods with similar prices to nutrient-poor foods; or to reduce the intake of nutrient-poor foods to compensate for the higher costs of foods with higher nutrient density in order to decrease the risk of various health disorders (23, 49).

Some strengths and limitations of this study should be considered. The main strength of the study is the finding of the nutrient density of individual foods and diets their monetary cost using the NRF9.3 index score. We could identify nutrient-dense and nutrient-poor foods and diets consumed by children of primary school age. This is the unique and innovative research in this age group, nationally and internationally as there are very few studies investigating cost of the diet in relation to nutrient density and no studies investigated diet cost in relation to anaemia and iron status. Foods costs were determined using the most popular supermarkets across South Africa, with constant prices in all provinces. Integration of these prices with dietary intake data has allowed us to estimate the diet costs at the individual level. However, we used cross-sectional data, and causality can therefore not be inferred, and the actual expenses might not be reflected. Dietary intake data may be affected by the inherent limitations of dietary assessment, such as misreporting of food intakes, which is known to be common in children and adults, and thus may affect the nutrient density and cost of individual diets.

In conclusion, results of our study suggest that consumption of foods and diets with lower nutrient density is associated with lower Hb and PF concentrations. The nutrient density-to-price ratio of the diets was significantly lower for anaemic versus non-anaemic children, indicating that for approximately the same monetary value, anaemic children consumed less nutritious foods. Thus, public health interventions and policies, education and empowerment of children and their caregivers to make better and healthier food choices, particularly in terms of nutrient-dense foods, can be a potential long-term strategy to improve nutritional anaemia and iron status.

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AUTHOR CONTRIBUTIONS

T.V.Z. and M.F. conceptualized this study and contributed to the writing of the paper. M.V. analysed the data and wrote the draft article. S.M.H., J.B., M.v.d.H., C.T-K. and M.C.S. were responsible for data collection in the original studies and contributed to the writing of the paper. All authors read and approved the final manuscript.

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Supplement Table S1: Dietary Reference Intakes (DRIs) of macro-and micro-nutrients that was used for the calculation of NRF9.3

Subscore	Nutrient	Age groups (years old)		
		Children 5-9	Males 9-12	Females 9-12
NR9*	Protein (g/kg/day)**	0.76	0.76	0.76
	Fibers (g)***	25	31	26
	Vitamin A (µg)**^	275.0	445	420
	Vitamin C (mg)**	25.0	45	45
	Vitamin D (µg)**	10.0	10.0	10.0
	Vitamin B12 (µg)**	1.0	1.5	1.5
	Iron (mg)**	4.1	5.9	5.7
	Zinc (mg)**	4	7	7
	Calcium (mg)***	800	1300	1300
LIM*	Saturated fat (g)**	10% of total energy		
	Added sugar (g)**	10% of total energy		
	Sodium (mg)**	1200	1500	1500

*NR9 – Nutrients to encourage; LIM – nutrients to limit; ** EAR - Estimated Average Requirement; AI*** – Adequate Intake; § EER - Estimated Energy Requirement; ^RAE - Retinol activity equivalent.

Supplement 2

The NRF index algorithms for nutrients NR9 and LIM nutrients sub-scores and for the combined NRF9.3 nutrient profile model

I. The algorithm of NRF per 100 g of food is the arithmetic difference between the summary of percent EAR values (%EAR) for the NR9 (NRn100g sub-scores) and the sum of %AI for the LIM (LIMn100g sub-scores). The NRn100g sub-scores are based on average of percent EAR for n index nutrients (14). The LIMn100g sub-scores are the average of percentage maximum AI values for the nutrients to limit (22, 14).

For the calculation of the NRF9.3_{100g} score for each food item consumed most by children the following steps was performed:

1. To determine the nutrient rich sub-score based on 100 g of food (NRn100g):

- the ratio of the each NR9 nutrient to the EAR (as the reference value) was added together in percentage values:

$$NRn_{100g} = \sum_{1-9} (NR9i/EARi) \times 100.$$

Where the NR9i is the content of nutrient i in 100 g of food;

EARi is the Estimated Average Requirement values for nutrient i ;

n is the number of nutrients (8, 14).

2. To determine the nutrient to limit sub-score based on 100 g of food (LIMn100g):

- the ratio of each LIM nutrient to the AI (as the reference value) was added together and expressed in percentage values:

$$LIMn_{100g} = \sum_{1-3} (LIMi/AIi) \times 100,$$

where the LIMi is the content of nutrient i in 100 g of food;

AIi is the Adequate Intakes values for nutrient i (Table 1);

n is the number of nutrients (8).

3. Finally:

$$NRF9.3_{100g} = NRn_{100g} - LIMn_{100g}$$

II. The algorithm of NRF per 100 kJ is the arithmetic difference between the nutrient rich score based on 100kJ of food (NRn_{100kJ}) and the nutrient limit score based on 100kJ of food (LIMn_{100kJ}).

For the calculation NRF9.3_{100kJ} score for each food item the following steps was done:

$$1. NRn_{100kJ} = (NRn_{100g}/ED) \times 100;$$

$$2. LIMn_{100kJ} = (LIMn_{100g}/ED) \times 100;$$

$$3. NRF9.3_{100kJ} = NRn_{100kJ} - LIMn_{100kJ}.$$

The Energy density (ED) was defined as the amount of energy per 100 g of food (kJ/100 g) (14). It was expressed as nutrient rich sub-score per 100 kJ: $NRn_{100kJ} = (\text{nutrient rich sub-score per 100 g/energy density}) \times 100$.

The NRD9.3 score consists of a total nutrient rich 9 sub-score (TNR9) and a total limiting 3 sub-score (TLIM3). The TNR9 sub-score is the sum of percentages of the EAR in the diet of participants for each of the nine qualifying nutrients:

$$TNR9 = \sum_{1-9} (TNRi / EARi) \times 100\%$$

The TLIM3 sub-score is the sum of the percentages of the AI for each of the three disqualifying nutrients:

$$TLIM3 = \sum_{1-3} (LIMi / AIi) \times 100\%$$

The NRD9.3 score of a diet was calculated by subtracting the TLIM3 sub-score from the TNR9 sub-score: $NRD9.3 = TNRn9 - TLIMn3$.

CHAPTER 6: CONCLUSIONS AND RECOMMENDATIONS

6.1 Introduction

School-age children are at high risk of anaemia and ID due to their increased nutrient requirements, because of accelerated physical and intellectual development, and poor dietary iron intake or iron absorption (Lozoff *et al.*, 2006).

The overall aim of this research study was to determine the relationship between dietary intake and anaemia and iron status and to estimate the cost of a diet in the pooled group (n=578) of South African 5- to 12-year-old school children. The data used for this pooled analysis were derived from baseline data of three independent intervention studies on primary school children living in three different areas of the KwaZulu-Natal and North West provinces, which has been previously reported (Baumgartner *et al.*, 2012; Taljaard *et al.*, 2013; Van der Hoeven *et al.*, 2016). Since two intervention studies selected children with poor iron status, our pooled sample was not representative of the study populations from which the children were recruited, and no generalisation of the anaemia and ID prevalence in school children were made. However, we focused on the relationship between dietary intake and anaemia and iron status in this study sample.

This concluding chapter provides a summary and discussion of all the main findings as well as the strengths and limitations of the study. Recommendations for future research will also be made.

6.2 Anaemia, iron deficiency and nutrient intake

In the pooled study sample, 13.8% of the children were anaemic and 27.7% were ID. The majority of the children (more than 80%) did not meet the EER. The percentage of energy derived from protein was within the AMDR (between 10% and 30% of total energy intake); however, it was just above the minimum range for all the children. In terms of micronutrient intake, almost half of the children did not meet the EAR for vitamin A and vitamin B12, and more than 50% did not meet the requirements for calcium, vitamin C, vitamin D and vitamin E. In terms of dietary iron intake, only 18% of the children did not meet the requirements. The low percentage of children who did not meet the EAR for iron can probably be due to the mandatory fortification of maize meal and wheat flour. When comparing children according to their anaemia and ID status in the pooled sample, it was observed that anaemic and ID children had significantly lower intakes of vitamin A and vitamin C, but iron intake did not differ. However, for the four study sites separately, the highest frequency of anaemia and ID (27% and 42%) was observed in the study site where the highest proportion of children

did not meet the EAR for vitamin A and vitamin B12 (91% and 75%, respectively), while for only 23% of children in this study site iron intake was below the EAR. Thus, these findings raised the question if the other nutrients, such as vitamin A and vitamin B12, may affect dietary iron absorption and, thus, also relate to anaemia and iron status of children.

6.3 Nutrient patterns

In order to improve the understanding of the synergistic role of nutrients related to anaemia and ID, nutrient pattern analysis was used. Factor analysis identified four nutrient patterns: a 'plant protein, carbohydrate, iron and B-vitamins' pattern; an 'animal protein, saturated fat and zinc' pattern; a 'vitamin A and vitamin B12' pattern; and a 'calcium and fibre' pattern.

Comparing children according to their anaemia and ID status across the tertiles of nutrient patterns scores showed that significantly fewer anaemic (26%) versus non-anaemic (34%) children fell in the top tertile of the 'vitamin A and vitamin B12' nutrient pattern, which supports our findings mentioned above (section 5.2). Furthermore, logistic regression analysis showed that the 'vitamin A and vitamin B12' nutrient pattern was inversely associated with anaemia [OR 0.63 (0.49-0.91), $p=0.035$]. This nutrient pattern probably reflects the content of these nutrients in some specific foods, particularly in foods from animal origin (i.e. organ meat, such as liver, which are rich in both of these nutrients), as well as in fruits and vegetables rich in carotenoids, which is a precursor of vitamin A (García-Casal, 2006; Schonfeldt *et al.*, 2013). Both vitamins A and B12 are known to facilitate and enhance non-haem iron absorption from cereal- and plant-based diets (Fishman *et al.*, 2000; García-Casal, 2006).

The higher proportion of anaemic children (41%) versus non-anaemic (30%) was found in the top tertile of the 'plant protein, carbohydrate, iron and B-vitamins' nutrient pattern score. Although logistic regression analysis did not show that this nutrient pattern was significantly associated with anaemia and ID, the results showed that the biggest proportion of anaemic children adhered to this pattern. Grouping of nutrients in this nutrient pattern can mostly be explained by intake of fortified staple foods (i.e. maize porridge and bread). Consumption of the fortified staple foods can make a significant contribution towards daily intake of various nutrients, including iron, and plays an important role in preventing and combating micronutrient deficiencies (Steyn *et al.*, 2008). However, a variety of foods need to be consumed. When the diet mostly consists of cereal- and plant-based foods and lacks variety of foods (e.g. animal sources), there is a greater risk of developing ID and nutritional anaemia, as indicated in our findings and supported results of other studies (Hurrell & Egli, 2010; Schonfeldt *et al.*, 2013), therefore, highlighting the importance of dietary diversity

6.4 Dietary diversity

We calculated the DDS based on nine food groups, for 1-day and 3-day dietary recall periods, respectively. In the pooled group, there was a statistically significant difference in the mean DDS and the proportion of children with low dietary diversity between the two reference recall periods although the difference in the values was relatively small. When comparing children according to anaemia and ID status, we found that a significantly lower proportion of anaemic (versus non-anaemic) and ID (versus non-ID) children consumed foods from specific food groups. For example, fewer anaemic children (versus non-anaemic) consumed foods from the 'vegetables and fruits other than vitamin A-rich' and 'ASF' food groups regardless of the dietary recall period. However, for the 'meat, fish, and seafood' and 'eggs' food groups, the difference between anaemic and non-anaemic children was significant only for the 3-day recall period, indicating that these foods are eaten less frequently and highlighting the importance of multiple-day dietary recall information. Foods from the 'organ meat' group were not consumed by anaemic children. Also, fewer ID children (versus non-ID) ate foods from the 'organ meat' group irrespective of the dietary recall period, indicating the significance of organ meat intake as possible strategy to reduce the risk of developing anaemia or ID. However, for the 'vegetables and fruits other than vitamin A-rich' and 'legumes, nuts and seeds' food groups, the difference between ID and non-ID children was significant only for the 3-day recall period. Furthermore, logistic regression analysis showed the inverse relationship between the consumption of fruits and vegetables, other than vitamin A-rich, ASFs and organ meat, to anaemia and ID. Consumption of fruits and vegetables may improve the bioavailability of non-haem iron (Balarajan *et al.*, 2011; Ghose & Yaya, 2018). This can be explained by the vitamin C content in most fruits and vegetables, which may play an important role in enhancing the bioavailability of iron from cereal- and plant-based foods, thus preventing nutritional anaemia (Péneau *et al.*, 2008). The haem iron in ASF also may enhance the absorption of non-haem iron from cereal- and plant-based diets almost twice if consumed together (Kristensen *et al.*, 2005; Kennedy *et al.*, 2013). Also, consumption of ASF, particularly organ meat (e.g. liver, kidney), which are rich in nutrients such as vitamins A and B12, may facilitate and enhance iron absorption from the diet, thus playing an important role in managing nutritional anaemia (Fishman *et al.*, 2000; Pettit *et al.*, 2011). Our results emphasise the importance of the multiple-day dietary recall to get information on foods that are not frequently consumed by the study population (i.e. 'meat, fish, and seafood'). This finding can probably explain why some studies did not find the association between dietary diversity and anaemia or ID when they used a 1-day recall period only (Gwetu *et al.*, 2016; Saaka *et al.*, 2017).

In terms of overall dietary diversity, approximately 40% of the children in this study consumed a low diverse diet from four or less food groups. Also, more anaemic children (versus non-anaemic) consumed a diet of low variety, regardless of the dietary recall period. Moreover, logistic regression analysis showed a significantly positive relationship between the consumption of low diverse diet and anaemia, irrespective of the dietary recall period, and ID for the 3-day recall period. Overall, our results indicate that the children who consumed a diet with low variety were more likely to be anaemic and ID. A diet with low variety of foods may cause inadequate nutrient intake, including iron, which is important to maintain adequate iron status and to prevent ID and anaemia (Torheim *et al.*, 2010; Abriha *et al.*, 2014). Consumption of a greater variety of foods is considered beneficial for the improvement of the nutritional quality of the diet compared to a monotonous diet and may help to alleviate multiple micronutrient deficiencies (Oldewage-Theron & Kruger, 2011; Labadarios *et al.*, 2011).

Thus, our finding proposes that, in order to improve anaemia and iron status among primary school-age children, a diverse diet should be promoted with emphasis on consumption of vegetables, fruits and ASF (including organ meats) in addition to the 'starchy staples' (such as maize meal and bread that are fortified according to a National Food Fortification Program) which was consumed by all the children in our study.

6.5 Diet cost

The Nutrient Rich Foods (NRF9.3) index score was calculated for the foods consumed by the children, with the aim of identifying nutrient-dense and nutrient-poor foods (Drewnowski, 2010). The most nutrient-dense food groups with the highest mean NRF9.3 score were organ meats, vitamin A-rich vegetables and fruits, fruits other than vitamin A-rich, fish (mostly canned sardines), and DGLV, but these food groups were the least consumed in our study group (12.2%, 13.8%, 15.2%, 15.5%, 27.7%, respectively). Conversely, foods from the sugar (consumed by 85.7 % of children), fats and oils (67.2%), salty snacks (39.5%), carbonated soft drinks (37.5%), and sweets and jam (30.6%) food groups were the least nutrient-dense, but often consumed. The inclusion of nutrient-dense foods from a variety of food groups is necessary to enhance recommended nutrient intake and improve the quality of the diet and construct of a healthy balanced diet (Tetens *et al.*, 2007; Drewnowski, 2010). Underconsumption of nutrient-dense foods and overconsumption of nutrient-poor foods, such as low-cost starches, added sugars, and vegetable fats, may lead to inadequate intake of micronutrients (Drewnowski & Eichelsdoerfer, 2009). However, the latter can be cheaper sources of foods.

Using the nutrient density and cost of foods to calculate the nutrient-to-price ratio enabled us to identify which foods are the best sources of nutrients to be encouraged per unit cost. Results showed that not all nutrient dense foods were the most expensive foods in terms of nutrient-to-price ratio. Particularly, the most iron-dense foods with the highest nutrient-to-price ratio from the ASF food group were chicken liver, followed by canned sardines and eggs, thus, providing the best nutrient value for money. Also, among other foods, DGLV and legumes were the cheapest sources of iron. While chicken liver and DGLV, for instance, may provide almost similar iron density per unit price, the bioavailability of iron from ASF are higher than from plant sources (Hurrell & Egli, 2010). Indeed, ASF are an important source of high quality protein and provide a variety of essential nutrients required in children's diet, that is, vitamin A (found in liver) and vitamin B12, considered as facilitators and enhancers of non-haem iron absorption (Ross, 2010; Schönfeldt *et al.*, 2013). Yet, the high cost of nutrient-dense foods may be one of the factors that might play a role in food selection, however, in this study we identified foods that are nutrient-dense and do not cost that much. Thus, our results are stressing the importance of proper nutrition education.

We further investigated the relationship of nutrient density and cost of the diets with anaemia and ID. Fewer anaemic children were found in the top quartile (4.9%) of the nutrient density diet (NRD9.3 score) compared to the lower quartile (25%). Also, the NRD9.3 score was positively associated with Hb and PF concentrations. Furthermore, children with anaemia and ID had significantly low NRD9.3 scores compared to non-anaemic and non-ID children. The diet cost did not differ significantly between anaemic and non-anaemic children. However, the nutrient-to-price ratio of the diet was significantly lower for anaemic versus non-anaemic children, indicating a lower nutrient density for approximately the same amount of money spent.

Our results suggest that by specially selecting foods that provide a higher nutrient density and substitute foods of poorer nutrient density, healthy diets can be obtained at no additional cost. This may help children with poor iron status to consume a diet richer in specific nutrients but without affecting the total cost of the diet (Dubois *et al.*, 2017). Thus, improving diet quality is not necessarily related to increased diet costs. In this regard, the nutrient density-based models may be used in combination with food cost to identify those foods that provide more of the targeted nutrients per unit cost. The higher nutrient density of the diet can be used as an indication of the higher dietary quality, and can be used to compare study groups with various health disorders (Fulgoni *et al.*, 2009; Glanz *et al.*, 2009).

6.6 Strengths and limitations of the study

Strengths

A strength of this cross-sectional study is that the dietary data used in this study were collected by means of three 24-hour recalls and a relatively big pooled sample (n=578) of 5- to 12-year-old school children. Having multiple 24-hour recalls for each participant reduced the effect of random measurement error (Gibson *et al.*, 2017). Furthermore, since there is a paucity of dietary studies on school-age children in South Africa, especially studies which have included fortified nutrients used in NFFPs, our results may add to the existing knowledge and information of the dietary intake of school-age children. Foods and diet costs were determined using supermarket price data with constant prices in all provinces of South Africa. Integration of these prices with dietary intake data has allowed us to estimate the diet costs at the individual level.

Limitations

Dietary intake data may be affected by the inherent limitations of dietary assessment, such as misreporting of food intakes. This is known to be common in children and adults, and thus nutrients may therefore be under- and over reported, then the prevalence of inadequate intakes for a population also may be misreported (Archer *et al.*, 2013; Mitka, 2013). Other study recommended to calculate the actual iron intake of individuals or groups using from 9- to 12-days as the optimal study period (Bingham, 1987). Also must be considered that the information in any food composition database gives an average indication of the nutrient content of foods (Southgate, 2000; Wolmarans *et al.*, 2013). In relation to the cost, the prices of foods included in the present study, were determined using standard supermarket prices; however, this might not reflect the actual expenses, which may be influenced by seasonal variations in food prices, or price variations in small stores and vendors, especially as some of the participants were from rural areas. Additionally, the study was cross-sectional, and unable to identify causal relationships, between exposure (diet) and outcome (anaemia and iron status).

6.7 Conclusion

The observed association between the 'vitamin A and vitamin B12' nutrient pattern and lower odds of anaemia in the studied children highlights the potential role of inadequate intake of vitamin A and vitamin B12 in the aetiology of anaemia: both these vitamins are known to facilitate and enhance iron absorption. The identification of nutrient patterns, rather than individual nutrients, may be a better way of exploring the cumulative and synergistic effects

of various nutrients on maintaining and improving nutritional anaemia and ID. Thus, we need to not only focus on iron rich food but also understand and motivate the importance of foods that will provide the nutrients in question, such as vitamin A and vitamin B12, in the prevention and management of anaemia and ID.

The inverse association between low dietary diversity and anaemia underlines the importance of promoting dietary diversification, with a focus on higher consumption of vegetables, fruits, and ASFs, especially organ meats. This may have a positive effect on maintaining and improving anaemia and iron status in children whose iron intake mostly comes from fortified staple foods (electrolytic iron in the South African situation). While consumption of fortified staple foods can certainly add to a healthy diet, these foods only may not be enough to improve anaemia and iron status in studied children. The issue of the type of iron fortificant, which is used in maize meal and wheat flour, and the recommended fortification premix values, must be considered.

Finally, the results of our study showed that the cost of low-nutrient density diets of anaemic and ID children were similar to the cost of the nutrient-dense diets of children with sufficient iron status. This result suggests that, for approximately the same amount of money spent, anaemic children in this study consumed less nutritious foods. Thus, it should be possible to plan a nutrient-dense diet with no extra cost, using low-cost options of foods (from each food group) that are rich in the nutrients to be encouraged in study children. Nutrient profiling is a new approach to rank foods and diets according to their nutrient density, cost and nutrient-to-price ratios. The adoption of healthier nutrient-dense diets may be achieved by taking into account this approach, which allows consumers to identify and select optimal diets at an affordable cost. This emphasises the importance of food and nutrition policies, specifically policies which may assist in making 'healthier' or more nutrient-dense food choices easier.

6.8 Recommendations for the future research

To determine the causality of diverse and nutrient-dense diets and nutritional anaemia and ID, including foods with enhancers and facilitators of dietary iron absorption, the information from the present study on dietary diversity and nutrient density may be considered for short- or long-term intervention strategies that need to be conducted. In this regard, it is recommended:

- to investigate if the increased consumption of animal-source foods and fruits and vegetables that are rich in the nutrients known as the iron enhancers and facilitators will improve the nutritional anaemia and iron status in children that continue intake of

their usual diet, i.e. using the school feeding as a vehicle. This recommendation can be achieved by conducting randomised controlled trials with an adequate power and appropriate period, selecting a population with an average low dietary diversity. The DDS equal to or less than 4, calculated from three non-quantitative 24-recalls, can be used as a reference for the screening process.

- to develop and validate a tool (i.e. dietary quality index) to screen the populations and specifically identify these that are at high risk of nutritional anaemia and iron deficiency. This recommendation can be achieved by using a combination of the nutrient profiling models approach and food-based dietary guidelines principles.
- to develop and validate (test) a nutrition education tool based on nutrient density concept in order to improve nutritional knowledge of and enable to identify and select foods with the higher nutrient density to improve anaemia and iron status. This recommendation can be achieved by conducting a nutrition education trial implemented within school environments, and also coupled with community and family members, as the caregivers, that responsible for food choices, food preparation and food purchases.

6.9 Recommendations for the stakeholders

The following recommendations are made in this regard:

- anaemia and ID in children of primary school age from vulnerable groups need to be controlled; in order to detect and address anaemia already at an early age the haemoglobin concentration of children can be tested under the supervision of community health specialists in collaboration with other stakeholder institutions;
- if anaemia or ID is found, it is then recommended that children enrol in programmes focusing on the diet – thus given more iron-rich foods and foods that are rich in iron absorption enhancers and facilitators – which can then be planned by the community nutritionist according the NSNP of South Africa guidelines in collaboration with food supervisors from a school-based committee to oversee the daily meal served to learners;
- the promotion of a screening tool to determine the populations of primary school children with a low dietary diversity, using a DDS cut-off point less than or equal to 4 from one qualitative 24-hr dietary recall;

- considering the fact that South Africa has a National Food Fortification Programme in place, which aims to address nutrient deficiency, and that all primary school children consumed fortified starchy staples according to the NSNP of South Africa guidelines, our finding strengthens the point that a diverse diet should be promoted despite or in addition to fortification efforts;
- in order to improve dietary diversification, the promotion of increased consumption of the variety of fruits and vegetables and animal-source foods within schools and communities in addition to intake of the usual diet are recommended; in this regard rural communities could grow home or community fruits and vegetables gardens, and could also increase growing of small domestic animals, such as poultry, as a simple and affordable means of satisfying specific needs of nutrient-dense foods;
- it is therefore important that proper support be provided to the NSNP of South Africa; to recommend using of nutrition education tools that underline the nutritional contribution of the nutrient-dense foods to the diet of South African primary school-age children; the combination the principles of the nutrient profiling models and the South African Food Based Dietary Guidelines, can be used. This stresses the importance of proper nutrition education of school teachers, personal and chefs, parents and caregivers not only on importance of individual food intake but in the context of nutrient density of the meals, and plan appropriate meals at schools according the NSNP of South Africa guidelines; also to make better and healthier food choices, particularly in terms of nutrient-density and cost of foods, selecting the nutrient-dense foods in order to substitute foods with a lower nutrient-density which can make it possible to consume a diet richer in specific nutrients without affecting the cost of the total diet.
- the importance of specific sources of nutrient-dense foods and substitution or decreased consumption of low nutrient-dense sweet and salted foods or cold drinks must play an important role in the nutrition education and need to be used by the all stakeholders in the food system. It is also recommended that children are taught the importance of food variety and foods combinations in addition to the starchy staples in order to make better food choices in terms of high nutrient-dense foods for managing nutritional anaemia and ID. The principles of the Traffic Lights Food Guide developed by Temple and Bourne (2010) could be used.

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ANNEXURE A: ETHICS APPROVAL CERTIFICATE OF STUDY



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2018/06/09

ETHICS APPROVAL CERTIFICATE OF STUDY

Based on approval on 01/06/2016 by Health Research Ethics Committee (HREC) after the review at the meeting held on 12/04/2016, the North-West University Institutional Research Ethics Regulatory Committee (NWU-IRERC) hereby approves your study as indicated below. This implies that the NWU-IRERC grants its permission that provided the special conditions specified below are met and pending any other authorisation that may be necessary, the study may be initiated, using the ethics number below.

Study title: Dietary intake in relation to iron status in 5-12 year old primary school children and estimated cost of a nutrient rich diet.	
Study Leader/Supervisor:	Dr T van Zyl
Student:	MV Visser
Ethics number:	N W U - 0 0 0 2 7 - 1 6 - A 1
	<small>Institution Study Number Year Status</small>
	<small>Status: S = Submission; R = Re-Submission; P = Provisional Authorisation; A = Authorisation</small>
Application Type: Single study	Risk: Minimal
Commencement date: 2016-06-01	
Continuation of the study is dependent on receipt of the annual (or as otherwise stipulated) monitoring report and the concomitant issuing of a letter of continuation up to a maximum period of three years.	

Special conditions of the approval (if applicable):

- Translation of the informed consent document to the languages applicable to the study participants should be submitted to the HREC (if applicable).
- Any research at governmental or private institutions, permission must still be obtained from relevant authorities and provided to the HREC. Ethics approval is required BEFORE approval can be obtained from these authorities.

General conditions:

While this ethics approval is subject to all declarations, undertakings and agreements incorporated and signed in the application form, please note the following:

- The study leader (principle investigator) must report in the prescribed format to the NWU-IRERC via HREC:
 - annually (or as otherwise requested) on the monitoring of the study, and upon completion of the study
 - without any delay in case of any adverse event or incident (or any matter that interrupts sound ethical principles) during the course of the study.
- Annually a number of studies may be randomly selected for an external audit.
- The approval applies strictly to the proposal as stipulated in the application form. Would any changes to the proposal be deemed necessary during the course of the study, the study leader must apply for approval of these amendments at the HREC, prior to implementation. Would there be deviated from the study proposal without the necessary approval of such amendments, the ethics approval is immediately and automatically forfeited.
- The date of approval indicates the first date that the study may be started.
- In the interest of ethical responsibility the NWU-IRERC and HREC retains the right to:
 - request access to any information or data at any time during the course or after completion of the study;
 - to ask further questions, seek additional information, require further modification or monitor the conduct of your research or the informed consent process.
 - withdraw or postpone approval if:
 - any unethical principles or practices of the study are revealed or suspected,
 - it becomes apparent that any relevant information was withheld from the HREC or that information has been false or misrepresented,
 - the required amendments, annual (or otherwise stipulated) report and reporting of adverse events or incidents was not done in a timely manner and accurately,
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- HREC can be contacted for further information or any report templates via Ethics-HRECAppl@nwu.ac.za or 018 299 1206.

The IRERC would like to remain at your service as scientist and researcher, and wishes you well with your study. Please do not hesitate to contact the IRERC or HREC for any further enquiries or requests for assistance.

Yours sincerely

Linda du Plessis

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ANNEXURE B: CONTENT AND STYLE GUIDELINES FOR THE NUTRITION - THE INTERNATIONAL JOURNAL OF APPLIED AND BASIC NUTRITIONAL SCIENCES



NUTRITION

The International Journal of Applied and Basic Nutritional Sciences

AUTHOR INFORMATION PACK

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DESCRIPTION

Founded by [Michael M. Meguid](#) in the early 1980's, *Nutrition* presents advances in **nutrition** research and science, informs its readers on new and advancing technologies and data in **clinical nutrition** practice, encourages the application of outcomes research and meta-analyses to problems in patient-related nutrition; and seeks to help clarify and set the research, policy and practice agenda for **nutrition science** to enhance human well-being in the years ahead.

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INTRODUCTION

Nutrition provides an international forum for professionals interested in the **applied and basic biomedical nutritional sciences, and publishes papers both of clinical interest and of scientific import.** Investigators are encouraged to submit papers in the disciplines of nutritionally related biochemistry, genetics, immunology, metabolism, molecular and cell biology, neurobiology, physiology, and pharmacology. Papers on nutrition-related plant or animal sciences which are not of direct relevance to man, whereas occasionally of interest are not the main focus of the Journal.

Nutrition publishes a wide range of articles, which includes original investigations, review articles, rapid communications, research letters, case reports and special category manuscripts. Manuscripts must be prepared in accordance with the "Uniform Requirements for Manuscripts Submitted to Biomedical Journals" developed by the International Committee of Medical Journal Editors (*N Engl J Med* 1991;324:424-428). All submissions are peer reviewed.

Original Investigation (3000-5000 words including tables, figures and references)

Original investigations are considered full-length applied (human) or basic (bench work) research reports. They cover topics relevant to clinical and basic studies relevant to man in the following areas nutritionally related biochemistry, genetics, immunology, metabolism, molecular and cell biology, neurobiology, physiology, and pharmacology. Studies in adult and pediatric populations are welcome. The work presented in the manuscript must be original; studies confirming previous observations will be considered. Other considerations of a paper's publishability are its importance to the science, the soundness of the experimental design, the validity of methods, the appropriateness of the conclusions and the quality of presentation.

Rapid Communication (1000-3000 words including tables, figures and references)

Papers representing concise and original studies of scientific importance are considered. In the cover letter the author should justify the request for Rapid Communication. The review process is 10 days, authors are allowed one revision if accepted, and the final version of the paper appears in the next available issue of the journal.

Research Letter (up to 1000 words, including up to 10 references and 1 figure or table)

A Research Letter contains new data or a clinical observation, in a format that allows for rapid publication.

Review Article (up to 5000 words including tables, figures and references)

In-depth, comprehensive state of the art reviews on a nutritional topic are welcomed. Reviews may be invited by the Editor or may be unsolicited viewpoints.

Case Report (up to 2500 words including tables figures, and references) Case Reports include case studies of 4 or fewer patients that describe a novel situation or add important insights into mechanisms, diagnosis or treatment of a disease.

Editorial (up to 1000 words including tables, figures and references)

Editorials express opinions on current topics of interest, or provide comments on papers published in *Nutrition* or other journals. Editorials are generally solicited by one of the Editors.

Correspondence (Letter to the Editor) (1000 words including tables, figures and references)

Opinion pieces concerning papers published in *Nutrition* are particularly welcomed and all submissions are subject to editing. Letters commenting on past-published papers are sent to the corresponding author for a response. Letters are selected for their relevance and originality; not all letters submitted can be published.

PREPARATION

NEW SUBMISSIONS

Submission to this journal proceeds totally online and you will be guided stepwise through the creation and uploading of your files. The system automatically converts your files to a single PDF file, which is used in the peer-review process.

As part of the Your Paper Your Way service, you may choose to submit your manuscript as a single file to be used in the refereeing process. This can be a PDF file or a Word document, in any format or layout that can be used by referees to evaluate your manuscript. It should contain high enough quality figures for refereeing. If you prefer to do so, you may still provide all or some of the source files at the initial submission. Please note that individual figure files larger than 10 MB must be uploaded separately.

Authors please note: We please ask you to use line numbering throughout the manuscript text, to facilitate clear and rapid peer review

References

There are no strict requirements on reference formatting at submission. References can be in any style or format as long as the style is consistent. Where applicable, author(s) name(s), journal title/book title, chapter title/article title, year of publication, volume number/book chapter and the pagination must be present. Use of DOI is highly encouraged. The reference style used by the journal will be applied to the accepted article by Elsevier at the proof stage. Note that missing data will be highlighted at proof stage for the author to correct.

Formatting requirements

There are no strict formatting requirements but all manuscripts must contain the essential elements needed to convey your manuscript, for example Abstract, Keywords, Introduction, Materials and Methods, Results, Conclusions, Artwork and Tables with Captions.

If your article includes any Videos and/or other Supplementary material, this should be included in your initial submission for peer review purposes.

Divide the article into clearly defined sections.

Figures and tables embedded in text

Please ensure the figures and the tables included in the single file are placed next to the relevant text in the manuscript, rather than at the bottom or the top of the file. The corresponding caption should be placed directly below the figure or table.

Peer review

This journal operates a double blind review process. All contributions will be initially assessed by the editor for suitability for the journal. Papers deemed suitable are then typically sent to a minimum of two independent expert reviewers to assess the scientific quality of the paper. The Editor is responsible for the final decision regarding acceptance or rejection of articles. The Editor's decision is final. [More information on types of peer review.](#)

REVISED SUBMISSIONS

Use of word processing software

Regardless of the file format of the original submission, at revision you must provide us with an editable file of the entire article. Keep the layout of the text as simple as possible. Most formatting codes will be removed and replaced on processing the article. The electronic text should be prepared in a way very similar to that of conventional manuscripts (see also the [Guide to Publishing with Elsevier](#)). See also the section on Electronic artwork.

To avoid unnecessary errors you are strongly advised to use the 'spell-check' and 'grammar-check' functions of your word processor.

Article structure

Subdivision - unnumbered sections

Divide your article into clearly defined sections. Each subsection is given a brief heading. Each heading should appear on its own separate line. Subsections should be used as much as possible when cross-referencing text: refer to the subsection by heading as opposed to simply 'the text'.

Introduction

State the objectives of the work and provide an adequate background, avoiding a detailed literature survey or a summary of the results.

Material and methods

Provide sufficient detail to allow the work to be reproduced. Methods already published should be indicated by a reference: only relevant modifications should be described.

Theory/calculation

A Theory section should extend, not repeat, the background to the article already dealt with in the Introduction and lay the foundation for further work. In contrast, a Calculation section represents a practical development from a theoretical basis.

Results

Results should be clear and concise.

Discussion

This should explore the significance of the results of the work, not repeat them. A combined Results and Discussion section is often appropriate. Avoid extensive citations and discussion of published literature.

Conclusions

The main conclusions of the study may be presented in a short Conclusions section, which may stand alone or form a subsection of a Discussion or Results and Discussion section.

Appendices

If there is more than one appendix, they should be identified as A, B, etc. Formulae and equations in appendices should be given separate numbering: Eq. (A.1), Eq. (A.2), etc.; in a subsequent appendix, Eq. (B.1) and so on. Similarly for tables and figures: Table A.1; Fig. A.1, etc.

This should include 1) title of paper (**use no abbreviations, limit: 120 characters with spaces**), 2) running head of fewer than 55 characters with spaces, 3) full names of all authors with highest academic degree(s); 4) affiliations of all authors; 4) role of each author in the work (see Authorship); 5) a word count for the entire manuscript (including figures and tables), and the number of figures and tables, 4) the complete mailing address (including telephone, fax, and e-mail address of the corresponding author for e-mailing of proofs and reprint requests).

Abstracts should be no more than **250 words**. The structured abstract for an original investigation should be organized as follows:

Objective: The abstract should begin with a clear statement of the precise objective or question addressed in the paper. If a hypothesis was tested, it should be stated.

Research Methods & Procedures: The basic design of the study and its duration should be described. The methods used should be stated, the statistical data/methods provided and referenced.

Results: The main results of the study should be given in narrative form. Measurements or other information that may require explanation should be defined. Levels of statistical significance should be indicated, including other factors crucial to the outcome of the study.

Conclusion(s): State only conclusions that are directly supported by the evidence and the implications of the findings.

Graphical abstract

Although a graphical abstract is optional, its use is encouraged as it draws more attention to the online article. The graphical abstract should summarize the contents of the article in a concise, pictorial form designed to capture the attention of a wide readership. Graphical abstracts should be submitted as a separate file in the online submission system. Image size: Please provide an image with a minimum of 531 × 1328 pixels (h × w) or proportionally more. The image should be readable at a size of 5 × 13 cm using a regular screen resolution of 96 dpi. Preferred file types: TIFF, EPS, PDF or MS Office files. You can view [Example Graphical Abstracts](#) on our information site.

Authors can make use of Elsevier's [Illustration Services](#) to ensure the best presentation of their images and in accordance with all technical requirements.

Highlights

Highlights are mandatory for this journal. They consist of a short collection of bullet points that convey the core findings of the article and should be submitted in a separate editable file in the online submission system. Please use 'Highlights' in the file name and include 3 to 5 bullet points (maximum 85 characters, including spaces, per bullet point). You can view [example Highlights](#) on our information site.

Keywords

5–7 key words or phrases should be provided which should be selected from the body of the text and not duplicate title words.

Abbreviations

Define abbreviations that are not standard in this field in a footnote to be placed on the first page of the article. Such abbreviations that are unavoidable in the abstract must be defined at their first mention there, as well as in the footnote. Ensure consistency of abbreviations throughout the article.

Acknowledgments

Collate acknowledgements in a separate section at the end of the article before the references and do not, therefore, include them on the title page, as a footnote to the title or otherwise. List here those individuals who provided help during the research (e.g., providing language help, writing assistance or proof reading the article, etc.).

Formatting of funding sources

List funding sources in this standard way to facilitate compliance to funder's requirements:

Funding: This work was supported by the National Institutes of Health [grant numbers xxxx, yyyy]; the Bill & Melinda Gates Foundation, Seattle, WA [grant number zzzz]; and the United States Institutes of Peace [grant number aaaa].

It is not necessary to include detailed descriptions on the program or type of grants and awards. When funding is from a block grant or other resources available to a university, college, or other research institution, submit the name of the institute or organization that provided the funding.

If no funding has been provided for the research, please include the following sentence:

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Units

Follow internationally accepted rules and conventions: use the international system of units (SI). If other units are mentioned, please give their equivalent in SI.

Math formulae

Please submit math equations as editable text and not as images. Present simple formulae in line with normal text where possible and use the solidus (/) instead of a horizontal line for small fractional terms, e.g., X/Y. In principle, variables are to be presented in italics. Powers of e are often more conveniently denoted by exp. Number consecutively any equations that have to be displayed separately from the text (if referred to explicitly in the text).

Footnotes

Footnotes should be used sparingly. Number them consecutively throughout the article. Many word processors build footnotes into the text, and this feature may be used. Should this not be the case, indicate the position of footnotes in the text and present the footnotes themselves separately at the end of the article.

Artwork

Electronic artwork

General points

- Make sure you use uniform lettering and sizing of your original artwork.
- Preferred fonts: Arial (or Helvetica), Times New Roman (or Times), Symbol, Courier.
- Number the illustrations according to their sequence in the text.
- Use a logical naming convention for your artwork files.
- Indicate per figure if it is a single, 1.5 or 2-column fitting image.

Color artwork

Please make sure that artwork files are in an acceptable format (TIFF (or JPEG), EPS (or PDF), or MS Office files) and with the correct resolution. If, together with your accepted article, you submit usable color figures then Elsevier will ensure, at no additional charge, that these figures will appear in color online (e.g., ScienceDirect and other sites) regardless of whether or not these illustrations are reproduced in color in the printed version. **For color reproduction in print, you will receive information regarding the costs from Elsevier after receipt of your accepted article.** Please indicate your preference for color: in print or online only. [Further information on the preparation of electronic artwork.](#)

Illustration services

[Elsevier's WebShop](#) offers Illustration Services to authors preparing to submit a manuscript but concerned about the quality of the images accompanying their article. Elsevier's expert illustrators can produce scientific, technical and medical-style images, as well as a full range of charts, tables and graphs. Image 'polishing' is also available, where our illustrators take your image(s) and improve them to a professional standard. Please visit the website to find out more.

Figure captions

Ensure that each illustration has a caption. A caption should comprise a brief title (**not** on the figure itself) and a description of the illustration. Keep text in the illustrations themselves to a minimum but explain all symbols and abbreviations used.

Tables

Please submit tables as editable text and not as images. Tables can be placed either next to the relevant text in the article, or on separate page(s) at the end. Number tables consecutively in accordance with their appearance in the text and place any table notes below the table body. Be sparing in the use of tables and ensure that the data presented in them do not duplicate results described elsewhere in the article. Please avoid using vertical rules and shading in table cells.

References

Citation in text

Please ensure that every reference cited in the text is also present in the reference list (and vice versa). Any references cited in the abstract must be given in full. Unpublished results and personal communications are not recommended in the reference list, but may be mentioned in the text. If these references are included in the reference list they should follow the standard reference style of the journal and should include a substitution of the publication date with either 'Unpublished results' or 'Personal communication'. Citation of a reference as 'in press' implies that the item has been accepted for publication.

AUTHOR INQUIRIES

Visit the [Elsevier Support Center](#) to find the answers you need. Here you will find everything from Frequently Asked Questions to ways to get in touch.

You can also [check the status of your submitted article](#) or [find out when your accepted article will be published](#).

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ANNEXURE C: Content and style guidelines for the Public Health Nutrition journal**Public Health Nutrition**

Public Health Nutrition (PHN) provides an international, peer-reviewed forum for the publication and dissemination of research with a specific focus on nutrition-related public health. The Journal publishes original and commissioned articles, high quality meta-analyses and reviews, commentaries and discussion papers for debate, as well as special issues. It also seeks to identify and publish special supplements on major topics of interest to readers.

SCOPE

The scope of *Public Health Nutrition* includes multi-level determinants of dietary intake and patterns, anthropometry, food systems, and their effects on health-related outcomes. We welcome papers that:

- Address **monitoring and surveillance** of nutritional status and nutritional environments in communities or populations at risk
- Identify and analyse behavioral, sociocultural, economic, political, and environmental **determinants of nutrition-related public health**
- Develop **methodology** needed for assessment and monitoring
- Inform efforts to improve **communication of nutrition-related information**
- **Build workforce capacity** for effective public health nutrition action
- Evaluate or discuss the effectiveness of **food and nutrition policies**
- Describe the development, implementation, and evaluation of **innovative interventions and programs** to address nutrition-related problems
- Relate diet and nutrition to **sustainability** of the environment and food systems

Papers that do not fall within the scope as described above may be directed to more appropriate journals. We prefer papers that are innovative (do not repeat research already undertaken elsewhere) and relevant to an international readership.

PUBLISHING ETHICS

PHN adheres to the Committee on Publication Ethics (COPE) guidelines on research and publications ethics. The Journal considers all manuscripts on the strict condition that:

1. The manuscript is your own original work, and does not duplicate any previously published work;
1. The manuscript has been submitted only to the journal - it is not under consideration or peer review or accepted for publication or in press or published elsewhere;
1. All listed authors know of and agree to the manuscript being submitted to the journal; and
1. The manuscript contains nothing abusive, defamatory, fraudulent, illegal, libellous, or obscene.

Text taken directly or closely paraphrased from earlier published work that has not been acknowledged or referenced will be considered plagiarism. Submitted manuscripts in which such text is identified will be withdrawn from the editorial process. Any concerns raised about possible plagiarism or other violations of ethical guidelines in an article submitted to or published in PHN will be investigated fully and dealt with in accordance with the COPE guidelines.

DETAILED MANUSCRIPT PREPARATION INSTRUCTIONS

Language

Papers submitted for publication must be written in English and should be as concise as possible. We recommend that authors have their manuscript checked by an English language native speaker before submission, to ensure that submissions are judged at peer review exclusively on academic merit.

We list a [number of third-party services](#) specialising in language editing and / or translation, and suggest that authors contact as appropriate. Use of any of these services is voluntary, and at the author's own expense.

Spelling should generally be that of the *Concise Oxford Dictionary* (1995), 9th ed. Oxford: Clarendon Press. Authors are advised to consult a current issue in order to make themselves familiar with PHN as to typographical and other conventions, layout of tables etc.

Authorship

The Journal conforms to the International Committee of Medical Journal Editors (ICMJE) definition of authorship. Authorship credit should be based on:

1. Substantial contributions to conception and design, data acquisition, analysis and/or interpretation;
2. Drafting the article or revising it critically for important intellectual content; and
3. Final approval of the version to be published.

The contribution of individuals who were involved in the study but do not meet these criteria should be described in the Acknowledgments section.

Ethical standards

All submissions must abide by the guidelines in the World Medical Association (2000) Declaration of Helsinki: Ethical Principles for Medical Research Involving Human Subjects, with notes of clarification of 2002 and 2004 (<http://www.wma.net/en/30publications/10policies/b3/>), the Guidelines on the Practice of Ethics Committees Involved in Medical Research Involving Human Subjects (3rd ed., 1996; London: The Royal College of Physicians) and the Guidelines for the Ethical Conduct of Medical Research Involving Children, revised in 2000 by the Royal College of Paediatrics and Child Health: Ethics Advisory Committee (Arch Dis Child (2000) 82, 177–182).

PRISMA Checklist

For systematic reviews and meta-analyses, PHN requires completion of the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) checklist (www.prisma-statement.org/). This policy includes all systematic reviews, including those for observational studies. A completed copy of the checklist should be submitted along with the manuscript, with page numbers noted as required. When a given item has not been addressed, authors must provide an explanation.

Editors and reviewers will not evaluate manuscripts based on the number of items checked off in the checklist. The purpose of the PRISMA guidelines is to recommend a critical set of items that should typically be reported in a manuscript. The guidelines are meant to improve transparency by helping authors improve the quality of their reporting. More clarity in reporting will facilitate review of your manuscript and increase its value to readers.

Cover Letter

Authors are invited to submit a cover letter including a short explanation of how the article advances the field of public health nutrition in terms of research, practice, or policy, and of its relevance to an international readership. The text for the cover letter should be entered in the appropriate box as part of the online submission process.

Title Page

Authors must submit a title page online as a separate file to their manuscript, to enable double-blind reviewing. For the same reason, the information on the title page should not be included in the manuscript itself. The title page should include:

1. The title of the article;
2. Authors' names, given without titles or degrees;
3. Name and address of department(s) and institution(s) to which the work should be attributed for each author, with each author's institution(s) identified by a superscript number (e.g. A.B. Smith¹);
4. Name, mailing address, email address, telephone and fax numbers of the author responsible for correspondence about the manuscript;
5. A shortened version of the title, not exceeding 45 characters (including letters and spaces) in length;
6. Disclosure statements, as outlined below. These must be included on the title page and not in the manuscript file, to enable double-blind reviewing; if the paper is accepted, they will be inserted into the manuscript during production.

Acknowledgments

Here you may acknowledge individuals or organizations that provided advice and/or support (non-financial). Formal financial support and funding should be listed in the following section.

Financial Support

Please provide details of the sources of financial support for all authors, including grant numbers. For example, "This work was supported by the Medical Research Council (grant number XXXXXXX)". Multiple grant numbers should be separated by a comma and space, and where research was funded by more than one agency the different agencies should be

transparency is the best course of action. Perceived conflicts of interest are as important as actual conflicts of interest, and undeclared conflicts (perceived as well as actual) can undermine the credibility of both the journal and the authors.

So that others can make judgements about potential conflicts, please provide details of all known financial and non-financial (professional and personal) relationships with the potential to bias the work. Where no known conflicts of interest exist, please include the following statement: "None."

Authorship

Please provide a very brief description of the contribution of each author to the research. Their roles in formulating the research question(s), designing the study, carrying it out, analysing the data and writing the article should be made plain.

Ethical Standards Disclosure

Manuscripts describing experiments involving human subjects must include the following statement: "This study was conducted according to the guidelines laid down in the Declaration of Helsinki and all procedures involving human subjects/patients were approved by the [name of the ethics committee]. Written [or Verbal] informed consent was obtained from all subjects/patients." Where verbal consent was obtained, this must be followed by a statement such as: "Verbal consent was witnessed and formally recorded."

Manuscript Format

The requirements of PHN are in accordance with the Uniform Requirements for Manuscripts Submitted to Biomedical Journals produced by the ICMJE, and authors are encouraged to consult the [latest guidelines](#), which contain useful, general information about preparing scientific papers. Authors should also consult the [CONSORT guidelines](#) for reporting results of randomised trials.

For detailed instructions regarding mathematical modelling, statistical analysis and nomenclature requirements, please refer to the [Appendix](#) to these instructions.

Typescripts should be prepared with 1.5 line spacing and wide margins (2 cm), the preferred font being Times New Roman size 12. At the ends of lines, words should not be hyphenated unless hyphens are to be printed. Continuous line and page numbering is required.

MANUSCRIPTS SHOULD BE ORGANISED AS FOLLOWS:

Abstract

Each paper must open with a structured abstract of not more than 250 words. The abstract should consist of the following headings: Objective, Design, Setting, Subjects, Results, Conclusions. All the headings should be used, and there should be a separate paragraph for each one. The abstract should be intelligible without reference to text or figures.

Keywords

Authors should list at least four keywords or phrases (each containing up to three words).

Introduction

It is not necessary to introduce a paper with a full account of the relevant literature, but the introduction should indicate briefly the nature of the question asked and the reasons for asking it.

Methods

For manuscripts describing experiments involving human subjects, the required ethical standards disclosure statement must be included on the title page only as described above. It will then be inserted into this section of the manuscript during production.

Results

These should be given as concisely as possible, using figures or tables as appropriate. Data should not be duplicated in tables and figures.

Discussion

While it is generally desirable that the presentation of the results and the discussion of their significance should be presented separately, there may be occasions when combining these sections may be beneficial. Authors may also find that additional or alternative sections such as 'conclusions' may be useful.

References

References should be numbered consecutively in the order in which they first appear in the text using superscript Arabic numerals in parentheses, e.g. 'The conceptual difficulty of this approach has recently been highlighted^(1,2)'. If a reference is cited more than once, the same number should be used each time. References cited only in tables and figure legends should be

numbered in sequence from the last number used in the text and in the order of mention of the individual tables and figures in the text.

Names and initials of authors of unpublished work should be given in the text as 'unpublished results' and not included in the References. References that have been published online only but not yet in an issue should include the online publication date and the Digital Object Identifier (doi) reference, as per the example below.

At the end of the paper, on a page(s) separate from the text, references should be listed in numerical order using the Vancouver system. When an article has more than three authors only the names of the first three authors should be given followed by 'et al.' The issue number should be omitted if there is continuous pagination throughout a volume. Titles of journals should appear in their abbreviated form using the [NCBI LinkOut page](#). References to books and monographs should include the town of publication and the number of the edition to which reference is made. References to material available on websites should follow a similar style, with the full URL included at the end of the reference, as well as the date of the version cited and the date of access.

Examples of correct forms of references are given below.

Journal articles

1. Rebello SA, Koh H, Chen C *et al.* (2014) Amount, type, and sources of carbohydrates in relation to ischemic heart disease mortality in a Chinese population: a prospective cohort study. *Am J Clin Nutr* 100, 53-64.
1. Villar J, Ismail LC, Victora CG *et al.* (2014) International standards for newborn weight, length, and head circumference by gestational age and sex: the Newborn Cross-Sectional Study of the INTERGROWTH-21st Project. *Lancet* 384, 857-868.
1. Alonso VR & Guarner F (2013) Linking the gut microbiota to human health. *Br J Nutr* 109, Suppl. 2, S21-S26.
1. Bauserman M, Lokangaka A, Gado J *et al.* A cluster-randomized trial determining the efficacy of caterpillar cereal as a locally available and sustainable complementary food to prevent stunting and anaemia. *Public Health Nutr*. Published online: 29 January 2015. doi: 10.1017/S1368980014003334.

ANNEXURE D: CONTENT AND STYLE GUIDELINES FOR THE AMERICAN JOURNAL OF CLINICAL NUTRITION

Instructions to Authors

[Statement of Scope](#)
[Criteria for Manuscript Acceptance](#)
[Summary of Requirements](#)
[Major Sections of the *AJCN*](#)
[Research Registration](#)
[Required Checklists](#)
[Format and Style Requirements](#)
[Units and Abbreviations](#)
[Nomenclature](#)
[Microbiological Culture Deposition](#)
[Correcting Proofs](#)
[Charges to Authors](#)
[Tips for Authors](#)

Statement of Scope

The purpose of *The American Journal of Clinical Nutrition (AJCN)* is to publish original research studies relevant to human and clinical nutrition. Well-controlled clinical studies that describe scientific mechanisms, efficacy, and safety of dietary interventions in the context of disease prevention or a health benefit will be considered. Public health and epidemiologic studies relevant to human nutrition, and innovative investigations of nutritional questions that employ epigenetic, genomic, proteomic, and metabolomic approaches are encouraged. Solicited editorials, book reviews, solicited or unsolicited review articles, invited controversy position papers, and letters to the Editor that relate to prior *AJCN* articles are essential components of the *AJCN*. All submitted material with scientific content will undergo peer review by the Editors or their designees before acceptance for publication.

Symposia or workshop articles may be published as supplements to the *AJCN* and are funded by their sponsors. The *AJCN* welcomes queries about the publication of supplements. The *AJCN* uses a 2-part acceptance process for supplements. The first step involves editorial acceptance of the topic and content as provided by the

symposium organizer; please send the following material to DTEarly@nutrition.org for consideration by the AJCN Editorial Office: title, location, and date of the meeting; the names and affiliations of potential guest editors; the sponsor(s) of the meeting; the sponsor(s) of the publication; and the agenda/program from the meeting along with the names of the speakers. The second step involves anonymous peer review of the individual articles. To be considered for publication, supplement articles must be received within 3 mo of each symposium or workshop. Each manuscript should not exceed 15 text pages, exclusive of tables, figures, and references; must adhere to *AJCN* style and format, and will be reviewed according to the same scientific standards used to evaluate original research articles.

For more information on supplements and symposia in *The American Journal of Clinical Nutrition*, please see [here](#).

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Criteria for Manuscript Acceptance

The *AJCN* can publish only about 20% of the more than 1800 original submissions received per year. Submitted manuscripts may be rejected without detailed comments after initial review by at least two *AJCN* editors if the manuscripts are considered inappropriate or of insufficient scientific priority for publication in the *AJCN*. All other manuscripts undergo a complete review by at least two consulting editors or other selected experts. Criteria for acceptance by the *AJCN* include originality, validity of data, clarity of writing, strength of the conclusions, and potential importance of the work to the field of clinical nutrition. Indicate explicitly in your cover letter what is truly new in the present work compared to work already published in the field. Because Cochrane assessments are now readily available on the web via PubMed, *AJCN* no longer considers it necessary to disseminate them by secondary publication in the Journal. Submitted manuscripts will not be reviewed if they do not conform to standard English usage and to the "Uniform Requirements for Manuscripts Submitted to Biomedical Journals" (Internet: <http://www.icmje.org/>), which is also available free of charge from the Secretariat Office, *Annals of Internal Medicine*.

American College of Physicians, Independence Mall West, Sixth Street at Race, Philadelphia, PA 19106-1572. As recommended in the Committee on Publication Ethics Code of Conduct for Journal Publishers, and supported by the International Committee of Medical Journal Editors, when ASN is made aware of cases of suspected research and publication misconduct, ASN holds the right to publish an Expression of Concern during an investigation, and, depending on the outcome of the investigation, to retract articles.

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Summary of Requirements

Each manuscript component should begin on a new page in the following sequence:

- Title page
- Abstract
- Text
- Acknowledgments
- References
- Tables: each table on a separate page, complete with title and footnotes
- Legends for figures
- Figures

Identify on the title page the author who will be responsible for correspondence regarding the manuscript. The signed Authors' Statement and Copyright Release Form and copies of any documents granting permission needed to reproduce material in print and electronic form or to use illustrations of identifiable subjects should be scanned and uploaded to the submission system

at <http://www.editorialmanager.com/ajcn> or e-mailed to ajcnsubmit@nutrition.org. If scanning is not possible, then the Authors' Statement and Copyright Release Form and any necessary documents may be faxed to (240) 404-6798. As recommended by

Required Checklists

Depending on the design of the study, one of the health research reporting checklists referenced at the Equator Network (<http://www.equator-network.org/reporting-guidelines/>) must accompany the first version of each manuscript as a “supplemental file” in the online manuscript submission system. Page or line numbers must be included to indicate where the checklist items are located in your paper. Participant flow charts should be included whenever possible, especially accompanying CONSORT, PRISMA, and STROBE checklists. The ARRIVE checklist is appropriate for manuscripts with animal research. If none of the checklists apply to your study, please explain in your cover letter why none is needed.

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Format and Style Requirements

Articles are copyedited according to *AJCN* style policy, the “Uniform Requirements for Manuscripts Submitted to Biomedical Journals,” and the style manual of the Council of Science Editors (Scientific style and format: the CSE manual for authors, editors, and publishers. 8th ed. Chicago: The University of Chicago Press; 2014).

Authorship

Scientific conduct

Each author must have participated sufficiently, intellectually, or practically in the work to take public responsibility for the content of the article, including the conception, design, and conduct of the experiment, and for the data interpretation. An article with corporate (collective) authorship must specify the key persons responsible for the article; others contributing to the work should be recognized separately. The Editors may require authors to justify the assignment of authorship.

Conflict of interest

Authors must disclose in the Acknowledgment section any possible conflicts of interest. For detailed guidelines, please see https://academic.oup.com/journals/pages/authors/authors_faqs/conflicts_of_interest. Authors who wish to make use of the ICMJE Form for Disclosure of Potential Conflicts of Interest (available from <http://icmje.org/conflicts-of-interest/>) may copy the Disclosure Statement from that form and paste it in to the Acknowledgments section of the manuscript file.

Instructions for manuscript preparation

Initial manuscript submissions

Prepare your manuscript, including figure legends and tables, in Word format. The manuscript should be formatted as follows: 216 x 279 mm (8½ x 11 in) or ISO A4 (212 x 297 mm), with margins of at least 2.5 cm; use double-spacing and 12-point type throughout. Do not justify the right margin. **The abstract and text pages should have consecutive line numbers in the left margin beginning in the abstract and ending before the reference section.** Number pages consecutively in the upper right-hand corner of each page, beginning with the title page. Foreign authors are advised to have their manuscripts reviewed by a scientific colleague who is fluent in English so that the manuscripts will conform to US English usage and grammar.

Revised manuscript submissions

Submit manuscript text, including figure legends and tables, in a Word file; tables must be included in the text file; do not submit tables in separate files. Submit each figure in a separate file according to the specifications listed in the section titled, Figures. Changes in the manuscript text must be marked with red font. This requirement does not apply to changes made to figures or supplemental material. Deleted text should be completely removed. Do not use the “track changes” feature in Word.

Fonts

For optimum legibility we recommend that you use only certain fonts in your document: Times, Times New Roman, Courier, Helvetica, Arial, and the Symbol font for special characters. For review purposes references will be copied from the manuscript file into the submission system and linked to the online source of the cited abstract or article.

Title page

The title page should contain: 1) **Title** of the article, beginning with a key word if possible, with only the first letter of the first word capitalized; 2) **Author Names** (first name, middle initial, last name); 3) **Author Affiliations** (departmental and institutional) at the time the research was done. Indicate which authors are associated with which institutions by listing the appropriate author initials in parentheses after each affiliation listed. 4) **Authors' last names**—listed separately for PubMed indexing; please consider this carefully, in particular for authors with names that include hyphens and prefixes. Punctuation and spacing are generally disregarded when indexing, and the name will usually be indexed under the first letter to appear in the name. 5) Any authors' changed affiliations - should be included in a separate line on the title page. 6) **Disclaimers**, if any, and **not Conflict of Interest**; 7) **Corresponding Author** name, mailing address, telephone number, and e-mail address; 8) the **Sources of Support** including grants, fellowships, and gifts of materials (eg, chemicals, experimental diets); 9) **Short running head** of not more than 50 characters (count letters and spaces); 10) **Abbreviations** list and their definitions for all abbreviations used in the text if there are 3 or more; and 11) **Clinical Trial Registry** number and website where it was obtained.

Abstract A properly constructed and informative abstract is helpful for the initial editorial review of the submitted manuscript. Original research articles must include a structured abstract that contains no more than 300 words, is written in **complete sentences**, and includes the following headings:

- **Background:** Provide 1 or 2 sentences that explain the context of the study.
- **Objective:** State the precise objective, the specific hypothesis to be tested, or both.

- **Design:** Describe the study design, including the use of cells, animal models, or human subjects. Identify the control group. Identify specific methods and procedures. Describe interventions, if used.
- **Results:** Report the most important findings, including results of statistical analyses.
- **Conclusions:** Summarize in 1 or 2 sentences the primary outcomes of the study, including their potential clinical importance, if relevant (avoid generalizations).

Review articles, special articles, and reports should include an unstructured abstract (no more than 300 words) that states the purpose of the article and emphasizes the major concepts and conclusions. Any abbreviations used in the abstract should be defined in the abstract at first mention.

Below the abstract, provide and identify 5–10 keywords or short phrases, including the subject group, that will help to increase the discoverability of your manuscript; do not use adjectives. Terms that are fundamental to your manuscript but are not included in your manuscript title or abstract are especially important to include to increase discoverability by indexing services such as PubMed.

Please note that during manuscript submission, you will be asked to supply keywords to assist the editors in locating suitable reviewers for your manuscript. Keywords for reviewer searches should include the terms most fundamental to your manuscript, and may differ from your list of keywords for publication.

Text

Use active voice whenever possible. Use past tense when describing and discussing the experimental work on which the article is based. Reserve present tense for reference to existing knowledge or prevailing concepts and for stating conclusions from the experimental work. Clearly differentiate previous knowledge and new contributions. Do not use level when referring to a concentration. Use metric units of measure; SI units are no longer required.

The text of observational and experimental articles should be divided into sections with the following headings: Introduction, Subjects (or Materials, for cell or animal studies) and Methods, Results, and Discussion. Long articles may require subheadings within some sections. Authors should consult recent issues of the *AJCN* for guidance on the formatting of other types of articles, book reviews, and editorials.

Introduction

Clearly state the purpose of the article. Summarize the rationale and background for the study or observation, giving only strictly pertinent references. Do not include methods, data, results, or conclusions from the work being reported. The introduction should be limited to 1.5 manuscript pages.

Subjects (or Materials) and Methods

Describe clearly your selection of the experimental and control subjects and provide eligibility and exclusion criteria and details of randomization. Describe the methods for, and success of, any masking (blinding) of observations. Report any complications of experimental treatments. Identify the methods, apparatus (manufacturer's name in parentheses), and procedures in sufficient detail to allow other researchers to reproduce the results. Define all group designations parenthetically at first mention [for example, "control (CON) and high-fat (HF) groups"] and include definitions for these abbreviations in the abbreviation footnote on the title page. Do not use trademark names, such as Teflon, as generic terms. Give references for established methods, including statistical methods; provide references and brief descriptions of methods that have been published but are not well known; and describe new or substantially modified methods, giving reasons for using them and evaluating their limitations. Identify precisely all drugs and chemicals used, including generic names, dosages, and routes of administration. If trade names for drugs and chemicals are included, give the manufacturer's name and location.

Ethics. When reporting experiments on human subjects, indicate that the procedures followed were in accordance with the ethical standards of the responsible institutional

or regional committee on human experimentation or in accordance with the Helsinki Declaration of 1975 as revised in 1983. Do not use patients' names, initials, or hospital identification numbers. When reporting experiments on animals, indicate approval by the institution's animal welfare committee and state whether the National Research Council's guide for the care and use of laboratory animals was followed.

Statistics. Describe statistical methods with enough detail to enable a knowledgeable reader with access to the original data to verify the reported results. When possible, quantify findings and present them with appropriate indicators of measurement error or uncertainty (eg, CIs, SDs, or SEs), even for differences that were not significant. Report the numbers of observations. Specify any general-use computer programs used, including the version number and the manufacturer's name and location. Include general descriptions of statistical methods in the Subjects (or Materials) and Methods section and specific descriptions in each table and figure legend. Indicate whether variables were transformed for analysis. Provide details about what hypotheses were tested, what statistical tests were used, and what the outcome and explanatory variables were (where appropriate). Indicate the level of significance used in tests if different from the conventional 2-sided 5% alpha error and whether or what type of adjustment is made for multiple comparisons.

The AJCN prefers replicate analyses. The Journal does not have a specific policy on simplicate sample analysis, but we will assess these on a case by case basis. Reviewers and Editors might disagree with the authors' approach, and we cannot guarantee the outcome. The Journal recommends strongly that at least a subset of samples be analyzed at least in duplicate in order that the degree of measurement error can be estimated. Furthermore, we refer the author to established approaches for dealing with such circumstances: Allison, D. B., Allison, R. L., Faith, M. S., Paultre, F., & Pi-Sunyer, F. X. (1997). Power and money: Designing statistically powerful studies while minimizing financial costs. *Psychological Methods*, 2(1), 20-33.

When data are summarized in the Results section, specify the statistical methods used to analyze them. Avoid nontechnical uses of technical statistical terms, such as

random (which implies a randomizing device), *normal*, *significant*, *correlation*, *sample*, and *parameter*. Define statistical terms, abbreviations, and symbols not listed under "Abbreviations for statistical terms" below. If there are 3 or more abbreviations used in the text, prepare an abbreviation footnote. The footnote should be associated with the first abbreviated term in the text and should be an alphabetized listing of all author-defined abbreviations and their definitions. Detailed statistical analyses, mathematical derivations, and the like may sometimes be suitably presented as one or more supplemental files.

Results

Present your results in a logical sequence in the text, tables, and figures. Do not present specifics of data more than once and do not duplicate data from tables or figures in the text; emphasize or summarize only important observations. Do not present data from individual subjects except for very compelling reasons. Report losses to observation (such as dropouts from a clinical trial). Use boldface for the first mention of each table or figure.

Discussion

The Discussion should not exceed 4 typewritten pages except in unusual circumstances as approved by the Editor. Emphasize concisely the important aspects of the study and the conclusions that follow from them. Do not repeat in detail data or other material given in the Introduction or Results. Include the implications of the findings and their limitations and relate the observations to other relevant studies. Link conclusions with the goals of the study and avoid unqualified statements and conclusions that are not completely supported by the data. Avoid claiming priority and alluding to work that has not been completed. State new hypotheses and recommendations when warranted by the results and label them clearly as such.

Acknowledgments

1) Acknowledge only persons who have made substantive contributions to the study. Authors are responsible for obtaining written permission from everyone acknowledged by name and for providing to the Editor a copy of the permission, if requested. 2) **Conflict of Interest (COI) Statement:** Authors must disclose any financial or personal relationships with the company or organization sponsoring the research at the time the research was done. Such relationships may include employment, sharing in a patent, serving on an advisory board or speakers' panel, or owning shares in the company. If an author or authors have no potential conflicts of interest, please state this. The COI Statement must include all authors. 3) **Authors' contributions to the manuscript** – Each author is required to list his or her contribution to the work, with a description of the contribution.

Please use the following descriptors: 1) designed research (project conception, development of overall research plan, and study oversight); 2) conducted research (hands-on conduct of the experiments and data collection); 3) provided essential reagents or provided essential materials (applies to authors who contributed by providing animals, constructs, databases, etc, necessary for the research); 4) analyzed data or performed statistical analysis; 5) wrote paper (only authors who made a major contribution); 6) had primary responsibility for final content; 7) other (use only if categories above are not applicable; describe briefly); 8) for single-authored papers, please state: The sole author had responsibility for all parts of the manuscript.

Please do not include "obtained funding" (the initials of authors who received grants may be included in the footnote regarding support on the manuscript's title page). Although not all manuscripts will necessarily include all descriptors, all manuscripts, including reviews, must indicate who is responsible for design, writing, and final content. An example of a properly formatted author contribution statement is as follows: "AX, RFG, and PGY designed research; RFG and QC conducted research; PT analyzed data; AX, PGY, and QC wrote the paper; PGY had primary responsibility for final content. All authors read and approved the final manuscript."

References

Number references consecutively in the order in which they are first mentioned in the text. For a standard journal article with more than 10 authors, please list first 10 authors before using “et al.”; list all authors when 10 or fewer. In the text, identify references by Arabic numerals in parentheses (1), not superscript. References cited in tables or in legends to figures should be numbered according to the first citation of the table or figure in the text. Supplemental Material should have a separate reference section.

It is rarely necessary to cite more than 50 references in an original research article. Try to avoid citing published abstracts as references [if a published abstract is cited, include “(abstr)” at the end of the reference]. Abstracts from scientific meetings not published in peer-reviewed journals may not be used as references. Unpublished observations and personal communications (written, not oral) may not be used as references but may be inserted in parentheses with the names of the responsible researchers and the year of the observation or communication. Authors are responsible for obtaining written permission from everyone so cited and for providing to the Editor a copy of the permission, if requested. Doctoral dissertations may be used as references. Include manuscripts accepted but not yet published; designate journal name followed by “(in press).” Report foreign titles in the original language, identify the language, and provide the English translation in parentheses. The references must be verified by the author against the original documents.

Journals

1) Journal article published electronically ahead of print: Authors may add to a reference, the DOI (“digital object identifier” number unique to the publication) for articles in press. It should be included immediately after the citation in the References.

Bergholdt HKM, Nordestgaard BG, Ellervik C. Milk intake is not associated with low risk of diabetes or overweight-obesity: a Mendelian randomization study in 97,811 Danish individuals. *Am J Clin Nutr* 2015 Jul 8 (Epub ahead of print; DOI: [doi:10.3945/ajcn.114.105049](https://doi.org/10.3945/ajcn.114.105049)).

2) Standard journal article: list all authors when 10 or fewer; when >10, list only the first 10 and add "et al." Abbreviate journal titles according to *Index Medicus* style, which is used in MEDLINE citations. Jeffery RW, Wing RR, Sherwood NE, Tate DF. Physical activity and weight loss: does prescribing higher physical activity goals improve outcome? *Am J Clin Nutr* 2003;78:684–9.

3) Corporate author

National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III). Third Report of the National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III) final report. *Circulation* 2002;106:3143–421.

Books and other monographs

4) Personal authors

Shils M, Shike M, Olson J, Ross AC. *Modern nutrition in health and disease*. 9th ed. Baltimore: Lippincott Williams & Wilkins, 1998.

5) Committee report or corporate author

National Research Council. *Recommended dietary allowances*. 10th ed. Washington, DC: National Academy Press, 1989.

Food and Nutrition Board, Institute of Medicine. *Dietary reference intakes for vitamin C, vitamin E, selenium and carotenoids*. Washington, DC: National Academy Press, 2000.

6) Chapter in book

Young VR, Tharakan JF. Nutritional essentiality of amino acids and amino acid requirements in healthy adults. 2nd. ed. In: Cynober LA, ed. *Metabolic and therapeutic aspects of amino acids in clinical nutrition*. Boca Raton, FL: CRC Press, 2004:439–70.

ANNEXURE E: CERTIFICATE OF THE LANGUAGE EDITING

Dr. JACKIE DE VOS**Academic copy editor / Akademiese teksredakteur**

BA (Psychology & Communication studies), BA(Hons (Psychology) (NWU)
 MEd, PhD (Educational Psychology) (NWU)
 BA(Hons (Translation) (UNISA)
 MA (Linguistics) (UNISA)
 BA (Sielkunde & Kommunikasie studies), BA(Hons (Sielkunde) (NWU)
 MEd, PhD (Opleedkundige Sielkunde) (NWU)
 BA(Hons (Vernakulêre) (UNISA)
 MA (Linguistiek) (UNISA)

**LANGUAGE EDITING**

8 November 2018

To whom it may concern

This letter serves to confirm that the following thesis (in article format) was edited (from January to November 2018):

“Dietary intake in relation to iron status in 5- to 12-year-old primary school children and estimated cost of a nutrient rich diet”

The onus rests on the client(s) to work through the proposed track changes and to accept or reject proposed changes. Clients should also ensure that all sources have been cited.

Yours sincerely,

Dr Jackie de Vos