The iron status of a healthy South African adult population

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Introduction: Iron deficiency is associated with significant morbidity and mortality, can present with or without haematological changes and is a major cause of microcytic anaemia. In South Africa and Africa in general, there is a paucity of studies on the iron status of healthy adult non pregnant females and males > 18 years of age. The aim of the study was to determine the prevalence of iron deficiency in a healthy South African population.

Methods: A total of 651 healthy adults > 18 years were included in the study. Blood samples were taken for the determination of iron status, haematological and inflammatory parameters. A ferritin level of < 30 μg/L was used to define iron deficiency and these subjects were further divided into those with and without anaemia. Diet and menstrual history in females was further investigated.

Results: Overall, the prevalence of anaemia was 12.6% and iron deficiency was found in 78% of anaemic subjects. The prevalence of iron deficiency was 39.8% in all participants and females and Black Africans had a very high prevalence of 56.6% and 50.7% respectively. Significant (p < 0.05) differences were found in concentrations of ferritin, haemoglobin, iron, transferrin, transferrin saturation, MCV and MCH between the groups.

Conclusion: Anaemia is a minor health problem but a large proportion of subjects with iron deficiency do not present with anaemia. The prevalence of iron deficiency was high especially in females and Black African participants.

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1. Introduction

Iron is an essential trace element that plays a crucial role in various metabolic functions. It is a structural component of haem-containing proteins like haemoglobin (Hb) and myoglobin which bind oxygen for delivery to the tissues. It is also involved in energy production, redox reactions and DNA synthesis. However, free iron is toxic and therefore it is tightly regulated by its binding and incorporation into proteins [1].

A reduction in the total body iron is referred to as iron deficiency (ID) and it can occur as a result of reduced intake, decreased absorption or increased losses of iron [2,3]. ID progresses through three stages namely depleted iron stores, iron deficient erythropoiesis and finally iron deficiency anaemia (IDA) [4]. In iron depletion, the total body iron is decreased but erythropoiesis is unaffected whereas in iron deficient erythropoiesis, due to insufficient supply of iron to erythroid tissues, erythropoiesis is affected. However, the Hb is still within normal limits and thus referred to as iron deficiency without anaemia (IDWA). The last and severe stage of ID presents with anaemia [5]. ID is the most common nutritional deficiency and the top ranking cause of anaemia in the world [6]. Children, pregnant and lactating women are considered at high risk because of the increased iron requirements. Non-pregnant women of reproductive age are also at risk of ID due to the loss of blood through menstruation [3].

In adults, anaemia is defined as an Hb concentration of below 13 g/dL in males and below 12 g/dL in non-pregnant females [7]. Anaemia is easy to diagnose, however to confirm that the anaemia is a result of ID can prove to be difficult. The gold standard for the diagnosis of ID is a bone marrow aspirate (BMA) which is highly specific but invasive and expensive [8]. Serum ferritin is the most specific non-invasive biochemical test to diagnose ID, as it reflects total body iron stores. A serum ferritin level below 12 μg/L is generally accepted as consistent with depleted iron stores [9]. However, Mast et al. found that the sensitivity and specificity of ferritin below 12 μg/L compared to BMA was 25% and 98% respectively. The sensitivity increased to 92% and the specificity remained the same when a cut-off of 30 μg/L was used [10]. Importantly, this cut-off may only be used in the absence of inflammation as ferritin is an acute phase protein and levels will be higher in the presence of...
inflammation [5,8]. The WHO and Centres for Disease Control and Prevention (CDC) Joint Report recommends that ferritin, Hb, mean cell volume (MCV), soluble transferrin receptors and zinc protoporphyrin are the best indicators of iron status in all populations. The performance of other tests such as iron, transferrin and transferrin saturation are improved when used in conjunction with the above tests [8].

Importantly, IDA is associated with significant morbidity and mortality as it can affect neurocognitive development and function, work productivity and even result in death [11]. In 2000, Nojilana et al. [12] estimated that 0.4% of all deaths in South Africa were a result of IDA. Various studies examined the prevalence of IDA in the South African population; however, these studies have been conducted mostly in children, pregnant women and the elderly population [12–15]. There is a paucity of studies on the iron status of healthy adult non pregnant females and males over 18 years of age [16–18]. Thus, the aim of this study was to determine the prevalence of iron deficiency in a healthy population representative of South Africa.

2. Methods

2.1. Study population

From a database of the ‘Establishing Adult Reference Intervals for Selected Analytes in South Africa’ study consisting of 1433 subjects, 651 adults meeting the inclusion criteria were included in our study. The study involved the recruitment of healthy individuals aged 18 years and older from the general public as well as students, hospital and laboratory staff. The protocol and standard operating procedures used in the main study are published elsewhere [19]. All participants completed a questionnaire and written informed consent was obtained. The questionnaire included the following: (i) demographic details i.e. age, gender and ethnicity; (ii) personal habits i.e. smoking and alcohol history; (iii) physical activity and exercise; (iv) dietary information; (v) current medication; (vi) menstrual history in females. A questionnaire and written informed consent was obtained. The questionnaire included the following: (i) demographic details i.e. age, gender and ethnicity; (ii) personal habits i.e. smoking and alcohol history; (iii) physical activity and exercise; (iv) dietary information; (v) current medication; (vi) menstrual history in females. The study was approved by the Health Research Ethics Committee of Stellenbosch University, Cape Town, South Africa (S12/05/147).

We excluded the following individuals: those not born in South Africa; with recent results pointing to a severe disease; a recent illness or hospitalisation 2 weeks prior to recruitment; who donated blood up to 3 months prior to recruitment; known carriers of Hepatitis B, Hepatitis C or Human Immunodeficiency Virus (HIV); pregnant or within one year of childbirth; and those who participated in a research study involving an investigational drug in the past 12 weeks. Additional exclusion criteria included: missing data for ferritin, iron, transferrin, transferrin saturation, highly sensitive C-reactive protein (hsCRP), Hb, MCV and mean cell haemoglobin (MCH); and participants with hsCRP concentrations ≥10 mg/L and WCC <11 (×10^9).

2.2. Biochemical analysis

Blood samples were collected, following a 12–14 h fast, after subjects were seated for 20–30 min. Biochemical parameters were analysed at an ISO 15189 accredited Pathology practice (PathCare Reference Laboratory, Cape Town, South Africa).

The measurement of serum ferritin was performed on the Beckman Coulter Access immunoassay system (Beckman Coulter Inc. Brea, CA, USA). The assay is a paramagnetic particle, chemiluminescent immunoassay and the calibrator is traceable to the World Health Organisation (WHO) 3rd International standard for ferritin (IS 94/572). The assay has a linear range of 0.2–1500 μg/L. Transferrin and hsCRP measurements were performed on the Beckman DXC automated analyser (Beckman Coulter Inc. Brea, CA, USA). Transferrin was measured in serum using an immunoturbidimetric method that utilises anti–human transferrin antibodies. The assay’s linear range is 0.75–7.5 g/L. Hs-CRP was determined in serum by an immunoturbidimetric assay utilising anti-CRP antibody coated latex particles with a linear range of 0.08–80 mg/L. Serum iron was measured using a colorimetric assay that utilises 2,4,6-tripyridyl-s-triazine (TPTZ) as a chromogen. TPTZ reacts with ferrous iron to form a blue coloured complex that is measured photometrically. The linear range of the assay is 2–179 μmol/L. Transferrin saturation was calculated according to the formula: % saturation = [iron / (transferrin × 25.6)] × 100.

The Beckman Coulter A+ T diff2 haematology analyser (Beckman Coulter Inc. Brea, CA, USA) was used for the determination of Hb, MCV, MCH and WCC using EDTA whole blood. This analyser is based on the Coulter principle of sizing and counting for quantitative determination of Hb, MCV, MCH and WCC.

2.3. Definitions & statistical analysis

Serum ferritin was used to define iron deficiency. A cut-off level of 30 μg/L for serum ferritin was chosen to distinguish between participants with ID (ferritin <30 μg/L) and those who are iron replete (IR) (ferritin >30 μg/L). For the diagnosis of anaemia, an Hb of <13 g/dL in males and <12 g/dL in females was used (WHO criteria). We used the laboratory reference ranges to define microcytic hypochromic anaemia as an Hb below cut-off for gender as well as an MCV <81 fl (microcytosis) and MCH <28 pg (hypochromia). Subjects with ID (ferritin <30 μg/L) were further subdivided into two groups namely IDA (Hb below cut-off for gender) and IDWA (Hb above cut-off for gender).

Data was analysed using Microsoft Excel® (Microsoft, Redmond, WA, USA) and SPSS® v20 statistical software (SPSS, Chicago, IL, USA). The Shapiro Wilk test was performed and non-parametric tests were applied as not all the data fitted a Gaussian distribution. Descriptive data is reported as median values and interquartile ranges (IQR). The Mann Whitney U test was used for comparisons between the groups. Statistically significant differences were indicated by p <0.05.

3. Results

3.1. Characteristics of study population

Of the 651 participants included, 410 (63%) were female and 241 (37%) male. The majority of participants were of Caucasian origin (55.5%) followed by Mixed-ancestry (33.9%) and Black African (10.6%). The age of all participants ranged from 18 to 76 years with a median age of 30 (22–44) years.

3.2. Prevalence of anaemia

The prevalence of anaemia in all participants was 12.6%. Female participants had a much higher prevalence of anaemia (18.3%) compared to 2.9% in male participants. Anaemia was more prevalent (31.9%) in Black African participants compared to 17.2% and 6.1% in the Mixed-ancestry and Caucasian respectively. Microcytic hypochromic anaemia was present in only 22 (26.8%) participants of which 20(91%) were female. However, ID (ferritin <30 μg/L) was found in 64 (78%) of the participants with anaemia. The cause of anaemia was not determined in the 18 (22%) subjects in the IR group (Table 1).

3.3. Iron status of study participants

We found a prevalence of 39.8% for ID in this study population. Black African participants had the highest prevalence of ID (50.7%) compared to 43.4% and 35.5% in the Mixed ancestry and Caucasian participants respectively (Table 1). Table 2 shows the medians and IQRs of iron status and haematological parameters. There was a statistically significant (p <0.001) difference in the concentrations of ferritin, transferrin, iron, transferrin saturation, Hb, MCV and MCH between the ID and IR groups (Table 2).
ID was more prevalent in female participants (56.6%) compared to males (11.2%). The median age in the ID group was 26 (21–36) years (Fig. 1) and these women had a lower median ferritin concentration of 43 (28–54) μg/L (Fig. 2) compared to the IR group with a median age and ferritin of 61 (40–100) μg/L (Fig. 2) respectively. In addition, the Hb in the ID group was lower than the IR group [12.5 (11.9–13) μg/dL] (Fig. 2) compared to the IR group with a median age and MCH concentrations (Table 3).

### 3.4. Prevalence of IDA

Overall, the prevalence of IDA in this population was 9.8% (Table 1). Almost all (97%) of the participants with IDA were female with an IDA prevalence of 15.1%. Black African participants had the highest prevalence of IDA (26.1%) compared to 14% and 4.2% in the Mixed ancestry and Caucasians respectively. A statistically significant difference (p < 0.001) was observed when Black Africans were compared with Caucasians and Mixed ancestry participants with Caucasians (Table 1). The median ferritin and Hb concentrations were significantly lower in the IDA group compared to the IDWA group. A statistically significant difference (p < 0.05) was also observed between the IDA and IDWA groups for iron, transferrin, transferrin saturation, MCV and MCH concentrations (Table 3).

#### 3.5. Diet and menstrual history in females

Most (34.1%) of the women in the ID group consumed meat 1–2 times a week whereas in the IR group (39.9%) consumed meat 3–4 times a week. There were also twice as many women in the ID group who do not eat meat (5.6% vs. 2.8%) and fish (12.5% vs. 5.6%) compared to the IR group. There were more women (44.9%) that consumed vegetables every day in the IR group compared to the ID group (11.6%).

The questionnaire was used to determine if women had regular/irregular cycles or in menopause as well as the use of hormonal therapy. More women with ID were still menstruating compared to those with normal iron stores. Most (53.9%) of the women with ID reported regular menstrual cycles, 14.7% had irregular cycles, 21.1% were on oral or depot contraceptives and 8.2% were menopausal. In the IR group 30.3% had regular cycles, 5.6% irregular cycles, 34.8% on contraceptives and 11.6% were menopausal.

#### 4. Discussion

As data on iron status in South African adults is lacking, the purpose of this study was to determine the iron status of relatively healthy South African adults participating in the ‘Establishing Adult Reference Intervals for Selected Analytes in South Africa’ study. The prevalence of 12.6% for anaemia found in this population is considered a minor public health problem according to the WHO/CDC classification [5]. This prevalence is much lower than the 17.5% found in the South African National Health and Nutrition Examination Survey (SANHANES) performed in 2012 [16]. Similar to the SANHANES study, we found a higher prevalence of anaemia in females compared to males. In the SANHANES study the adult population was defined as subjects older than 15 years, whereas in our study the participants were older than 18 years. Additionally, we followed a strict recruitment strategy to ensure a healthy population was included; however the same does not apply to the SANHANES study where all subjects, irrespective of their health status, were eligible for inclusion in the study.

There is a lack of reliable epidemiological data for ID because its prevalence is often predicted using the prevalence of anaemia [8,20]. The WHO estimates that if the prevalence of IDA in the population evaluated is >20% then ID is present in 50% of the population [20]. In our study the prevalence of IDA in females was 15.1%, however ID was present in 56.6% of females. This demonstrates that using the WHO estimate would have resulted in an underestimation of the prevalence of ID in female participants. In a South African study of urban non-pregnant adult

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**Table 1**

Iron status in different ethnic groups.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Total (n = 651)</th>
<th>African (n = 69)</th>
<th>Mixed ancestry (n = 221)</th>
<th>Caucasian (n = 361)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Iron deficiency, n(%)</td>
<td>64(9.8)</td>
<td>18(26.1)</td>
<td>31(14)</td>
<td>15(4.2)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Iron replete, n(%)</td>
<td>18(2.8)</td>
<td>4(5.8)</td>
<td>7(3.2)</td>
<td>7(1.9)</td>
<td>NS</td>
</tr>
<tr>
<td>All participants, n(%)</td>
<td>82(12.6)</td>
<td>22(31.9)</td>
<td>38(17.2)</td>
<td>22(6.1)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>339(93.9)</td>
<td></td>
</tr>
</tbody>
</table>

ac, significant difference between African and Caucasian; bc, difference between Mixed ancestry and Caucasian; NS no significant difference.

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**Table 2**

Iron status and haematological parameter medians (IQR).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Iron deficient (n = 259)</th>
<th>Iron replete (n = 392)</th>
<th>Abnormal range</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ferritin (μg/L)</td>
<td>14(8–21)</td>
<td>83(50–131)</td>
<td>&lt;30</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Transferrin (g/L)</td>
<td>2.9(2.7–3.3)</td>
<td>2.5(2.2–2.8)</td>
<td>&lt;3.6</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Iron (μmol/L)</td>
<td>1.37(1.5–1.84)</td>
<td>1.8(1.46–2.24)</td>
<td>&lt;9 (F)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Transferrin saturation (%)</td>
<td>19(13–26)</td>
<td>28(22–36)</td>
<td>&lt;20</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Hb (g/dL)</td>
<td>12(12–13.2)</td>
<td>13.3(12.8–13.8)</td>
<td>&lt;12 (F)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>MCV</td>
<td>88(84–91)</td>
<td>91(88–94)</td>
<td>&gt;81</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>MCH (μg)</td>
<td>30(28–31)</td>
<td>31(30–32)</td>
<td>&gt;28</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

IQR = interquartile range.

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**Fig. 1.** Box plot illustrating the age of female participants in the ID and IR groups (p < 0.001).
female health care workers, 10% were found to have possible IDA based on the presence of microcytic hypochromic anaemia [18]. Our population was also recruited from urban areas and consisted of students, health care workers as well healthy volunteers from the general population. If we had used a similar approach to Lawrie et al., the estimated prevalence of IDA would be 3.4% in all participants and 4.9% in females. Interestingly, microcytic hypochromic anaemia was found in the same female health care workers, 10% were found to have possible IDA based on our criteria. It is clear that the use of haematological parameters alone does not provide a reliable assessment of iron status in females. We, however, cannot draw any conclusions for men because of the very small number of male participants with IDA (0.8%) in our study.

Microcytic anaemia is not specific for ID and is also found in patients with anaemia of inflammation, thalassaemias and sideroblastic anaemia [21]. Haematological parameters in combination with biochemical tests such as serum ferritin and transferrin saturation provide an accurate assessment of the iron status [8,22]. In the SANHANES study, the prevalence of iron deficiency, as determined by ferritin <15 μg/L, and IDA in women aged 16–35 years, was 15.3% and 9.7% respectively; however, there is no available data for the prevalence of ID in men [16]. Although it seems that our prevalence of ID and IDA is higher than reported in the SANHANES, we used a higher cut-off for ferritin (30 μg/L) in our study. Secondly, we did not categorize participants into age groups and thus our prevalence is for female participants of all ages. Lastly, our exclusion criteria reduced the effect of inflammation on ferritin and therefore our findings are a more reliable indication of ID compared to SANHANES where subjects with chronic diseases and inflammation may have been misclassified.

Wolmarans et al. [17] found that 27.4% had low iron status in a study conducted in non-pregnant adult South African women 18–55 years of age. This prevalence is lower than our study; however, some of the women may have been missclassified due to the presence of infection. CRP was elevated in 16% of women and almost half of them reported a cold at the time of blood collection [17]. Although we excluded participants with a recent illness and a CRP concentration >10 mg/L, the possibility of misclassification of participants still exists.

An elevated serum ferritin is found in inflammatory states which includes subjects with mixed anaemia i.e. anaemia of chronic disease (ACD) and IDA; therefore, a higher cut-off should be used for diagnosing ID. Ferritin concentrations of up 100 μg/L are considered diagnostic of ID in inflammatory states [3,23]. An alternative marker such as soluble transferrin receptors (sTfR) may be of clinical value in inflammatory states. The concentration of sTfR is increased in ID but it is not affected by inflammation [24,25]. However, elevated sTfR concentrations can be found in conditions with increased erythropoiesis such as haemolytic anaemia [26]. Although sTfR is considered highly sensitive for ID, ferritin is considered to be more specific [10,25]. Current available ferritin assays are traceable to the high order WHO international internal standard and this can allow the use of the same cut-offs and comparisons between different studies. In contrast the lack of standardisation of sTfR assays has led to variable cut-offs and limited comparisons between studies [25,26]. In addition, the lack of available routine automated assays and the cost of sTfR prevent its use in resource limited settings especially in Africa [24]. The expression of hepcidin, a peptide hormone that regulates cellular iron export, is increased in inflammation and decreased in ID [27,28]. The measurement of hepcidin may be helpful in inflammatory conditions; however, its use is limited by the lack of available routine and standardised assays [3]. In our setting and throughout Africa, the cost of hepcidin assay would be prohibitive.

There are two forms of iron, haem and non-haem, which are obtained from the diet. Haem iron, which is well absorbed, is an excellent

### Table 3

<table>
<thead>
<tr>
<th>Parameter</th>
<th>IDA (n = 64)</th>
<th>IDWA (n = 195)</th>
<th>Abnormal range</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ferritin (μg/L)</td>
<td>8(4–14)</td>
<td>17(11–23)</td>
<td>&lt;30</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Transferrin (g/L)</td>
<td>3.2(2.9–3.5)</td>
<td>2.9(2.7–3.1)</td>
<td>&gt;3.6</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Iron (μmol/L)</td>
<td>8.8(5.6–13.2)</td>
<td>15.4(11.3–19.1)</td>
<td>&lt;9 (F)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Transferrin saturation (%)</td>
<td>12(7–15)</td>
<td>20(15–27)</td>
<td>&gt;20</td>
<td>0.007</td>
</tr>
<tr>
<td>Hb (g/dL)</td>
<td>11.4(10.9–11.6)</td>
<td>12.9(12.4–13.5)</td>
<td>&lt;12 (F)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>MCV (fl)</td>
<td>83(79–87)</td>
<td>89(87–92)</td>
<td>&gt;81</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>MCH (pg)</td>
<td>27(26–29)</td>
<td>30(29–31)</td>
<td>&gt;28</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

IQR = interquartile range; IDA = iron deficiency anaemia; IDWA = iron deficiency with anaemia.
source of iron found in red meat, fish and poultry whereas non-haem iron found in vegetables and iron fortified foods is inefficiently absorbed [27]. It is not surprising that ID was more prevalent in women who consumed less meat and fish. The women with ID were also much younger and the majority were still menstruating compared to those with IR stores. Iron losses are more in women of reproductive age because of menstruation and hence they require more iron than postmenopausal women and men.

Recent research has shown that there are inter-ethnic variations in the genes involved in iron metabolism which are associated with a low iron status and anaemia. However these studies have been conducted mostly in European and Asian populations [29]. To our knowledge there is only one study conducted in South Africa on Black African females that has explored the association between genetic variants from genome wide association studies and low iron status. This study found that there was no association between the previously described variants and low iron status in Black African females [30]. Black African participants in our study had a higher prevalence of ID and IDA compared to other ethnic groups. We cannot explain if this is attributed to dietary and environmental factors or inter-ethnic genetic variations. This highlights the need for further genomic studies in the South African population to identify the inter-ethnic genetic variations associated with a low iron status.

Our study has some limitations. Due to the small number of African participants, we cannot draw any conclusions regarding the differences observed between the ethnic groups. We also did not determine if these differences are due to inter-ethnic variations of genes involved in iron homeostasis, which may have affected the results. Additionally, we did not compare our findings with BMA which is considered the gold standard for ID. We also did not measure sTfR which may have allowed us to include the participants with CRP concentrations > 10 mg/L. Finally, we did not examine the association between ID and alcohol as well as BMI. However we had numerous strengths, the most important being the recruitment of healthy participants using very strict criteria. We also excluded subjects with possible infection and evaluated dietary and menstrual patterns. Also, to our knowledge, this is the first South African study looking at the iron status of healthy males.

5. Conclusion

We evaluated a healthy South African adult population and using a cut-off of ferritin < 30 μg/L we found a 39.8% prevalence of ID. Although we found a higher prevalence than previous studies comparisons cannot be made because of differences in the population studied and study design. In addition, we examined an urban population and these findings may be different in a rural population. The use of haematological parameters as screening tests for ID is flawed and will result in a gross underestimation of the problem because the proportion of subjects with ID that have anaemia is rather small. In addition, IDA is the last and severe stage of ID and it contributes significantly to the burden of disease and mortality. ID was a bigger problem in females and this highlights the need for health care providers to increase awareness and in selected cases screen by measuring serum ferritin. This approach will allow for early detection of subjects with ID and may help to decrease the prevalence of IDA found in pregnant women. The importance of dietary iron cannot be ignored; we demonstrated that women with ID consumed less iron rich foods. ID is a preventable problem and in our setting a feasible intervention is to increase awareness amongst health care workers and provision of focused nutritional education at each health visit especially at primary health care level. There is a need for further studies which are more representative of the South African population at large.

Conflict of interest

None.

Authorship.

All authors have made substantial contributions to the following: (1) the conception and design of the study, acquisition of data, analysis and interpretation of data, (2) drafting of the article and revising it critically for intellectual content, (3) final approval of submitted version.

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