Determinants of Anaemia Among Women of Reproductive Age In South Africa: A Healthy Life Trajectories Initiative (HeLTI)

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Abstract

Background – Anaemia continues to be a major public health problem among women of reproductive age (WRA). A thorough understanding of anaemia risk factors is necessary to design better interventions. This paper examines the determinants of anaemia among WRA in South Africa.

Methods- We included baseline data from 480 women participating in the pilot-phase of a randomized controlled trial (HeLTI). We measured haemoglobin (Hb) status using the Hemocue. Plasma iron status markers (ferritin and soluble transferrin receptor (sTfR)), markers of inflammation (C-reactive protein (CRP)) and alpha-1-acid glycoprotein (AGP)) and retinol binding protein (RBP) were assessed using the multiplex method. We used multivariate logistic regression to describe associations with anaemia and structural equation modelling (SEM) to characterise direct and indirect pathways influencing haemoglobin concentrations.

Results- The prevalence of anaemia, iron deficiency (ID), and iron deficiency anaemia (IDA) was 39.4%, 38.1% and 21.6% respectively. The multiple logistic regression showed that ID (OR: 2.62, 95% CI: 1.72, 3.98), iron deficiency erythropoiesis (IDE) (OR: 1.62, 95% CI: 1.07, 2.46), and elevated CRP (OR: 1.69, 95% CI: 1.04, 2.76), increased the odds of being anaemic. SEM analysis revealed Hb was directly and positively associated with adjusted ferritin (0.0031 per mg/dl; $p \leq 0.001$), and CRP (0.015 per mg/dl; $p \leq 0.05$), and directly and negatively associated with soluble transferrin receptor sTfR (-0.042 per mg/dl; $p \leq 0.001$). While contraception use had both a direct (0.34; $p \leq 0.05$) and indirect (0.11; $p \leq 0.01$) positive association with Hb. Additionally, chicken and beef consumption had a positive indirect association with Hb concentrations (0.15; $p \leq 0.05$) through adjusted ferritin.

Conclusion-A key driver of anaemia in our setting is ID, however the presence of inflammation also increases the risk of anaemia. To address anaemia, interventions should aim to improve the diet quality of women, in particular access to iron rich foods. We recommend the use of multi-micronutrient supplements with a lower dose of iron and other micronutrients which would ensure that women receive the same benefits as with iron folic acid, while alleviating anaemia of inflammation.

Background

Anaemia is a major public health concern among women of reproductive age (WRA) (1). In WRA, anaemia may result in reduced work capacity and predisposes an individual to various infections (2). Anaemia in pregnancy has been linked to an increase in low birth weight, perinatal and neonatal mortality, and preterm birth (3). Moreover, anaemia in pregnancy has also been associated with increased maternal mortality (4). The World Health Organisation estimates that anaemia affects approximately 42% of pregnant women and 29.4% of non-pregnant WRA worldwide (5).

South Africa has policies in place to address anaemia and iron deficiency (ID); such as the mandatory iron fortification of maize meal and wheat flour and the *Guidelines for maternity care in South Africa* recommendation for daily iron supplementation in pregnancy (6, 7). However, the prevalence of anaemia and ID remains high. In the 2012 South African National Health and Nutrition Survey conducted among WRA (15–35 years) the prevalence of anaemia, ID, and iron deficiency anaemia (IDA) was 23%, 16% and 10%

respectively. According to the 2016 South African Demographic Health Survey, 31% of WRA (15–49 years) are anaemic (8). The above national data in conjunction with a systematic review (8) point to the fact that approximately 23%-33% of South African WRA are likely to enter pregnancy with anaemia (9).

The aetiology of anaemia is complex. The biological causes for anaemia as outlined by the WHO framework ((10) include iron, other micronutrient deficiencies (vitamin A, vitamin B12 and folate) and genetic disorders (11). Vitamin A has been proposed to play a role in regulating plasma iron levels, with decreasing vitamin A levels resulting in decreasing plasma iron (12–14). The presence of inflammation in an individual may also result in anaemia by causing poor iron absorption, loss of erythrocytes and/or ineffective erythropoiesis(15, 16). ID has long since been identified as the major contributor anaemia in most settings. It is estimated that ID contributes to at least half of all anaemia cases (17). As a result, and in the absence of individual data on iron status, the prevention and treatment for populations at risk of anaemia is typically directed at programs to improve iron status. However, intervening with routine iron supplementation may result in potential adverse effects in settings where individuals may already be iron replete and/or with heavy burdens of infectious disease, whereby iron supplementation may result in severe aggravation of infections (9) (18). Additionally, Symington et al in a study done in South Africa reported that iron supplementation alone may not be effective in reducing the prevalence of anaemia(9).

Furthermore, studies have highlighted other significant underlying risk factors for anaemia (10), that are likely to contribute by either decreasing the availability and consumption of micronutrient rich foods or by increasing the risk of infectious diseases (11). These are social and environmental risk factors such as socioeconomic status, food insecurity, education and poor sanitation (16). In addition, substantial to the risk of anaemia are individual characteristics in WRA such as women's use of hormonal contraception and body mass index (19). Several studies have shown that adiposity is associated with increased odds of inflammation (20–22). This obesity-related inflammation has been shown to reduce iron absorption from the gut, contributing towards ID. It is the complexity of addressing all risk factors that remains challenging, at least for now, in our efforts to curb anaemia and other micronutrient deficiencies at a public health level.

Data on the risk factors for anaemia among non-pregnant WRA remain sparse in South Africa(23). National data on dietary-related and sociodemographic risk factors for anaemia, ID and IDA were investigated by Mchiza et al using the South African National Health and Nutrition Examination Survey (SANHANES-1) (23), but did not include relevant biodemographic factors such as contraception use or parity. Moreover, iron status was not adjusted for inflammation, neither did they explore the complex interrelationships between anaemia, ID, biodemographic factors, inflammation factors, socioeconomic factors, and dietary factors. Nevertheless, for South Africa to achieve the World Health Nutrition Target of a 50% reduction in anaemia by 2025, further evidence is needed on the specific risk factors for anaemia among non-pregnant WRA within this setting. Therefore, the aim of this paper was two-fold: (i) to determine the prevalence of ID and anaemia in young women; and (ii) identify factors associated with anaemia and determine the direct and indirect effects of inflammation, socioeconomic, biodemographic and nutritional factors on anaemia.

Methods

Study design, setting and population

The South African data used in the study is from the pilot baseline study of the HeLTI trial (Healthy Lives Trajectories Initiative), a multinational trial which aims to develop and evaluate an integrated continuum of care (4 phases) starting preconception and extending through pregnancy, infancy, and childhood. The goal is to optimise women's physical and mental health, reduce childhood obesity, the risk of non-communicable diseases, as well as improve child development (24). The methods used in this study have been previously described in detail (25). Data was collected from the pilot phase of the main trial and data collection took place (June 2018 to July 2019) at the South African Medical Research Council (SAMRC) Developmental Pathways to Health Research Unit (DPHRU), located within the Chris Hani Baragwanath Academic Hospital (CHBAH), a tertiary hospital in Soweto (Southwestern Township). Generally healthy, non-pregnant WRA of African descent were recruited from Soweto, a historically disadvantaged urban area of 200 km² in the city of Johannesburg, Gauteng province. Women were eligible for inclusion if they were aged 18–26 years; proficient in local languages and if they had been residing in their home in Soweto for at least 3 months. Exclusion criteria were diagnosis of type-1 diabetes; cancer or epilepsy; or not able or willing to provide written informed consent. Due to South Africa's high prevalence of human immune virus (HIV) infection (23.2% of women aged 15–49 years (26), women who were HIV positive were included in the study for the sample to be a better representation of the general population. As a result, HIV was self-reported and CD4 and viral load were not assessed.

Data collection

Haematological biomarkers

Venous blood samples were analysed for iron status indices ferritin and soluble transferrin receptor (sTfR) vitamin A status (retinol binding protein, (RBP)) and the inflammation/infection markers C-reactive protein (CRP) and alpha-1-acid glycoprotein (AGP) using the Q-Plex™ Human Micronutrient Array (7-plex, Quansys Bioscience, Logan, UT, USA) (25) (27). Our outcome variable of interest, ferritin concentration was adjusted for inflammation as measured via AGP and CRP concentrations using the correction factors proposed by Thurnham et al. (7). Participants were categorised as being iron deficient if their inflammation-adjusted plasma ferritin concentration was < 15 µg/L as recommended by the WHO (28).

Haemoglobin concentrations were measured from capillary blood using a calibrated Hb 201 + HemoCue® system (HemoCue Johannesburg, South Africa). Hb values were adjusted for altitude and the point-of-care cut-off Hb < 12 g/dL was used to diagnose anaemia (29). IDA was defined as ferritin < 15 µg/L and Hb < 12g/dl. Iron deficient erythropoiesis (IDE) was defined as sTfR ≥ 8.3 mg/L. As plasma RBP is suppressed in the presence of inflammation, RBP was also adjusted for inflammation using correction factors proposed by Thurnham et al. (7).

Physical measurements

Weight (kg) and height (cm) of participants were measured to calculate body mass index: BMI = weight (kg)/(height [m])². Weight was measured to the nearest 100g and height to the nearest 0.1cm. Mid-upper arm circumference (MUAC) was measured to the nearest 0.1cm using a plastic measuring tape. Measurement was taken at the mid-point of the upper arm, between the acromion process and the tip of the olecranon. A MUAC ≤ 24cm was used to define undernutrition [26].

Living conditions

Surveys conducted to assess sociodemographic, bio-demographic and nutrition factors were 1. sociodemographic to assess education and employment status; 2. general health which included medical and reproductive history, HIV status, tobacco, and alcohol use; 3. food insecurity and a frequency food questionnaire. Field teams visited household to assess and record the household type of residence, household density and the number of household assets. The following surveys were used: the United States (US) Centre for Disease Control (CDC) Global Adult Tobacco Survey (30), the WHO Alcohol Use Disorders (WHO-AUDIT) Test (31), and food insecurity was assessed using an adapted Community Childhood Hunger Identification Project (CCHIP) index (32).

Statistical analysis

Study data were collected and managed using REDCap electronic data capture tools hosted at the University of Witwatersrand (33). Data were tested for normality by visual inspection of Q-Q plots and histograms, and the Shapiro–Wilk test. Normally distributed data are expressed as means $\pm$ SD; non-normally distributed data are expressed as medians (interquartile range [IQR]). Descriptive analyses were used to report socioeconomic, bio-demographic, inflammation, and nutritional characteristics of the study sample. Bivariate associations between anaemia and socioeconomic, bio-demographic, inflammation and nutrition were examined. Multivariate logistic regression was used to examine the factors associated with anaemia and inflammation adjusted ID. Models were constructed with the use of block stepwise regression whereby variables were entered into the model in blocks in order of anticipated importance, WHO framework and the author's judgement. None of the included variables showed multicollinearity, with variance inflation factors < 2 for each model.

Structural equation modelling was applied to examine the specific causal models and assess the comparative strength of direct and indirect relationship among independent variables with ID and anaemia (haemoglobin concentration). SEM was the analysis of choice as it allows for a pictographic representation of hypothesis-driven relationships between variables such as potential mediators, confounders, and latent variables (34). The multivariable analyses were guided by an *a priori* model (Fig. 1 &2), based on expert knowledge, literature, and results from the logistic regression. We then hypothesised relationships *a priori* among the variables. From this framework SEM was used to estimate the associations in the different pathways between biodemographic factors, inflammation factors, sociodemographic factors, socioeconomic, nutritional factors and the two outcomes anaemia and ID. Direct, indirect, and total effects were calculated using non-linear combination estimates. To evaluate the best fitting model for our data, we reported goodness of fit indices including root mean squared error of approximation (RMSEA), comparative fit index (CFI), Tucker-Lewis index (TLI), and standardized root mean squared residual (SRMR). All data analyses were performed using Stata statistical software, version 14.1 (StataCorp, College Station, TX: Stata Corporation).

Results

Characteristics of women

The characteristics of women in the study sample are presented in Table 1. The median age of the participants was 21 years (IQR 19-23). Half the women were nulliparous and the median age at first delivery for women with children was 19 years (IQR 17-21). Most of the women had a medium household asset score. More than half the women (67.5%) reported consuming chicken and beef at least once a week. Inflammation was present in 37.3% of the women. Mean haemoglobin concentration was 12.2 g/dl and anaemia was present in 39.4% of the women. The prevalence of ID and IDA was 38.1% and 21.6% respectively. Vitamin A deficiency based on RBP was observed in 3.5% of the participants.

Multivariate analysis

Table 2 depicts the results of a multivariable logistic regression model for factors associated with anaemia. The results show three risk factors for anaemia among WRA: elevated CRP levels (inflammation indicator), IDE and ID. Women with elevated CRP had 1.69 greater odds for anaemia, compared to their counterparts (odds ratio (OR):1.69, 95% confidence interval (CI): 1.04, 2.76; $p \leq 0.05$). In comparison to non-ID women, women who were ID were 2.62 times more likely to be anaemic (OR: 2.62, 95% CI: 1.72, 3.98; $p \leq 0.001$), while compared to their non- IDE counterparts, women who had IDE were 1.62 times more likely to be anaemic (OR:1.62, 95% CI: 1.07,2.46; $p \leq 0.05$).

Table 3 shows multivariable regression analyses for factors associated with ID. Vitamin A deficient women were more than eight times more likely to have ID (OR:8.75, 95% CI: 2.62, 28.98; $p < 0.001$), while IDE women were 5.41 times more likely to have ID (OR:5.41, 95% CI: 3.52, 8.33; $p < 0.001$). In comparison to their counterparts, women who used contraception were 0.61 times less likely to be ID (OR:0.61, 95% CI: 0.38, 0.98; $p \leq 0.05$), and women who were multiparous were 0.36 times less likely to be ID (OR:0.36, 95% CI: 0.16, 0.81; $p \leq 0.014$). Compared to women who did not consume chicken and beef monthly, those who consumed chicken and beef monthly were 0.56 less likely to have ID (OR:0.56, 95% CI: 0.36,0.88; $p \leq 0.001$).

Structural Equation Modeling

Results from the SEM analyses for the association between biodemographic, inflammation, socio-economic and dietary variables with anaemia are shown in Table 4 and figure 1. The main factors associated with Hb levels were use of contraception, corrected ferritin, weekly chicken, and beef consumption and sTfR levels. Corrected ferritin had a positive direct and total association with Hb ($p \leq 0.001$), sTfR also had a negative direct and total association with Hb concentration ($p \leq 0.001$). Chicken and beef consumption had an indirect positive association with Hb ($p \leq 0.05$), mediated by adjusted ferritin ($p \leq 0.05$). Contraception use had a positive direct ($p \leq 0.05$ and total ($p \leq 0.05$) association with Hb as well as an indirect association with Hb ($p \leq 0.001$), mediated by adjusted ferritin. CRP had a significant negative direct and total association with Hb ($p \leq 0.05$), while the indirect association was insignificant ($p = 0.872$). Furthermore, BMI was directly associated with CRP ($p \leq 0.001$) as well as directly associated with parity ($p \leq 0.001$). A direct positive association was also observed between contraception use and parity ($p \leq 0.001$). As indicated by the fit statistics below on table 4 we infer that our hypothesized model fits the data.

The main factors associated with ferritin levels are depicted in table 5 and figure 2. RBP had a direct and total association with corrected ferritin ($p \leq 0.001$). Contraception use had a positive direct association with ferritin levels ($p \leq 0.001$) and an indirect association with ferritin levels through a pathway mediated by parity

($p \leq 0.001$) and RBP ($p \leq 0.05$). The total association of contraception use on ferritin was significant ($p \leq 0.001$). While parity had a positive indirect ($p \leq 0.05$) association with ferritin through a pathway mediated by RBP ($p \leq 0.05$). Additionally, contraception use was positively associated with parity ($p \leq 0.001$) and BMI was positively associated with CRP ($p \leq 0.001$) and parity ($p \leq 0.05$). Parity had a positive and direct association with RBP ($p \leq 0.05$), and contraception use had a positive and direct association with RBP ($p \leq 0.05$) through a pathway mediated by parity ($p \leq 0.001$). As indicated by the fit statistics below on table 5, we infer that our hypothesized model fits the data.

Discussion

We carried out this study to understand the influence (as well as the interactions) of socioeconomic, biodemographic, nutritional and inflammation factors on anaemia among non-pregnant WRA in South Africa. We found that the prevalence of anaemia, ID and IDA was 39.4%, 38.1% and 21.6% respectively. From this it is evident that ID is the main contributor to anaemia, as 54.8% of anaemia is a result of ID. This result agrees with the established approximation that 50% of anaemia cases are due to ID. This approximation has been supported by studies by Stoltzfus et al and Stevens et al, who showed that approximately 50% of anaemia was amenable to iron supplementation (4, 35). Moreover, recent data from a meta-analysis on iron fortification concluded that ID was the main contributor to anaemia in many geographical settings(36).

However, some studies have shown that in areas of high infection burden, inflammation is the main contributor to anaemia (37, 38). For example a study done in Sierra Leone, another Sub Saharan country with high inflammation, showed that 45% of non-pregnant WRA were anaemic but the driver of anaemia was not ID but inflammation(39). Like Sierra Leone, South Africa is grappling with a high infection burden because of the tuberculosis/HIV pandemic that is concurrently occurring with a rise in non-communicable disease and obesity (40–42). Confirming this, 37.3% of the WRA in our study had elevated inflammation. We, therefore, expected the prevalence of IDA to be lower and for anaemia of inflammation to play a more dominant role. In contrast, our results show ID was the major contributor to anaemia. Further analysis with multivariate regression model confirmed that IDE and ID were significant risk factors for anaemia. SEM results also showed that low iron stores were associated with low haemoglobin levels. However, the multivariate logistic results revealed that the presence of inflammation, denoted by CRP, also significantly increased the risk of being anaemic. Finally, the SEM analysis showed a direct association between elevated CRP and haemoglobin concentration. This indicates that although ID is the key driver, anaemia of inflammation is present. An understanding of the proportion of anaemia that is attributable to iron deficiency and the proportion attributable to inflammation is particularly important to the design and implementation of public health programs. Therefore, in our setting, it may be prudent to replace IFA supplements with MMS that contains a lower dose of iron. This would ensure that women would get the same benefits as with IFA in reducing IDA, as well as additional benefits of alleviating anaemia of inflammation (43).

The SEM results also revealed that contraception use was directly associated with higher haemoglobin concentrations and indirectly affected haemoglobin levels through its protective effect on ferritin levels. Women who used contraception in our study had higher ferritin levels. It is important to note that the majority of the WRA in our study were using hormonal contraceptives and hormonal contraception use is reported to cause less bleeding during menstruation, which in turn results in less blood being lost in menstruation (44,

45). Supporting this, a study conducted in multiple countries reported that those who used hormonal contraceptives had higher ferritin and Hb levels than those who did not(46). Furthermore, studies done in Tanzania and Ethiopia and other low- and middle-income countries showed that hormonal contraceptive use was associated with reduced risk in anaemia(47–49). Not surprisingly, contraception use in our study was higher among women who had more children, resulting in these multiparous women being protected against ID.

It is well established that anaemia and ID is patterned by socioeconomic status, with the poor being the most affected. Research points to the fact that improved socioeconomic status is linked to improved nutrition conditions(50). Our SEM analyses showed that weekly chicken and beef consumption (which is high in bioavailable iron) was indirectly protective against anaemia through its positive association with ferritin. Moreover, those who consumed chicken and beef weekly were less likely to be ID. Research has shown that many young South Africans are unemployed and do not consume beef as the cost of beef is high and often prohibitive (36), the economically disadvantaged in the population tend to consume less meat and if they do buy meat they purchase fat portions(51). Mchiza et al reported that those who consumed fat portions of meat were at increased risk of anaemia(47). It is therefore prudent that when developing interventions, we consider programs that aim to empower WRA economically to improve ID status and therefore anaemia in our context.

Our SEM analyses further revealed that low vitamin A stores are directly associated with low iron levels. Vitamin A deficiency was associated with a more than eight-fold increased risk in being ID. Studies in many parts of the world have shown that, because of generally poor diets ID co-exists with other micronutrient deficiencies, which could be the case in our low resource setting(12, 52). Nonetheless, a physiological interdependence between vitamin A deficiency and low iron status has been documented (53). Vitamin A has been proposed to play a role in regulating plasma iron levels, with decreasing vitamin A levels resulting in decreasing plasma iron (12–14). Some studies have shown that combined vitamin A and iron supplementation was effective in improving ID(14, 54–58) 71–78). Hence, IFA supplementation alone, may not be adequate to deal with anaemia and ID in WRA (59). Acknowledging this, the WHO has suggested that in areas of high prevalence of nutritional deficiencies MMS should replace IFA (60). Hence, as previously mentioned, administering MMS, which contains a lower dose of iron plus vitamin A (plus other micronutrients) instead of the traditional IFA could be more beneficial.

From our SEM analysis we see women with high a BMI are more likely to have elevated inflammation. Several studies have shown that adiposity is associated with increased odds of inflammation (20–22). In a nine country study, in seven countries obesity was consistently associated with elevated CRP (20). Furthermore, obesity is a state of low-grade systemic inflammation reflected by increased concentrations of pro-inflammatory cytokines and inflammatory markers (61). This obesity-related inflammation has been shown to reduce iron absorption from the gut, contributing towards ID. Several epidemiological studies have reported increased risk for ID in overweight and obese subjects (62–64) (65–68) (69, 70). However, our SEM analyses show no significant direct or indirect association of obesity with ID.

Our results show a relationship with BMI and parity with high BMI being associated with high parity; and women with higher parity being more likely to have higher ferritin and higher RBP levels. Research done by Abrams et al showed that child bearing is associated with weight gain (71). During pregnancy women gain

weight and adopt eating habits which they are unable to lose postpartum (72–74). This observation may indicate that the diet of multiparous WRA in our study could be richer in vitamin A and iron than that of their normal weight counterparts.

Strengths of the study include the use of several iron biomarkers, the use of both AGP and CRP as inflammation markers as well as the correction of ferritin for the presence of inflammation. Our study also made use of SEM an analytical approach which allows for simultaneous testing of multiple mediation pathways thereby avoiding the potential bias arising from neglecting the correlation between mediators. Though our analysis identified potential risk factors of anaemia and ID, yet because this is a cross sectional study, we cannot establish causality between any exposure of interest.

Conclusions

To address anaemia among WRA in South Africa intervention programs should also seek to improve young women's economic livelihoods, to improve their buying power and consequently their ability to buy iron rich food, thereby improving their diet quality. The use of MMS which contains a lower dose of iron and other micronutrients is recommended as it would ensure women would get the same benefits as with IFA plus additional benefits of alleviating anaemia of inflammation.

Abbreviations

WRA- women of reproductive age

CRP- C-reactive protein

HAS – household asset score

SEM- structural equation modelling

IDA – iron deficiency anaemia

IDE- iron deficiency erythropoiesis

HIV- human immune virus Initiative

AGP- alpha-1-acid glycoprotein

MUAC- mid upper arm circumference

sTfR- soluble transferrin receptor

ID – iron deficiency

BMI – body mass index

Hb – haemoglobin

Declarations

Ethical approval and participant consent

This study was conducted in accordance with the ethical principles laid down in the Declaration of Helsinki, and all procedures involving human participants were approved by the Human Research Ethics Committees of the North-West University, Potchefstroom (NWU-0042919-S1), and the University of the Witwatersrand, Johannesburg (M171137).

During the recruitment process, potential participants were visited in their homes and were informed in their home language about: (i) the objectives of the study; (ii) the use of the results; and (iii) the risks and benefits of the study. An informed consent form was supplied to potentially eligible women who were interested in being part of the study. The interested women were then invited to the study site for the signing of informed consent and data collection.

Author Contributions: Conceptualization L.J.W., C.M.S., S.L. and S.N.; methodology, T.M.S., L.J.W., L.M., C.M.S., S.L., and S.N.; investigation, T.M.S. and L.J.W.; data curation, L.J.W. and L.M.; formal analysis, T.M.S. and G.C.; supervision, L.J.W., L.M., C.M.S. and S.N.; resources, S.L., C.M.S. and S.N.; project administration, T.M.S. and L.J.W.; funding acquisition, C.M.S., S.L., and S.N.; writing /original draft preparation, T.M.S. writing/review and editing, T.M.S., J.B., L.J.W., L.M., C.M.S. and S.N. All authors have read and agreed to the published version of the manuscript.

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Availability of data and materials: The data set of the current study is available from the corresponding author based on reasonable request.

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Tables

Table 1. Characteristics of 18–25-year-old non pregnant women of reproductive age residing in Soweto, South Africa (n = 480).

Characteristics	Median (IQR) or n (%)
Anaemia Hb <12g/dl	189 (39.4)
Inflammation adjusted ID< 15 µg/L	183 (38.1)
Iron deficiency anaemia Hb <12g/dl ferritin < 15 µg/L	104 (21.6)
Age (y)	21 (19-23)
Socioeconomic	
Household asset score	
Low (1-3)	31 (6.5)
Medium (4-6)	304 (63.3)
High (7-11)	145 (30.2)
Years of education (y)	12 (7-12)
Dietary Factors	
At risk of food insecurity (yes)	227 (47.3)
Food insecure (yes)	132 (27.5)
Consumption of leafy green vegetables(yes)	121 (25.2)
Consumption of chicken and beef (yes)	324 (67.5)
Behavioral variables	
Smoking History (yes)	160 (33.3)
Current Alcohol consumption (yes)	329 (68.5)
Bio-demographics	
Parity	
nulliparous	244 (50.8)
primiparous	191 (39.8)
multiparous	45 (9.4)
Age at first delivery (y)	19 (17-21)
Age at start of menarche (y)	13 (12-15)
Contraception use (yes)	332 (69.2)
HIV positive self -reported (yes)	21 (4.4)
Anthropometry	
MUAC	
underweight≤24cm	94 (19.6)

BMI (kg/m ²)	
Underweight (<18.5 kg/m ²)	41 (8.5)
Normal weight (18.5–24.9 kg/m ²)	221(46.0)
Overweight (25–29.9 kg/m ²)	104(21.7)
Obese (≥30 kg/m ²)	114(23.8)
Biomarkers of inflammation	
CRP (mg/L)	1.39 (0.44-3.71)
AGP (g/L)	0.86 (0.72-1.01)
Inflammatory status	
Inflammation (CRP > 5 mg/L and AGP > 1 g/L)	179 (37.3)
Incubation (CRP >5 mg/L and AGP ≤ 1 g/L)	52 (10.8)
Early convalescence (CRP> 5 mg/L and AGP > 1 g/L)	43 (9.0)
Late convalescence (CRP ≤ 5 mg/L and AGP > 1 g/L)	84 (17.5)
Iron status biomarkers	
Inflammation-adjusted ferritin (µg/L) ¹	25.65 (8.04-54.96)
sTfR (mg/L)	7.51 (5.7-10.5)
IDE (sTfR > 8.3 mg/L)	201 (41.9)
Inflammation adjusted RBP µmol/L	1.49 (1.18-1.87)
Vitamin A status-RBP µmol/L	
< 0.7 deficiency	17 (3.5)
≥0.7-1.05 insufficiency	59 (12.3)
≥1.05 sufficient	404 (84.2)

Variables are either median (IQR) or n (%). MUAC, mid upper arm circumference; BMI, body mass index; AGP, α-1-acid glycoprotein; CRP, C-reactive protein; ID, iron deficiency; IDA, iron deficiency anaemia; sTfR, serum transferrin receptor; IDE, iron deficient erythropoiesis, RBP, retinol binding protein. Ferritin values were adjusted for inflammation using the correction factors suggested by Thurnham et al. (7). Iron deficiency was defined as an inflammation-adjusted ferritin concentration <15mg/L. Anaemia was defined as altitude adjusted haemoglobin concentration <12g/dl.

Table 2. Multivariate Logistic Model of factors associated with anaemia in non- pregnant WRA in Soweto South Africa (n=480).

Variable	OR (95% CI)	P-value
Iron Status biomarkers		
IDE (sTfR >8.3mg/L)	1.62 (1.07,2.46)	0.023*
ID inflammation adjusted (ferritin < 15 µg/L)	2.62 (1.72,3.98)	0.001*
Inflammation markers		
CRP_above5 mg/L	1.69 (1.04,2.76)	0.035*
Bio-demographics		
Contraception use (yes)	0.74 (0.47,1.15)	0.18
Parity		
nulliparous	ref	
primiparous	0.82 (0.53,1.26)	0.37
multiparous	0.83 (0.41,1.70)	0.61

Anaemia (yes or no) was used as the outcome variable. Other variables were removed from the model for lack of significance. Anaemia was defined as Hb < 12 g/dl. Iron deficiency was defined as an inflammation-adjusted ferritin concentration < 15mg/L. Inflammation was defined as a C-reactive protein concentration >5 mg/L. Iron deficiency erythropoiesis was defined as IDE (sTfR > 8.3 mg/L). Significant results were signified with a*.

Table 3. Multivariate Logistic Model of factors associated with ID in non -pregnant WRA Soweto, South Africa (n=480).

Variable	OR (95% CI)	P-value
Dietary factors		
RBP $\mu\text{mol/L}$		
RBP sufficient ≥ 1.05	ref	
RBP deficient < 0.7	8.75 (2.62,28.98)	$<0.00^*$
RBP insufficient $\geq 0.7-1.05$	1.30 (0.694,2.44)	0.41
Weekly chicken and beef consumption	0.56 (0.36,0.88)	0.001*
Iron Status biomarkers		
sTfR $> 8\text{mg/L}$	5.41 (3.52,8.33)	$<0.001^*$
Biodemographic		
Ever used contraception(yes)	0.61 (0.38,0.98)	0.043*
Parity		
Nulliparous	ref	
Primiparous	0.75 (0.47,1.26)	0.23
Multiparous	0.36 (0.16,0.81)	0.014
Socio-economic Variables		
Household asset score		
High (1-4)	ref	
Low (5-8)	2.40 (0.97,5.92)	0.057
Medium (>8)	0.85 (0.53,1.36)	0.50

Iron deficiency (yes or no) was used as the outcome variable. Other variables were removed from the model because of a lack of significance. Iron deficiency was defined as an inflammation-adjusted ferritin concentration, 15mg/L . Inflammation was defined as a C-reactive protein concentration $>5\text{ mg/L}$ or a-1-acid glycoprotein concentration $>1\text{ g/L}$. Vitamin A deficiency and insufficiency was defined as an adjusted retinol-binding protein or retinol concentration $<0.7\text{ }\mu\text{mol}$ and $<1.05\text{ }\mu\text{mol}$ respectively. Significant results were signified with a*.

Table 4. Direct and indirect associations of socioeconomic, bio-demographic, inflammation, and nutritional characteristics with haemoglobin concentration in women of reproductive age.

	Hb	Ferritin	sTfR	CRP	HAS	Parity	Chicken & beef consumption	BMI
Ferritin								
Direct	0.0031***							
Indirect								
Total	0.0031***							
sTfR								
Direct	-0.042***							
Indirect								
Total	-0.042***							
Chicken and beef								
Direct		17.77*	-2.37					
Indirect	0.15*							
Total	0.15*	17.77*	-2.37					
CRP								
Direct	-0.015*	0.064						
Indirect	0.001		-0.036	-0.0036				
Total	-0.14*	0.064	-0.036	-0.0036				
HAS								
Direct							0.015	-0.011
Indirect	0.0037	0.37				0.0011		
Total	0.0037	0.37			0.003	0.0011	0.015	-0.011
Contraception								
Direct	0.34*	29.98***			0.003	0.42***		
Indirect	0.11**	4.48						
Total	0.45*	34.45**				0.42***		
Parity								
Direct		10.65						
Indirect	0.033							
Total	0.033	10.65						

BMI				
Direct			0.33***	0.011**
Indirect	-0.0044	0.14		
Total	-0.0044	0.14	0.33***	0.011**

mass index. *** $P \leq 0.001$, ** $P \leq 0.01$, * $P \leq 0.05$. Ferritin was adjusted for inflammation. The model statistics were the Root Mean Square Error of Approximation for :0.025, the Comparative Fit Index: 0.969, the Tucker-Lewis Index: 0.947 and SRMR :0.039.

Table 5. Direct and indirect associations of socioeconomic, bio-demographic, inflammation, and nutritional characteristics with ferritin concentration in women of reproductive age.

	Ferritin	RBP	CRP	Chicken and beef	Parity	Contraception	BMI
RBP							
Direct	25.48***						
Indirect							
Total	25.48***						
Chicken and beef							
Direct	15.85						
Indirect							
Total	15.85						
CRP							
Direct	0.035	0.0030					
Indirect	0.077						
Total	0.11	0.0030					
HAS							
Direct		0.015		0.015	0.0011	0.0030	-0.011
Indirect	0.71	0.000098	-0.0036				
Total	0.71	0.015	-0.0036	0.015	0.0011	0.0030	-0.011
Contraception							
Direct	31.5***				0.42***		
Indirect	1.04*	0.041*					
Total	32.59***	0.041*			0.42***		
Parity							
Direct		0.097***					
Indirect	2.47*						
Total	2.47*	0.097***					
BMI							
Direct			0.33***		0.12***		
Indirect	0.066	0.0021					

Abbreviations: RBP, retinol binding protein; CRP, C-reactive protein; HAS, household asset score; BMI, body mass index. *** $P \leq 0.001$, ** $P \leq 0.01$, * $P \leq 0.05$. The model statistics were the Root Mean Square Error of Approximation for :0.026, the Comparative Fit Index: 0.958, the Tucker-Lewis Index: 0.921 and SRMR: 0.035.

Figures

Figure 1

SEM framework for the anaemia outcome.

Figure 2

SEM framework for ID outcome