Nutrition 62 (2019) 194–200

Contents lists available at ScienceDirect

Nutrition

journal homepage: www.nutritionjrnl.com

Applied nutritional investigation

Nutrient patterns and their relation to anemia and iron status in 5- to 12-y-old children in South Africa

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ARTICLE INFO

Article History:
Received 27 August 2018
Received in revised form 27 December 2018
Accepted 23 January 2019

Keywords:
Anemia
Iron deficiency
Nutrient patterns
Primary school children
South Africa

ABSTRACT

Objective: The aim of this study was to assess nutrient patterns and their relation to anemia and iron status of school children using pooled data from three study populations in South Africa.
Methods: Data from 5- to 12-y-old children (N = 578) from three independent studies conducted in two provinces in South Africa were pooled. Data used in the analysis were dietary intake, hemoglobin, and plasma ferritin concentrations. Nutrient patterns were determined using factor analysis. Logistic regression analysis was performed to determine relationships of nutrient patterns with anemia and iron deficiency.
Results: In the pooled group, 13.8% of the children were anemic and 27.7% were iron deficient (ID). More than half of children did not meet the Estimated Average Requirement for various nutrients, including vitamins A, C, B_{12}, folate, and zinc, although only 17.7% of children had an iron intake below the requirements. Median intakes for vitamins A and C were lower for anemic than non-anemic children ($P = 0.03$ and 0.02, respectively) and for ID versus non-ID children ($P = 0.03$ and 0.046, respectively). Four nutrient patterns were identified: plant protein, carbohydrate, iron, and B vitamins; animal protein and saturated fat; vitamins A and B_{12}; and calcium and fiber. The vitamin A and B_{12} nutrient pattern was associated with lower odds of being anemic (odds ratio, 0.63; 95% confidence interval, 0.49–0.91; $P = 0.035$).
Conclusion: The present results highlighted the potential role of the combination of dietary vitamin A and B_{12} in the etiology of nutritional anemia in school-age children in South Africa. Nutrient pattern analysis may improve the understanding of the synergistic role of nutrients related to anemia and may assist in planning intervention strategies.

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Introduction

Global estimates show that anemia affects 600 million children and about 50% of these cases are children of primary school age [1]. The most common cause of anemia is iron deficiency (ID) [1]. Other causes include micronutrient deficiencies other than iron (e.g., folic acid, vitamin A, and B vitamins including vitamin B_{12}), poor health, and acute and chronic infections [2,3]. In children of school age, anemia is associated with reduced cognitive and motor development and has a negative effect on schoolwork performance [4].

The only national data available for South African children from 7 to 9 y of age is based on the 2005 National Food Consumption Survey (NFCS-FB-1), which indicated an anemia prevalence of almost 19% [5]. The results of smaller independent studies conducted after the NFCS-FB-1 were summarized by Taljaard et al. [6] and show that anemia remains a common problem in school children, with a prevalence ranging from 6.9% to 27%.

In low- and middle-income countries, including South Africa, the diets of the majority of children lack variety, which may result in insufficient intake of various nutrients important for growth and

This article was part of the first author's doctoral thesis, which was supported by study grants from the South African National Research Foundation, the Nestlé Nutrition Institute Africa and Association of African Universities. The funders had no role in study design, data collection and analysis, did not influence the results interpretation and decision to publish, or preparation of the manuscript. TVZ and MF conceptualized this study and contributed to the writing of the paper. MV analyzed the data and wrote the draft article. SMH, JB, MvdH, CTK, and CMS 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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https://doi.org/10.1016/j.nut.2019.01.016
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proper functioning of the body [7]. An adequate intake of dietary iron is important to maintain sufficient iron stores and to prevent iron deficiency anemia (IDA), an advanced stage of ID that is characterized by low hemoglobin production [4]. Individuals who mostly consume plant-based diets are at greater risk for developing ID owing to the low bioavailability of iron in plant foods [8,9]. A South African study in a rural disadvantaged community showed that <15% of children consumed foods from animal sources on the day of recall [10], which is a good source of various nutrients, placing these children at risk for nutritional anemia [11].

Fortification of staple foods with micronutrients of concern is one of the effective strategies to prevent nutritional anemia at a population level and has been introduced in some low- and middle-income countries, including South Africa [12]. In 2003, the South African Department of Health legislated mandatory fortification of frequently consumed staple foods—that is, wheat flour (used to make bread) and maize meal—with six vitamins: vitamin A, thiamine, riboflavin, niacin, pyridoxine, folic acid, and two minerals, iron and zinc [13]. Theoretically these fortified staple foods can make a significant contribution toward nutrient intake in children [14]. However, to our knowledge, there are no national data available on nutrient intake of school-age children in South Africa. A summary of dietary studies in children 6 to 15 y of age conducted between 2000 and 2015 reported that only a few studies included the fortified values for the fortified maize meal and bread in their dietary analysis and it was therefore difficult to determine whether nutrient intake improved after mandatary fortification of these staple foods [15].

Current knowledge on the association between dietary intake and nutritional anemia is focused mostly on single nutrients. Diets, however, consist of a combination of nutrients that have an interactive and synergistic effect on health, and thus it is relevant to assess the combined effect of nutrients [16]. Factor analysis is one of the modern reduction techniques whereby the number of variables (nutrients) are reduced to determine the whole nutrient pattern [16,17]

Information on nutrient patterns and their relationship with anemia and ID in school-aged children in South Africa is lacking. Therefore, in this study, we conducted a pooled analysis of existing data with the aim of assessing the relationship of nutrient patterns with anemia and ID by using the factor-analysis approach.

Participants and methods

The data used for this pooled analysis were previously reported and derived from the baseline point of three independent intervention studies with school children from different sites in South Africa [18–20]. 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) province. These areas are all 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, which provides children with a daily school meal. Parents or caregivers signed informed consent forms, and the learners gave verbal assent. Data collection included biochemical measurements, sociodemographic data, anthropometric measurements, and 24-h dietary recalls. A short summary of the three independent intervention studies is presented in Supplementary Table 1. For the purpose of the present study, only the relevant data of 578 participants were extracted from the original electronic data files and used electronically after written permission was obtained from the principal investigators. The following data were extracted from the databases: sociodemographic information; anthropometric measurements (height and weight); biochemical data: hemoglobin (Hb), plasma ferritin, and C-reactive protein (CRP); and dietary intake data (energy, and macro- and micronutrients). Cases for the pooled analyses were selected from the three original databases using the following inclusion criteria: age between 5 and 12 y; availability of three biochemical values, namely Hb, plasma ferritin, and CRP; and complete dietary intake data. Cases with missing data for any of these variables were excluded.

Dietary intake assessment

Dietary data were collected by means of the 24-h dietary recall method. Three 24-h recalls per child were completed on non-consecutive days for all children in one of the studies [19], but only on a subsample of children in the other two studies [18,20]. Both the child and the parent or caregiver responsible for the food preparation were present during the 24-h recall interviews, which were done by trained fieldworkers. Food portion sizes were estimated using plastic food models, household utensils, food packaging materials, and “dish-up and measure.” Where the amount of food consumed was reported in household measures or volume, it was converted to grams using the MRC Food Quantities Manual [21]. The South African Food Database (SAFOODS) was used to code the foods consumed by the children. The South African Medical Research Council's FoodFinder3 software program was used to convert food intake to energy, macronutrients, 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 South African Food Database [22]. The average daily energy and nutrient intake of the three 24-h recalls was calculated for each child. The percentage of children with an average daily intake below the Estimated Energy Requirements

Table 1

The characteristics of 5- to 12-y-old school children and biochemical indicators of anemia 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, y*	8.7 ± 1.3	10 ± 1[a]	9±1.9 [b]	8.2±0.9 [c]	<0.001
Sex Boys, % (n)	51.0 (296)	47 (48)	54 (54)	52 (194)	0.593
Girls, % (n)	49.0 (282)	53 (54)	46 (46)	48 (182)	
Hb (g/L)†	125 (124–26)	120 (118– 122)[a]	127 (124–129)[b]	126 (125–127)[b]	0.001
Plasma ferritin (μg/L)†	23.7 (14.4–36.9)	17.6 (9–29)[a]	23.1 (16.4–33.2)[b]	21.9 (13.6–34.4)[b]	0.006
Anemia, % (n)	13.8 (80)	26.5 (27)[a]	14 (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 anemia, % (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 (8)[b]	22.8 (86)[c]	0.01
Stunted, % (n)	27.2 (154)	12.7 (12)[a]	13 (13)[a]	34.4 (129)[b]	0.05
Underweight, % (n)	15 (87)	3.9 (4)[a]	24 (24)[b]	15.8 (59)[c]	0.01
Overweight, % (n)	6.1 (35)	12.6 (12)[a]	4 (4)[b]	5 (19)[b]	0.001

ANOVA, analysis of variance; BAZ, BMI-for-age z-score; CRP, C-reactive protein; HAZ, height-for-age z-score; Hb, hemoglobin; KZN, KwaZulu-Natal, NW, North West province; WAZ, weight-for-age z-score.

Anemia defined as Hb <115 g/L; iron deficiency as plasma ferritin <15 μg/L; iron deficiency anemia as Hb <115 g/L + plasma ferritin <15 μg/L; inflammation as CRP ≥5 mg/L; stunting, underweight, and overweight was defined as HAZ <−2 SD, WAZ <−2 SD,and BAZ >+1 SD, respectively.

Plasma ferritin values of children with CRP concentration ≥5 mg/L were adjusted for inflammation by multiplying plasma ferritin values with a correction factor of 0.65 [26].

ANOVA used for continuous variables were compared across groups; Pearson χ^2 for categorical data. Values with different letters in superscript (a,b,c) differed significantly using multiple comparisons (Bonferroni test).

*Reported as mean ± SD.

†Reported as mean (95% CI).

(EER) and, for micronutrients, the Estimated Average Requirements (EAR) was calculated [23,24].

Anthropometric measurements

Weight and height of the children were measured according to 2007 World Health Organization (WHO) recommended procedures, as described in previously published papers [18–20]. The 2007 WHO references values were used to calculate age- and sex-specific indicators of nutritional status, such as stunting, underweight, and overweight. Stunting was defined as a height-for-age z-score (HAZ) <-2 SD, underweight as weight-for-age z-score (WAZ) <-2 SD, and overweight as body mass index-for-age z-score (BAZ) $>+1$ SD [25].

Biochemical measurements

To determine biochemical values, blood samples were collected from children in the original studies. For the purpose of the present study, Hb, plasma ferritin, and CRP concentrations were used. Hb concentrations were measured on whole blood by using the direct cyanmethemoglobin method (Bio Rad Laboratories [PTY] Ltd, Hercules, CA, USA) by using Drabkin's solution and a standard miniphotometer in the Baumgartner et al. [18] study, whereas in the other two studies a hematology analyzer (Coulter Ac·T^{TM} 5 diff CP; Beckman Coulter, Brea, CA, USA) was used [19,20]. Plasma ferritin and CRP were measured using an automated chemiluminescent immunoassay system (CLIA, IMMULITE; DPC Buhlmann GmbH) in the Baumgartner et al. study, whereas in the other two studies a ferritin enzyme-linked immunosorbent assay kit (Ramco Laboratories Inc., Stafford, TX, USA) was used for plasma ferritin and an immunoturbidimetric method (Technicon RA-1000 auto analyzer; Technicon Instruments, Tarrytown, NY, USA) for CRP [19,20].

For the pooled data analysis, the plasma ferritin values of children with a CRP concentration $\geq$5 mg/L were adjusted for inflammation by multiplying plasma ferritin values with a correction factor (CF) of 0.65 [26]. Selected cutoff points of iron status markers were used to classify children as ID, anemic, or IDA. Anemia was defined as Hb $<$ 115 g/L; ID based on plasma ferritin $<$ 15 μg/L; and IDA was defined as a combination of Hb $<$ 115 g/L and plasma ferritin $<$ 15 μg/L [27,28].

Ethical approval for the present study

Ethical approval for the pooled data analysis was obtained from the Health Research and Ethics Committee of the North-West University. The appropriate ethical committees granted ethical permission for the three original studies [18–20].

Determination of nutrient patterns

Exploratory factor analysis was conducted as described by Field [29] to develop a number of nutrient patterns (called factors) that explained most of the variances in the observed nutrients (variables). The factor analysis was applied with the correlation matrix to standardize data. The reliability of the factor analysis was verified using the Kaiser-Meyer-Olkin (KMO) test. Factors were rotated by orthogonal Varimax rotation method to provide a simpler structure and to improve interpretation. Factors were retained and interpreted for further analysis based on their natural interpretation, “eigenvalues” of $>$1.00 and scree-plot construction. To define the extent to which each of the input nutrient variables contributes to the meaning of each of the factors, the nutrients (at least three variables) with factor loadings $\geq$0.53 on a given factor were retained for nutritional interpretation. Factor scores for each of the nutrient patterns were computed for each individual child and indicate the degree to which each child's nutrient intake conforms to the identified patterns. For each factor, children were grouped into three categories according to tertiles of factor scores, and the distribution of children across the tertiles of each pattern according to their anemia and ID status was determined.

Statistical analyses

The SPSS version 25 (SPSS Inc., Chicago, IL, USA) was used for all statistical analyses. The Kolmogorov–Smirnoff test was used to assess the normality of the data distribution. The Levene's test was used to test the homogeneity of variances. For normally distributed data, the results are expressed as either mean ± SD or mean and 95% confidence interval (CI). The dietary data were log-transformed, and after log-transformation, most were still not normally distributed. Dietary data are therefore reported as medians (25th; 75th percentile). The results for categorical data are presented as frequencies and percentages. Differences between two groups were tested using the independent *t* test for normally distributed data and the Mann–Whitney U test for non-normally distributed data. Differences between categorical data were determined using the Pearson χ^2 test with *z*-test with adjusted *P*-values (Bonferroni method). To determine differences across more than two groups for continuous variables, analysis of variances was used; if significant differences were detected, the Bonferroni test was used to identify which groups differed. For the analysis of covariance by means of the general linear model univariate procedure, energy intake, age, sex, and study site were entered as covariates. The Bonferroni test was used to identify which groups differed if significant differences were detected.

Binary logistic regression models were computed to identify the association of nutrient patterns with anemia and ID. We calculated two models for each nutrient pattern. Model 1 refers to the crude analysis, whereas model 2 includes the potential confounding factors, namely energy intake, age, sex, and study site. Odds ratio (OR) and 95% CI for the regression parameters are reported. Statistical significance was set at $P < 0.05$ and trend toward statistical significance at $P < 0.1$.

Results

Characteristics and anemia and iron status of children in the pooled group and by study sites

In all, 578 cases were pooled for analysis, consisting of study site 1 (KZN) n = 102, study site 2 (NW1) n = 100, and study site 3 (NW2) n = 376. The characteristics of children and the biochemical indicators of anemia and iron status for each of the study sites and the pooled group are presented in Table 1. The sex distribution was 51% boys and 49% girls, from 5 to 12 y of age. The mean age differed significantly across the three study sites ($P < 0.001$).

In the pooled group (N = 578), 13.8% of the children were anemic, 27.7% were ID, 19.8% presented with elevated CRP levels, and 27.2% were stunted. Differences across study sites were observed: In terms of anemia and iron status at the time of data collection, the KZN study site had a significantly higher proportion of anemic children (26.5%) compared with the other two study sites (14 and 10.4%, respectively; $P < 0.001$). In the KZN study site, 42.2% of the children were ID, which was significantly higher compared with the other two study sites (18.8 and 26.3%, respectively; $P < 0.001$). The proportion of children with raised CRP levels was higher in NW2 (22.8%) than in the other two study sites (19.6 and 8%, respectively, $P < 0.01$).

The NW2 study site had the highest proportion of stunted children (34.4%), NW1 had the highest proportion of underweight children (24%), and KZN had the highest proportion of overweight children (12.6%).

Energy and nutrient intake of children in the pooled group and by study sites

More than 80% of children did not meet the Estimated Energy Requirements (Table 2). Although the intake of protein was within the adequate range (between 10 and 30% of total energy intake), the mean percent of energy from protein was just above the minimum range of 10% in all three study sites. Overall, $>$50% of children did not meet the EAR for a variety of nutrients, including vitamins A, C, and B_{12}, folate, and zinc; although only 17.7% of children had an iron intake below EAR.

Energy and nutrient intake of children in the pooled group stratified by anemia and ID status

Within the pooled group, children were stratified by anemia and iron status (Supplementary Table 2). Results showed that energy intake did not differ significantly between the compared subgroups. Median intakes for vitamins A and C were lower for anemic than non-anemic children ($P = 0.03$ and 0.02, respectively), and for ID versus non-ID children ($P = 0.03$ and 0.046, respectively).

Nutrient patterns identified by factor analysis in the pooled group and by study sites

The exploratory factor analysis procedure was tested statistically and found Kaiser-Meyer-Olkin $>$0.8 (data not shown), which

Table 2
Dietary intake of 5- to 12-y-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*-value*
TE, kJ†	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 (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	3.7	<0.001
Plant protein, g†	26.9 (21.8; 32.3)	24.9 (21.4; 31.1)[a]	25 (19; 30.9)[a]	27.9 (23.1; 33.4)[b]	0.006
Animal protein, g†	18.4 (11.1; 26.8)	12.3 (5; 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)	42 (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	21.9	22.1	0.054
Carbohydrate, g†	243.7 (199.6; 289.5)	272.4 (210.7; 323)[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 (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 (133.7; 309.7)	171.7 (124.9; 255.6)[a]	297.8 (120; 318.9)[b]	216.9 (139; 300)[c]	0.001
Calcium <EAR, %	97.5	100	97	98	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[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[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; 803.9)[b]	496.8 (367.8; 862.6)[b]	<0.001
Vitamin A <EAR, %	47.8	91.2[a]	46[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	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	98	100	0.733
Vitamin E, mg†	7.3 (4.2; 12.5)	10.7 (10.2; 19.2)[a]	7 (2.2; 8.2)[b]	6.4 (4.1; 10.8)[c]	<0.001
Vitamin E <EAR, %	56.1	25.3[a]	80[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 (10.7; 19)[b]	15.1 (11.5; 20.9)[b]	0.037
Niacin <EAR, %	2.7	2.9	2	2.8	0.600
Vitamin B_6, mg†	2.7 (1.9; 3.7)	2.3 (1.8; 3.3)[a]	1.7 (0.9; 2.6)[b]	3 (2.2; 3.9)[c]	0.010
Vitamin B_6 <EAR, %	6.6	6.8[a]	12[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[b]	10.4[c]	<0.001
Vitamin B_{12}, 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 B_{12} <EAR, %	46.9	75.3[a]	42[b]	40.4[b]	0.045

EAR, Estimated Average Requirements; EER. Estimated Energy Requirements; KZN, KwaZulu-Natal, NW, North West province; RE, retinol equivalent; TE, total energy.
Values with different letters in superscript (a,b,c) differed significantly, multiple comparisons: Bonferroni test.
*Mann–Whitney U test for non-parametric variables; Pearson χ^2 for categorical data.
†Reported as median (25th; 75th percentile); all such values.

confirmed that the multivariate reduction technique is applicable to the study sample. The factor analyses were conducted for the three study sites separately and for the pooled group. Because similar nutrient patterns were observed in all study sites (data not shown), the final results are presented for the pooled group (Table 3). Estimates from a factor analysis performed on 23 nutrients (equal to the

Table 3
Extracted nutrient patterns and factor loadings identified by factor analysis in the pooled group of 5- to 12-y-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: Vitamins A and B_{12}	Factor 4: Calcium and fiber
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 B_6	**0.811**	0.321	0.137	-0.142
Folate	**0.779**	-0.136	0.276	0.383
Vitamin B_{12}	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**
% 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 the measure of 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 [29].

Table 4
Comparisons of proportions of children in subgroups of anaemia and iron deficiency status across the tertiles scores of four nutrient patterns

Tertiles of nutrient patterns	Anemic n = 80	Non-anemic 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[a]	30.9[b]		37	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	33.9		34.4	32.8	
			Vitamin A and B_{12} 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 fiber nutrient pattern			
T1	25.3[a]	28.9[a]	0.068	26.1[a]	32[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]	

ID, iron deficient; T, tertile.
Values are expressed as percentage of the subgroups for categorical variables; χ^2 test used for categorical variables.
Superscript letters in a row that are the same denote a subset of subgroups that did not differ significantly from each other; superscript letters in a row that differ denote a subset of subgroups that differ significantly from each other at the 0.05 significance level; statistical significance testing was performed using *z*-test with adjusted *P*-values (Bonferroni method).

number of nutrient variables) were reduced to four nutrient patterns (factors) based on the convergence of the scree plot and Kaiser's criterion. The four retained factors explained 60.2% of the total variance after Varimax rotation. Factor loadings, which are the equivalents to the correlations between the nutrients and nutrient patterns, and the names assigned to each nutrient pattern are presented in Table 3. The factor loading values did not change when the variables (nutrients) that were not prevalent in the nutrient patterns and did not show correlations with nutrient patterns were not included in the analysis [29]. The first nutrient pattern (factor 1) is mainly representative of plant protein, carbohydrate, B vitamins, and iron (27% of the total variance explained); the second nutrient pattern (factor 2) represents animal protein, saturated fat, and zinc (12% of the total variance explained); the third nutrient pattern (factor 3) is mainly representative of vitamins A and B_{12} (11.1% of the total variance explained); and the forth nutrient pattern (factor 4) mainly represents fiber and calcium (10.1% of the total variance explained). Factors were named according to the nutrients with higher loadings (>0.53, as dominant nutrients) that cluster around the same pattern [29]. Factor 1 was named plant protein, carbohydrate, iron and B vitamins nutrient pattern; factor 2 was the animal protein and saturated fat; factor 3 was vitamin A and B_{12}; and factor 4 was the calcium and fiber nutrient pattern.

The comparisons of the nutrient intakes of children across the tertiles for each nutrient pattern score showed that intake of dominant nutrients in each nutrient pattern was significantly lower in the first tertiles (T1) compared with the T2 and T3 (all $P < 0.001$), adjusted for energy intake, age, sex, and study site (data not shown).

We compared the distribution of anemic or ID children across the score tertiles for each nutrient pattern (Table 4). A significantly higher proportion of anemic children (41.3%) fell within the T1, and a lower proportion (26.2%) in the T3 of the vitamin A and vitamin B_{12} pattern compared with non-anemic children (30.3 and 34.3%, respectively; $P = 0.012$). Within the other nutrient patterns, we found no significant difference in the proportion of anemic or ID children.

However, there was a trend ($P < 0.1$) for a higher proportion of anemic children within T3 of both the plant protein, carbohydrate, iron, and B vitamins pattern and the calcium and fiber pattern. Furthermore, there was a trend for a lower proportion of ID children within T1 of the calcium and fiber pattern. Comparisons of the continuous factor scores between subgroups of children according to their anemia and ID status showed similar results (data not shown).

The results of the logistic regression analysis (Table 5) present the OR of anemia and ID and corresponding 95% CIs in the pooled group of children (N = 578) according to continuous factor scores. The vitamin A and vitamin B_{12} nutrient pattern scores were associated with lower odds of being anemic (OR, 0.63; 95% CI, 0.49–0.91; $P = 0.035$), and tended to be associated with lower odds of being ID. The plant protein, carbohydrate, iron, and B vitamins nutrient pattern scores tended to be associated with higher odds of being anemic or ID. No significant association was observed between other nutrient pattern scores and the odds of being anemic or ID.

Discussion

To determine the various nutrient combinations that could be associated with anemia and ID, we identified four nutrient patterns

Table 5
Odds of anemia and iron deficiency and corresponding 95% CIs in the pooled group of children (N = 578) according to continuous factor scores from a factor analysis

	Factor 1: Plant protein, carbohydrate, iron. and B vitamins	*P*-value	Factor 3: Vitamins A and B_{12}	*P*-value
		Anemia		
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 (1.10v1.32)	0.070	0.96 (0.95–1.31)	0.050

*Model 1: crude.
†Model 2: adjusted for energy intake, age, sex, and study site.

explaining 60.2% of the total variance in nutrient intakes. Results showed that the vitamin A and B_{12} nutrient pattern was inversely associated with anemia and tended to be inversely associated with ID. The plant protein, carbohydrate, iron, and B vitamins nutrient pattern tended to be positively associated with anemia and ID.

Almost half of the children in the pooled group had an inadequate intake of vitamins A and B_{12}. The KZN study site had the highest proportions of children with inadequate intake of these vitamins (91.2 and 75.3%, respectively) and also the highest proportions of children with anemia and ID. Moreover, comparisons of the subgroups of children according to their anemia and iron status showed that anemic and ID children had significantly lower dietary intake of vitamin A. These results are in agreement with the nutrient pattern analysis because a significantly higher proportion of anemic children fell within the lowest tertile of the vitamin A and B_{12} nutrient pattern compared with children without anemia. Literature shows that in school-age children, vitamin A deficiency was one of the main predictors of anemia [30,31]. Another study by Oliveira et al. [32] showed that insufficient dietary intake of vitamins A and B_{12} in children was one of the factors most strongly associated with anemia. An adequate supply of both vitamins A and B_{12} is needed for the production of red blood cells, and facilitates the absorption of non-heme iron from plant-based diets [33,34]. The vitamin A and B_{12} nutrient pattern probably reflects the combination of these nutrients within some specific foods, particularly organ meat, such as liver. In appropriate amounts, these foods are valuable sources of many essential micronutrients, including vitamins A and B_{12} [11]. Irregular consumption of foods, like organ meats (i.e., less than once per week), was significantly associated with anemia [35].

In the present study, the scores of the plant protein, carbohydrate, iron, and B vitamins nutrient pattern tended to be associated with higher odds for anemia and ID. This pattern is characterized by several of the micronutrients (riboflavin, niacin, pyridoxine, folic acid, and iron) that are used in the mandatory fortification of wheat flour (bread) and maize meal [13]. The high loadings for plant protein and carbohydrates further points to consumption of these two fortified staple foods in this nutrient pattern. Potentially, consumption of the fortified staple foods can make a significant contribution toward daily iron intake of South African children [14]. On average, children 5 to 12 y of age need to consume from 4.1 to 5.9 mg of iron every day [23]. The average daily portion size of fortified bread consumed by the children was 99 g (equal to three slices), which provides 3.4 g of iron. In addition, the average daily amount of fortified maize meal was 119 g (eaten as either a soft, stiff or crumbly porridge), which provides 3.5 g of iron (data not shown). In the pooled group, only 17.7% of children had an iron intake below the EAR, yet 13.8% were anemic and 27.7% ID. These results suggest that an adequate dietary iron intake does not necessarily equate to sufficient iron status. The use of electrolytic iron as fortificant in maize meal and wheat flour could have contributed to this finding because electrolytic iron as fortificant does not seem to be effective in reducing the rates of anemia or ID in children [36–38]. Also, we used the EAR of the Institute of Medicine, which suggests 18% bioavailability for iron in diets containing some meat products [23]. It may well be that the bioavailability of iron in the South African diet is lower, and the percentage of children with insufficient iron intake may therefore be an underestimation.

The high loading of plant protein in the plant protein, carbohydrate, iron, and B vitamins nutrient pattern suggests the presence of inhibitors of iron absorption, such as phytate and polyphenols, which are present in plant foods. Absorption of non-heme iron from plant foods or fortificant can be enhanced by vitamin C in the meal [39]. A high proportion of children in the present study had an intake of vitamin C below the EAR, and our results show that anemic children had significantly lower vitamin C intake than non-anemic children. Studies have further shown that the absorption of non-heme iron from plant foods will be enhanced by the addition of a small amount of animal protein in the same meal [40,41]

To our knowledge, studies evaluating the relationship of nutrient patterns with anemia and ID are scarce. In addition, only a few studies have explored the relationship of food patterns with anemia and ID, and these studies focused on adults [42–44]. Overall these studies suggest that food patterns characterized by a low intake of meat and vegetables are associated with an increased risk for suboptimal iron status or anemia. In the most recent study, Beck et al. [44] observed a low odds of suboptimal iron status based on ferritin concentrations in young women who followed a meat and vegetable pattern. We analyzed the data for food patterns (data not shown), but no clear picture emerged, probably because of the monotonousness of the children's diet.

Some strengths and limitations of this study should be considered. The identification of nutrient patterns, rather than individual nutrients, as used in this study may be a better way of exploring the cumulative and synergistic effects of various nutrients in the etiology of nutritional anemia. It is acknowledged that this study is cross-sectional and therefore unable to identify causal relationships between exposure (diet) and outcome (anemia and iron status). The time interval between the original studies and the time interval since data collection should have little impact on our results because the focus of this study was on associations of nutrient patterns with anemia and iron status, which are not time-bound, rather than anemia and iron status per se. Although the pooled data are from studies conducted between April 2009 and June 2012, the results on micronutrient intakes provide valuable information because limited data is available on dietary intake of school-age children in South Africa since the implementation of the national food fortification programme (NFFP) in 2003. It must be mentioned that the dietary intake data may be affected by misreporting of food intakes, which is known to be a common challenge in dietary assessment [45,46], and limitations inherent to the food composition database used to convert food intake data to nutrient intakes. Nutrient values in any food composition database reflect averages and therefore gives an approximate indication of the nutrient content of a food [47]. Also, the nutrient content of fortified bread and maize meal varies [48,49] and may differ from the values in the food composition database.

Conclusion

Results from the present study demonstrated that a vitamin A and B_{12} nutrient pattern was associated with lower odds of anemia in school-age children 5 to 12 y of age. These results highlight the potential role of inadequate intake of both vitamins A and B_{12} in the etiology of nutritional anemia. The vitamin A and B_{12} nutrient pattern probably reflects consumption of animal source foods that are rich in vitamins A and B_{12}, particularly liver. Nutrient pattern analysis is a novel and potentially powerful tool for exploring the relationship between nutrient intake and anemia status and can potentially contribute toward planning intervention strategies.

Acknowledgments

The authors acknowledge the research team members and all the participants of the single studies we obtained the data from. They also acknowledge S. Ellis and C. Ricci for their guidance on statistical analysis and interpretation.

Supplementary materials

Supplementary material associated with this article can be found in the online version at doi:10.1016/j.nut.2019.01.016.

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