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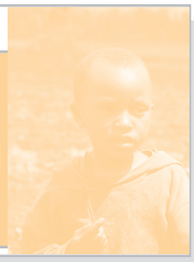
Folate and iron status of South African non-pregnant rural women of childbearing age, before and after fortification of foods



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Objective. To assess the effect of fortification of staple foods on the folate and iron status of women of childbearing age.

Design. A prospective cohort study was undertaken.

Setting. Dikgale Demographic Surveillance Site, a rural area in the Capricorn district of Limpopo Province.

Subjects. Non-pregnant women of childbearing age, 18 - 44 years (*N*=80).

Outcome measures. Serum folate, ferritin, vitamin B_{12}, red blood cell folate and full blood count.

Results. The prevalence of low serum folate (<3ng/ml) in the study population was 27.6% before fortification; after fortification, none of the women had low serum folate. Low red cell folate (<164 ng/ml) was observed in 26.4% of subjects before fortification, and in 1.9% of subjects after fortification. The prevalence of vitamin B_{12} deficiency (<145 pg/ml) was 6.3% during phase 1 of the study and increased to 11.3% during phase 2. Low haemoglobin levels were present in 7.5% of women before fortification, and in 5% of women after fortification. The percentage of women with low ferritin levels was similar before and after fortification (25%).

Conclusion. The study shows a significant improvement in folate status in women of childbearing age approximately 9 months after fortification of maize and wheat foodstuffs in South Africa, whereas no improvement in iron status as measured by serum ferritin was observed.

Folates are vital for cell division and homoeostasis because of the essential role of folate-containing co-enzymes in nucleic acid synthesis, methionine regeneration, and the shuttling, oxidation and reduction of one-carbon units required for normal metabolism and regulation.[1,2] Furthermore, folates play an important role in reducing the incidence of neural tube defects.[3] Prior to the fortification of foods, numerous worldwide studies reported high folate deficiencies in women of childbearing age.[4,5] In Limpopo Province, the prevalence of folate deficiency was reported to be 21% in non-pregnant women of childbearing age, and 48% in pregnant women.[6,7] Dietary surveys undertaken in the Capricorn district of Limpopo Province have shown that folate intake is below the recommended daily allowance (RDA).[8-10]

Together with iron and vitamin B_{12}, folate is involved in erythropoiesis – a process whereby haemopoietic tissue in the bone marrow produces red blood cells.[11] In addition to the high prevalence of folate deficiency in women of childbearing age, vitamin B_{12} and iron deficiencies have also been reported globally.[12,13] In the poorer populations of Africa, iron deficiency affects up to 50% of both women of childbearing age and infants, and is considered the leading nutritional deficiency worldwide.[13,14] In Limpopo Province, the prevalence of iron deficiency was reported to be 50.9%, while vitamin B_{12} deficiency was reported to be 16.4% among pregnant women.[15]

To increase the levels of folate and iron in the general population, compulsory fortification of foods with folic acid, iron and other nutrients has been implemented in many countries.[4,5] Several international studies have reported improved folate status after fortification of foods,[16,17] since synthetic folic acid in fortified foods is more bioavailable than naturally occurring folate.[18] However, the results of a study on the effect of iron fortification on iron status have been inconsistent.[19,20] It has been documented that, despite the improved folate status in populations after the introduction of fortified foods, some people are still at risk of developing folate deficiency due to non-dietary factors such as intestinal

malabsorption.[11] Additional folate demands resulting from pregnancy and lactation also predispose women to the development of folate deficiency.[21]

In South Africa, compulsory fortification of maize and wheat foodstuffs with folic acid, iron, vitamin A, thiamine, riboflavin, niacin, pyridoxine and zinc was implemented in October 2003.[22] To our knowledge, this is the first study aimed at determining the effect of fortification, especially on non-pregnant women of childbearing age, in South Africa.

Subjects and methods

Study area

The study was conducted at the Dikgale Demographic Surveillance Site (Dikgale DSS), a rural area with poor infrastructure, situated in the Capricorn district of Limpopo Province, ± 50 km northeast of its capital city, Polokwane. Dikgale DSS consists of 8 villages situated close to one another, with a total population of approximately 8 000 (Dikgale DSS Census, 2000/2001). Poor socio-economic status and a high rate of unemployment are prevalent in this area.[23]

The Dikgale DSS database (2000/2001) contains 1 649 women of childbearing age (18 - 44 years). A random sample of 120 women was selected in October 2002 and recruited by means of house visits. Lactating women, and those on chronic disease medication, using oral contraceptives, and who were tobacco and alcohol users, were excluded from the study. Pregnancy screening was carried out, using commercial pregnancy urine test-strips, and those women who were found to be pregnant were excluded from the study. Eventually, 100 women participated in the study (Phase 1: pre-fortification period). From October 2003, maize and wheat foodstuffs were fortified.[22] Approximately 9 months after fortification was introduced, a follow-up study (Phase 2: post-fortification period) was conducted on 80 of the same women who participated in the study prior to fortification. During the follow-up period, 20 women were unavailable to re-participate in the study, owing to pregnancy or absence from Dikgale DSS.

The Ethics Committee of the University of Limpopo (Turfloop Campus) and the Department of Health and Welfare Research Committee of Limpopo Province approved the study. The women were fully informed of the nature of the study, and informed consent was obtained from all women who participated at the beginning of the intervention and during the last phase of the study.

Blood collection and biochemical analysis

Before fortification was implemented, fasting blood samples were collected from non-pregnant women of childbearing age via venepuncture during November 2002 - April 2003. Full blood count (red blood cell count (RBC), haemoglobin (Hb), haematocrit (HCT), mean cell volume (MCV), mean cell haemoglobin (MCH) and mean cell haemoglobin concentration (MCHC)) were determined from whole blood on the A^{c}.T 5diff Beckman Coulter haematology analyser (Beckman Coulter, California). Fifty microlitres (50 µl) of whole blood was lysed with 1 000 µl of red cell folate lysing agent (Beckman Coulter, California) and stored at –80°C until analysis on an Access ImmunoAssay (Beckman Coulter, California). Plasma and serum obtained from centrifuged blood samples were stored as aliquots at –80°C for further analysis, which included serum ferritin (SF), vitamin B_{12} (VB12) and folate (SFol) on the ImmunoAssay machine. Red cell folate (RCF) was calculated as haemolysed folate × 21/ (HCT/100) and determined in 53 subjects only. Approximately 9 months after fortification, blood collection and analyses were repeated.

Normal levels of folate were ≥3 ng/ml, while serum folate deficiency was said to be severe if <2.5 ng/ml, or moderate if <3 ng/ml.[24] RCF deficiency was present if the level was <164 ng/ml, and considered normal when ≥164 ng/ml.[24] Women with SF levels between 12 µg/ml and 20 µg/ml were said to be moderately iron depleted, while those with levels <12 µg/ml were considered to be severely iron depleted. SF levels were considered normal when ≥20 µg/ml.[24] VB12 deficiency was said to be present in women whose levels were <145 pg/ml, and normal when ≥145 pg/ml.[24]

Statistical analysis

Statistical Package for Social Sciences (SPSS) for Windows (version 12) was used to analyse the data. Tests of normality (Kolmogorov-Smirnov and Shapiro-Wilk) were used to check the distribution of variables. Due to the non-Gaussian distributions of the variables, they were expressed as medians (interquartile (IQ) ranges). After being log-transformed, the variables before and after fortification were compared using a paired-sample *t*-test. Furthermore, median changes in the variables before and after fortification were computed. The percentages of subjects with variables below/above the cut-off points before and after fortification were determined and compared using the chi-square (χ^2) or Fisher's exact test. Statistical significance was set at a probability level (*p*-value) of 0.05.

Results

Table I presents medians (IQ ranges) and median differences (IQ ranges) of variables in women of childbearing age before and after fortification. Median levels of Sfol, RCF, VB12, RBC, Hb and HCT were significantly increased after fortification. No change in median levels of SF was observed after fortification,

while VB12 levels were significantly reduced (p=0.02). Table II shows the percentages of subjects with variables below or above the cut-off points. Apart from a significant reduction in the MCHC (p=0.002) levels after fortification, the other measured indices of full blood count (MCV and MCH) remained unchanged. The percentage of subjects with severe folate deficiency (Sfol <2.5 ng/ml) was 16.3%, while 11.3% of women were moderately depleted (Sfol 2.5 - 3 ng/ml). The prevalence of low Sfol was reduced from 27.6% to 0%, and low RCF was reduced from 26.4% to 1.9% after fortification, while no change in the prevalence of iron deficiency was observed. The percentage of women with VB12 deficiency almost doubled during this period. From Fig. 1, it can readily be seen that Sfol and RCF deficiencies were reduced by 100% and 92.8% respectively, whereas low RBC and Hb were reduced by 50% and 33.3% respectively, after fortification. No reduction in percentage of subjects with severe iron depletion (ferritin <12 µg/ml) was observed after consumption of foods fortified with electrolytic iron.

Discussion

A high prevalence of folate deficiency in women of childbearing age was observed prior to food fortification in this study. The results are similar to the findings of Ubbink *et al.*,[7] who reported a high prevalence of plasma folate deficiency (21%) among non-pregnant women of reproductive age in a rural population of Northern Province (now Limpopo Province). The high prevalence of folate deficiency may be due to the low dietary folate intake reported by dietary studies conducted in the study area.[8-10] The present findings are also consistent with several studies carried out in other countries.[5,16,25]

The introduction of a mandatory food fortification programme in South Africa in October 2003 resulted in improved folate status among women of childbearing age in the present study. This is consistent with several studies of folic acid fortification in various parts of the world, which have reported a 63 - 87% reduction in folic acid deficiency after fortification.[18,26,27] A 92.8% reduction in the prevalence of RCF deficiency from 26.4% to 1.9% after fortification, recorded in the present study, agrees with what has been reported by Jacques *et al.*,[4] who found that, among middle-aged/older populations, folate deficiency was reduced from 22% to 1.7%. These authors attributed the improved folate status to the increased bioavailability of synthetic folic acid in fortified foods. Folate deficiency has also been linked to poor socio-economic status leading to poor dietary intake[26] but, in the present study, no association was observed between folate deficiency and the women's socio-economic status (data not shown).

Iron deficiency is considered the leading nutritional deficiency worldwide.[14] The prevalence of iron deficiency is reported to affect up to 50% of both women of childbearing age and infants.[13] Before implementation of iron-fortified foods by national governments, iron supplementation was used as strategy to combat iron deficiency.[14] In the South African fortification programme, electrolytic iron was standardised at an amount of 43 mg/kg in wheat foodstuffs, 37 mg/kg in maize foodstuffs, and 14 mg/kg in unsifted maize meal.[28] Although iron forms part of the fortification programme in South Africa, our results indicate that no significant change in the iron status, as measured by serum ferritin, occurred.

Iron is important for the synthesis of Hb and myoglobin, the iron-containing enzymes essential for electron

Table I. Comparison of biochemical and haematological variables of women of childbearing age before and after fortification

Variables	RR*	Pre-fortification period	Post-fortification period	Median difference	p-value
Sfol (ng/ml)	≥3	3.58 (2.87, 4.22)	10.51 (8.53, 13.52)	7.23 (5.08, 9.33)	0.000‡
RCF (ng/ml)†	≥164	227.01 (153.77, 301.42)	429.29 (367.26, 610.33)	199.37 (130.05, 336.94)	0.000‡
SF (µg/ml)	≥20	35.25 (11.95, 59.0)	30.15 (11.77, 65.35)	0.65 (–8.40, 11.95)	0.99
VB12 (pg/ml)	180 - 914	266.00 (196.25, 394.00)	258.50 (182.0, 363.50)	–24.50 (–75.0, 27.25)	0.02§
RBC (10^8/µl)	4.0 - 6.20	4.56 (4.33, 4.80)	4.74 (4.49, 5.05)	0.18 (–0.03, 0.46)	0.03§
Hb (g/dl)	11.0 - 18.8	13.55 (12.63, 14.20)	14.0 (13.10, 14.60)	0.50 (–0.27, 1.10)	0.000‡
HCT (%)	35.0 - 55.0	39.95 (37.77, 41.77)	41.75 (39.10, 44.30)	1.90 (–0.57, 3.65)	0.04§
MCV (fl)	80.0 - 99.0	89.0 (82.0, 92.0)	88.0 (84.0, 92.0)	0.00 (–1.00, 2.00)	0.69
MCH (pg)	26 - 34	30.25 (27.83, 31.40)	29.75 (28.10, 31.07)	–0.50 (–1.00, –0.50)	0.48
MCHC (g/dl)	31 - 35	33.90 (33.36, 34.25)	33.48 (32.88, 33.85)	–0.41 (–0.67, –0.08)	0.002‡

* Reference range.
† Sample size.
‡ Indicates a significant difference at the level 0.01 (two-tailed).
§ Indicates a significant difference at the level 0.05 (two-tailed).

Table II. Comparison of percentages of women with variables below or above the cut-off points before and after fortification

Variables	Cut-offs	Pre-fortification Period (*N*=80) Below % (*N*)	Above % (*N*)	Post-fortification Period (*N*=80) Below % (*N*)	Above % (*N*)	*p*-values	RR
Sfol (ng/ml)	<2.5	16.3% (13)		0% (0)		0.001*	≥3
	2.5 - 3.0	11.3% (9)		0% (0)			
RCF (ng/ml)†	<164.0	26.4% (14)		1.9% (1)		0.000*	≥164
SF (µg/ml)	<12.0	25% (20)		25% (20)		0.74	≥20
	12 - 19.9	8.8% (7)		12.5% (10)			
VB12 (pg/ml)	<145.0	6.3% (5)		11.3% (9)		0.16	180 - 914
RBC (108/µl)	<4.0	5.0% (4)		2.5% (2)		0.41	4.0 - 6.20
HGB (g/dl)	<11.0	7.5% (6)		5.0% (4)		0.51	11.0 - 8.8
HCT (%)	<35.0	7.5% (6)		8.8% (7)		0.58	35.0 - 55.0
MCV (fl)	≥100		0% (0)		0% (0)	0.63	80.0 - 99.0
MCH (pg)	<26.0	11.3% (9)		13.8% (11)		0.55	26 - 34
MCHC (g/dl)	<31.0	1.3% (1)		2.5% (2)		0.84	31 - 35

* Indicates a significant difference at the level 0.01 (two-tailed).
† Sample size.

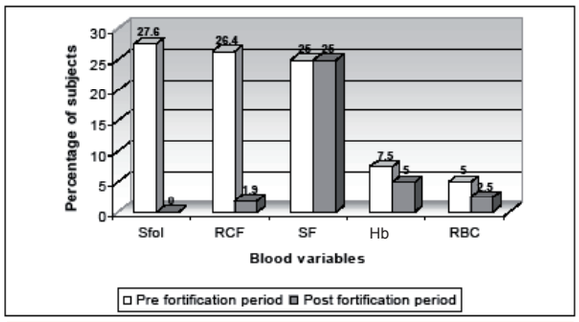

Fig. 1. Percentage of subjects with selected blood variables below the cut-off points before and after fortification.

transfer and oxidation-reduction reactions.[29] Iron bioavailability, defined as the amount of a nutrient ingested, absorbed and utilised for normal metabolic functions, is affected by numerous dietary and host-related factors.[29,30] Research over the last 45 years has produced highly variable results with respect to the bioavailability of iron powders (from 5% to 145% relative to the standard – ferrous sulphate).[13] This suggests that the elementary iron used in South Africa may not be readily available. Ascorbic acid is reported as the most effective enhancer of iron absorption,[31] and is supported by a study which showed that in infants, fortification of cereals with electrolytic iron and ascorbic acid improved their iron status.[19] Our study population has been reported to have low intakes of natural foods containing ascorbic acid but high intakes of food containing tannins, polyphenols, oxalates and phytate.[32,33] These dietary substances are inhibitors of iron absorption[32] and could be among the factors responsible for the high prevalence of iron deficiency before and after fortification.

However, interpretation of our results has to take into account that the sample size involved was small and the methods used to define iron status (Hb and SF) were not sufficient to define iron status. Ferritin is an acute-phase reactant, and inflammation[34] might have influenced the observed levels. On the other hand, Hb concentrations do not depend solely on iron levels, as folate deficiency (among others) can also result in low levels of Hb.[35] These findings nevertheless do justify the need for extensive studies on the iron used in the Department of Health's fortification programme. The awaited findings of the second National Food Consumption Survey may, however, give a clearer picture about the programme.

In South Africa, as in other countries, VB12 does not form part of the fortification programme. Low levels of VB12 were reported in 16.4% of pregnant women in the Capricorn district of Limpopo Province.[15] In our study, deficiency of VB12 was 6.3% and 11.3% during phase 1 and phase 2 of the study, respectively. Therefore, food fortification with VB12 should be considered, as suggested by Allen *et al.*[36]

In conclusion, this study shows significant improvement of folate status in women of childbearing age, after fortification of maize and wheat foodstuffs was introduced in South Africa – but a lack of improvement in iron status (as measured by SF and full blood count). The iron status results have to be interpreted with caution as ferritin is known to be an acute-phase reactant and, as such, is easily affected by infections and inflammations[34] – factors that were not considered in this study. A follow-up study is therefore needed, using more sensitive measures of iron status, to determine the efficacy of iron fortification in this

population. With regard to VB12, our findings support the recommendations made by other researchers[36,37] that food should be fortified with VB12.

This study was supported by a grant from the National Research Foundation and the University of Limpopo, Medical Science Programme. We thank registered nurses, Dikgale DSS fieldworkers and Mr S R Choma for assisting with laboratory analysis, and Mrs R Olwagen for advice on statistics.

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