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Effect of consumption of whole bread baked from wheat cultivated with micronutrient fertilizers on blood indices of iron

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Abstract

A 4-month-long, double-blind field study involving one village control community (n=160) receiving regular whole bread and other community (n=152) receiving whole bread baked from wheat cultivated with microfertilizers was conducted to test the effect of this approach on blood iron indices. Hemoglobin, serum iron and total iron binding capasity and serum ferritin were determined by cyanmethemoglobin, colorimetric and radioimmunoassay methods, respectively. Transferrin saturation was calculated. Hemoglobin concentration was increased only in 5-12 age groups of both experimental and control groups. Transferrin saturation was increased in all age groups of experimental village ($P<0.05$). Serum ferritin in both villages was decreased. Consumption of whole bread baked from cultivated wheat with microfertilizers increased transferrin saturation percentage in all age and gender groups and hemoglobin only in 5-12 age group. Agronomic methods may be useful in preventing iron deficiency anemia.

***Key words*:** Anemia, iron deficiency, micronutrients, bread, microfertilizer.

Introduction

Iron deficiency (ID) is the worlds most widespread nutritional disorder and affects about 5 billion people in developed and developing countries. ID leads to anemia, which afffects nearly 2 billion people worldwide. Overall, 39% of the preschool children and 52% of pregnant women are estimated anemic, more than 90% of whom live in developing countries [1-3]. Prevalence of ID in 5-14 age groups, non-pregnant women and adult men has been reported 38.8, 34 and 9.9 percent, respectively in Iran [4]. Iron deficiency anemia (IDA) results in destruction of educational resources and health cares, decrease of cost benefit because of increasing of morbidity and mortality ratio, reduces the psychological and physical capacity of individuals and entire population, bringing serious economic consequences and obstacles to national development [5, 6]. Conversely, treatment can raise national productivity levels by 20 percent [2].

While the traditional remedy of micronutrients deficiency was in form of food supplements, suitability of agricultural strategies for increasing micronutrient density in grain destined for human consumption is now being assessed as a sustainable, long-term solution [7]. For certain essential micronutrient elements (e.g., Zn, Ni and Se), increasing their supply to food crops can result in significant increases in their concentrations in edible plant products [8]. Concentrations of Zn and Fe in cereal grain increase with an increase in Zn and Fe fertilizer additions [9].

Even though no management practices to increase mineral nutrient concentration in grain destined for human consumption have been described, fertilization aimed at increasing grain nutrient density to allow good crop establishment when that seed is sown in nutrient-deficient soil is reported occasionally. However, comprehensive agronomic approaches, including specific fertilization strategies, aimed at enhancing seed nutrient concentration have yet to be pursued [7]. The objective of the present study was to assess the effect of whole bread baked from whole wheat cultivated with microfertilizers on blood indices of iron.

Materials and Methods

In this double blind field trial, study subjects were residing in two villages, who entered the study after securing a permit from governer of Kandovan district and Nutriton and Food Security Committee of Tabriz University of Medical Sciences and written informed consent. Two villages in Myaneh township, Tajaragh and Khanegha, were considered as experimental and control, respectively. Criteria for selecting were to have bakery, to be far from Myaneh city, to have health house center and to be far from each other. Population list of each village was providing by gender and age. Gender and age groups were categorized based on WHO categories with considering Hb rate [10]. Three hundred and fifty people of each village were selected by random sampling method. Physicians carried out exams and interview for each subject.

People were screened for exclusion criteria which included: renal failure, thalassemia, tuberculosis, infections, to be on diet, blood donation in two recent weeks, drug consumption, using of supplementary vitamins and minerals and menstruation over 7 days, as well as more than one time per month for women. Stool exams were carried out for subjects who remained and those with any parasite infestion were treated.

Micronutrient fertilizer used for wheat cultivation contained zinc (60 kg ha^{-1}) as $ZnSO_4$, iron (18.5 kg ha^{-1}) as $FeSO_4$, manganese (14.5 kg ha^{-1}) as $MnSO_4$, copper (10 kg ha^{-1}) as $CuSO_4$ and sulphur

(S) 350 kg ha^{-1}. Fifty thousand kilograms of the whole wheat was produced and converted into whole flour (100% extraction) at flour factory of Arde-Zorrat in Myaneh city. Same amount of regular wheat, which was cultivated in the same soil without adding micronutrient fertilizer, was converted into whole flour. Micronutrients and phytic acid content of both kinds of wheat were measured by atomic absorption and colorimetric methods, respectively.

Blood samples were taken in fasting state. After separating the needle, 1 cm^3 of the blood was added in a container including 0.2 cm^3 of 5% EDTA for Hb measurement by cyanmethemoglobin method in central laboratory of Myaneh. The subjects who had moderate or sever iron deficiency anemia were treated and were excluded of the study. The serums which were separated by centrifuging 3,000× g for 10 minutes were kept at –18°C. Serum iron, TIBC and serum ferritin were measured by colorimetric and radioimmuonoassay methods, respectively.

Inhabitants of experimental village consumed whole bread baked from fortified wheat and those of control village consumed whole bread baked from regular wheat for 4 months. After this period the same measurements mentioned above were carried out. Food consumption was carried out by one 24-hour recall and two days record of foodstuffs (2-week days and 1 weekend) for each subject.

Statistical analysis was performed with SPSS version 10 software and included means and SDs. Between-group comparisons were made by independent t-test. Basal and final values were compared by paired t-test. Regarding to serum ferritin, since there was no normal distribution, which skewed to the left, non-parameter tests were used (Wilcoxon for basal-final comparison and Man-Whitney test for between-group comparisons). For calculating nutrient intake, food processor software was used.

Results

Out of 700 subjects considered 525 subjects participated in the beginning of the study (257 of Tajaragh village and 268 of Khanegha village). However, 312 subjects gave blood at the end of the study. Subjects of 2-5 age groups were omitted in statistical analysis, because of few numbers. Categorizing of subjects to normal and suffering from ID had no effect on the results.

Table 1 shows the number of participants by sex and age groups at basal and after 4 months. Table 2 shows the amount of bread consumed in each village by sex and age groups. Iron and zinc intake are shown in Table 3.

Analysis of experimental and control wheat is shown in Table 4. Iron and zinc contents of fortified whole wheat were higher than those in regular whole wheat. Phytic acid:zinc ratio in experimental wheat was lower than in control wheat.

Comparison of the iron indices between experimental and control villages is showen in Table 5. Iron deficiency anemia (hemoglobin <12 g/dl and TS%<15% or ferritin <12 μg/l) prevalance decreased 50% in exprimental women of 13-60 age group (from 9 to 4.5%) (p<0.01). No change was observed among men. There were no IDA cases in other groups. In control village IDA prevalance was not changed after 4 months (8.2% in women 13-60 y of age and 6% in men 13-60 y of age). In the other control age and sex groups it was not seen any IDA case.

Table 1. The number of participants of the study by age and sex groups after 0 and 4 months.

Group	Experimental community		Control community	
	0 months	4 months	0 months	4 months
M and F, 2-5 y of age	9	4	12	3
F & M, 5-12 y of age	51	45	48	28
F & M, 12&13 y of age	26	16	13	10
F, 13-50 y of age	93	66	86	75
F, 50-60 y of age	5	2	3	2
M, 13-60 y of age	68	28	61	34
Total	252	160	223	152

Table 2. The amount of bread consumption (g d^{-1}) in the study communities.

Group	Experimental community	Control community
F & M, 5-12 y of age	350±30	342±25
F & M, 12&13 y of age	380±33	380±30
F, 13-60 y of age	440±35	435±28
M, 13-60 y of age	450±30	442±25

Table 3. Iron and zinc intake in experimental and control villages by age and sex groups.

Group	Iron (mg/d)		Zinc (mg/d)	
	Experimental	Control	Experimental	Control
F & M, 5-12 y of age	32.0±4.0	31.2±4.5	10.8±1.3**	8.3±1.6**
F & M, 12&13 y of age	35.5±3.5	35.1±4.0	15.8±1.8**	9.8±1.5**
F, 13-60 y of age	40.0±6.0**	36.0±4.5**	19.8±1.0**	10.0±1.3**
M, 13-60 y of age	50.0±3.1**	45.4±6.0**	23.2±1.2**	13.2±1.6**

Table 4. Specification of experimental and control wheat.

Index	Enriched wheat	Regular wheat
Calcium mg/100 g	50±10	40±20
Magnesium mg/100 g	140±20	90±30
Copper mg/100 g	45±0.07	0.38±0.05
Iron mg/100 g	4.5±0.08**	3.9±0.04**
Zinc mg/100 g	3.69±0.02**	1.79±0.01**
Phytic acid mg/100 g	710±20*	470±30*
PA/Zn ratio	21.9±1.3*	25.4±0.8*

*P<0.05, **P<0.01

Discussion

Mean hemoglobin concentration increased in the 5-12 y of age group in both villages (P<0.05), but did not change in other age and sex groups. Zlotkin and colleagues showed that fortification of flour with iron and zinc together increased Hb less than fortification with iron alone [11]. However, in their study subjects suffered from iron deficiency anemia, while in our study the initial mean Hb was high. In persons with low Hb, iron is absorbed as well and iron indices are improved fast [12]. Viteri et al. stated that fortification of sugar with iron resulted in increased hemoglobin concentration in 5-7 age group [13]. However, fortificant and vehicle were different than those used in our study.

Digkhuizen and coworkers showed that decrease in iron deficiency anemia and increase in Hb concentration in Indonesian children who received iron supplement alone were higher than in those who consumed iron–zinc supplement, because of iron–zinc interaction [14]. In our study there was not any interaction between iron and zinc, maybe due to low molar ratio Zn:Fe. Negative effect on iron absorption was observed when this ratio was 5:1,while no effect was shown with 1:1 [15].

Table 5. The iron indices in gender and age groups of experimental and control communities after 0 and 4 months.

Index	Group	Experimental community 0	Experimental community 4	Control community 0	Control community 4
Hb(g/dl)	F & M, 5-12 y	13.2±0.9*	13.6±1*	13.5±0.6*	13.9±0.6*
	F & M, 12&13 y	13.6±0.9	13.5±0.9	13.7±0.9	13.6±1.3
	F, 13-60 y	13.3±1.4	13.6±1.1	13.5±1.7	13.7±0.9
	M, 13-60 y	14.8±1.5	14.9±1.7	14.2±1.9	14.4±1.3
TS(%)	F & M, 5-12 y	17.5±9.7*	20.1±7.8*[a]	16.8±8.1	16.6±4.3[a]
	F & M, 12&13 y	17.1±5*	19.7±7.4*[a]	18.1±7.3*	15.9±5.7*[a]
	F 13-60 y	16.4±6.1*	18.3±5.4*[a]	16.7±4.5	17.1±5[a]
	M 13-60 y	17.7±7.7*	21.4±5.6*[a]	17.3±7.9	18.1±4[a]
Ferritin (μg/l)	F & M 5-12 y	34.8±22*	21.8±14.5*	34.7±22.9*	23.4±13.5*
	F & M, 12&13 y	31.1±21.9**	12.9±6**	28.4±20**	13.8±9.3**
	F, 13-60 y	25.1±3.1**	14.5±11.9**	28.4±42**	18.3±15.9**
	M, 13-60 y	39.9±37.5*[b]	26.8±30.2*[a]	77.2±52**[b]	56.6±42**[a]

*P<0.05, difference between initial and final results for each group
**P<0.05, difference between initial and final results for each group
a P<0.05, difference between groups at the end of experiment
b P<0.05, difference between groups at basal of experiment

TS% increased in all groups of the experimental village (P<0.05). Serum ferritin decreased in both villages after 4 months (P<0.01). Sandstrom reported that consumption of diet containing high phytate after 8 months resulted in serum ferritin decrease [16]. Decrease in both villages may be due to bran content of bread. We could not find another reason to ferritin decreasing , in spite of TS(%) increasing. Levrat–Lverny and colleagues showed that consumption of whole wheat enriched with phytic acid improved minerals bioavailability in rats, iron absorption from whole flour was 2-fold of that from white flour [17]. However, more research on human is needed. Some authors have reported negative effects of phytate on mineral status [18, 19]. Phytate was analysed in rat intestine by phytase [17]. Phytic acid usually is consumed with completed charbohydrates, so their fermentation results in low pH in colone and this situation is proper for phytase activation. In human, remained phytase of wheat in stomach impacts on phytic acid [17]. Effect of phytate on iron absorption is controversial. Difference in results of studies may be due to differences in body iron store, analytical methods, iron content and its chemical form in diet and other factors [20].

In our study, results of both wheat batches were nearly similar, except TS%. Perhaps it was due to the amount of iron in both kinds of wheat. Pean et al. showed that iron absorption from fortified bread (Fe content 24 $mgkg^{-1}$) was 10.5% [21]. Iron content of our control wheat was high (39 $mg\ kg^{-1}$). Meanwhile, phytic acid : zinc molar ratio in both wheat batches was higher than 15 . However, mineral absorption is proper in less than 15 ratio [22].

The field trial was not without problems, some of which could weaken or invalidate the results. The reduction in number of subjects could be responsible for explaining some of the results, just as Herman and colleagues have stated in their study [23]. Since fortification is primarily a preventive rather than a therapeutic measure for iron deficiency, and for ethical reasons, any individual with moderate or sever anemia (Hb<100 g^{-1}) was given a course of iron therapy. Despite these shortcomings, there was a 50% decline in IDA prevalance in women 13-60 y of age in the experimental village.

Conclusions

Consumption of whole bread baked from wheat cultivated with microfertilizers increased TS(%) in all age and sex groups but Hb only in 5-12 age groups. Further studies are needed to determine the effect of this bread on blood iron indices.

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