Spring 1979, Vol. 8,No.1

BREAD ENRICHMENT IN IRAN

Hemoglobin and Serum Protein Response of Adults to Iron Fortified Bread in Low Income Groups *

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ABSTRACT

Iron deficiency anemia is one of the untritional problems of the low income, undernouished populations of the world. In Iran, many rural and low socioeconomic urban groups suffer from iron deficiency anemia because of a low iron intake and the low level of public health. To overcome and to prevent iron deficiency, a program was suggested to enrich the bread with iron, calcium and some of the B vitamins.

A study was undertaken to test the efficacy of such a program. Fifty families in the 13th District of Tehran were contacted. All members of each household were considered as the subjects of the study. As participants, they received free bread in exchange for samples of blood. Enriched bread was baked by one bakery located on the same street as eighteen of the families. Regular bread was baked by another bakery near ten families.

At the beginning and after the termination of the program, blood samples were secured from individual members of the families. Hemoglobin, hematocrit, serum protein fractions were determined. During the two months,

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during which enriched bread was consumed, hemoglobin and some of the serum protein fractions were elevated.

Introduction

Iron deficiency anemia is one of the nutritional problems of the low income, undernourished, underprivileged population around the world. Prevalence of anemia among the poverty stricken groups in the world is due primarily to the fact that intake of both iron rich foods and animal protein is low in their diet. In most countries, cereals provide most of the calories and iron. In the Western diet, cereals contribute a third of the total calories and iron (1) while in the Easterm countries, cereals converted into high extraction flour in unleavened bread, comprise more than two-thirds of the caloric and iron intake (2,3).

Reports on food surveys have often uncovered the areas of the globe where the problem exists (4-6). A recent ten state survey of the United States (7) and a survey of the migrant farm workers of North America (8), as well as reports from South America, also indicate evidence of anemia (9). Iran is no exception as iron deficiency consistently presents a public health problem, especially among women during child bearing years, and in children. Reports from the southern province of Fars in Iran indicate that the incidence of iron deficiency among the rural population there is significant (10). Mofidi (6) has also reported on the incidence of iron deficiency anemia in rural Iran. Some of these studies attribute the anemia to a high intake of phytates (3, 11, 12).

In recent decades, many attempts have been made toward enriching staple foods with iron. Recently, the Food and Drug Administration of the United States increased the level of enrichment of wheat flour and bread to 25 mg iron/lb of bread and 40 mg/lb of flour (13, 14). This has led to a very controversial argument (17 - 20).

Availability of iron in the diet and in supplements has been tested to secure more information about the practicability of its fortification. Hussein et al (15) tested the availability of wheat iron vs. a dose of ferrous ascorbate. The iron salt was absorbed more than the wheat iron in iron deficient subjects. Callender and Warner (16) observed that the availability of iron was higher from a standard 5 mg dose of ferrous iron than from bread containing labelled ferric ammonium citrate; the addition of orange Juice en-

hanced iron absorption in all instances. In another report, Callender and Warner (17) observed that Fe^{59} in brown bread was absorbed to a single dose of ferrous iron in the form of ferrous sulfate. Elwood and coworkers (1) found that the availability of iron in ferric ammonium citrate is similar to that of iron in white flour; fruit juice enhanced the absorption of the iron salt. White and Gynne (18) studied the availability of iron using a general Western diet with bread and rolls fortified with ferrous sulfate and ferric phosphate. Iron was absorbed in sufficient amounts to meet the requirements.

Björn-Rasmussen and coworker (19) demonstrated that the presence of non-iron compounds in different foods may favor or inhibit iron absorption, giving rise to variation in absorbability. Cook and colleagues (20) reported that absorption of iron pyrophosphate and ferric orthophosphate was less than that of ferrous sulfate, whereas ferrous sulfate and reduced iron were about equally available. Senchak and colleagues (21) observed an increase in the availability of iron when the rice content of the diet was higher than wheat, there was less availability of iron when the rice content of the diet was lower than wheat; phosphorus seemed to affect iron absorption. The authors point out the importance of interactions between nutrients whenever the structure of the diet is altered. Swiss and Beaton (22) advocated a multiple fortification program (bread, cereals and baked products other than bread) to eradicate the risk of iron deficiency in Canada. Vaghefi et al (23) reported the results adding ferric ammonium citrate to bead dough in a mixture with yeast. The iron was biologically available and produced a mean increase in serum iron in young adult females.

This study was designed to test the practicability and the results of an iron enrichment program in a community under normal living conditions in a low income group.

Procedure

In a community northeast of the city of Tehran, where most of the inhabitants are recent migrants with low incomes, 50 families were contacted at random, interviewed and asked to participate in this study. Those who expressed willingness to cooperate were tested for hemoglobin. Those having hemoglobins lower than 12.00g percent were selected

and randomly divided into two groups of control and study. Care was taken to have an equal number of anemic subjects in both groups in respect to age and sex. The control group in which 100 individuals participated received regular bread baked by their neighborhood bakery. One hundred individuals in the study group received enriched bread from a second bakery in the vicinty of their homes. Special coupons marked with the name of the bakery were issued to the heads of the household, indicating the quantity of bead adequate for 24-hour consumption by the entire family. Arrangements were made with the bakeries, and bread was only given to the subjects in exchange for the coupons; the bakers were reimbursed accordingly. This method of control protected each group from getting bread from the wrong bakery. Leavening packages containing yeast, calcium, flour filler and tumeric were provided for the control bakery; yeast, calcium, flour filler, ferric ammonium sulfate and riboflavin were provided the study group's bakery. These were delivered to the bakeries by two different technicians who supervised the preparation of the dough every day. The bakers were not aware of the difference in the yeast mixtures. Composition of the yeast nutrient mixture is shown in Table I. Bread allowances were made for all members of the family in order to assure that those with low hemoglobin received the fortified bread.

Blood samples were taken at the beginning and at the end of the two months study period. Subjects were eliminated who had any illness or problems during this period; some were dropped because they did not secure bread from the assigned bakery; others were deleted who refuse to give blood at the end of the study. Thus, there were 66 people in the study and 31 in the control group who were included in the evaluation.

Fingertip blood was secured in two capillary tubes as well as in 0.02 ml pipettes for hemoglobin determination by the cyanomethemoglobin method. Serum was collected from the microhematocrit tubes and used for determining serum protein and protein fractions. Serum protein was determined by a micro modification of the Biuret method. Serum protein fractions were measured by electrophoresis using cellulose acetate strips. The protein fractions were estimated with a recording desitometer after the fractions were stained.

Results and Discussion

A formula containing ferric ammonium citrate, riboflavin and calcuim carbonate was developed in amounts corresponding to 4.98, 0.2 and 148.0 mg of the untrients per 100 g of flour, respectively. This mixture was combined with equal amount of flour. The final baked product provided approximately 4.15 mg iron, 0.17 mg riboflavin and 123.2 mg calcium per 100 g of bread (Table I).

As Table II shows, the level of hemoglobin in the male subjects did not change after two months in which the enriched bread was consumed. However, total protein was elevated significantly in the study group. When these changes were studied among the different age groups, male subjects below 13 years (Table II_2) and over 25 (Table II_3) showed a more pronounced change in their level of serum total protein. Serum albumin and α_1 globulin were lowered in the control group and serum γ globulin was elevated in the control as well as in the study group (Table II). Changes in the γ globulin were much more pronounced in males younger than 13 years in the study group (Table II_2) and in the control group between the ages of 12 and 26 years (Table II_1).

The hemoglobin level of female subjects in both control and study groups was unchanged (Table III). Serum total protein level was increased significantly in the study group. This change was more pronounced in the age group below 13 years (Table III_2).

Serum albumin was lowered in both control and study groups in female subjects (Table III). Serum globulins were elevated, especially γ globulin which was raised in both control and study groups. As shown in Table II, the changes in other globulin fractions were not consistent, the only noticeable change was in the γ globulin.

Comparing the different age groups, only the women of child bearing age showed a significant increase in their hemoglobin leved (Table III and Table III_3). Changes in serum total protein levels were evident in all age groups, especially in those younger than 13 years (Table III_2).

When all the subjects are considered together, the changes are more noticeable, as is shown in Table IV. Hemoglobin levels were elevated significantly ($P<0.01$) in the group that consumed enriched bread. Total protein was significantly elevated in both control and study groups.

This indicates that having access to free bread somehow improved the protein metabolism of these people, quite likely by augmenting the small quantity of protein in their typical daily diet. It could be interpreted that the diet of these people was not only deficient in iron, but also deficient in calories. The distribution of free bread enabled the subjects to utilize funds for securing bread to purchase other nutritious foods. It is believed this is what occurred, since the rise in serum total protein was evident in both the control and study groups.

This significant rise in serum γ globulin can be attributed to the fact that the study was carried out in the months of summer when diseases are prevalent, especially in the low income groups, and where the standared of public health are also low.

Because it is such a physically debilitating disease, the prevalence of iron deficiency anemia among the people of a nation may be detrimental to the man power as well as to the economy of the country. Data suggest that the deleterious effects of iron deficiency are not limited to physical fatigue and disability but also affect the intellectual performance in conjunction with the level of hemoglobin (24).

Data collected by Martinez-Torres and Layrisse (25) on iron absorption and fortification suggest that iron deficiency anemia throughout the world cannot be prevented soley by diet; an increase through iron fortification is considered to be the solution.

Iron may be involved in biochemical functions other than energy transport system and circulation. Robbins and Pederson suggest that iron may have a role in cell division (26). Thus, it may be assumed there are other harmful effects related to an iron deficiency that are yet unknown.

This study showed that when iron is added to bread it can be effective in raising the hemoglobin level of some individuals, probably those who are free of parasites. Because of some difficulties inherent in such studies, especially those in the low income brackets, we were not able to screen the subjects for intestinal parasites.

ACKNOWLEDGEMENTS

Those study was supported in part by funds from the school

of Public Health and the Institute of Public Health Research, University of Tehran. Iran Mayeh Co. generously provided the yeast and secured the cooperation of the local bakers in this study. The technical assistance of N. Agheli and M. J. Mohamadiha is sincerely appreciated.

Table I

Composition of Vitamin-Mineral Salt Mixture

Supplement	Enrichment vehicle added	Nutrient Enrichment in flour	Enrichment level in bread*
	----------	mg/100 g	----------
Ferric Ammonium Citrate	33.0	4.98	4.15
Riboflavin	0.2	0.2	0.17
Calcium Carbonate	370.0	148.0	123.2

* 100 g flour = 120 g bread. Nutrients in 100 g flour = 403.2 mg plus 96.8 mg flour filler.

Table II

Changes in blood components in male subjects during a two-month period consuming enriched bread and changes in the male controls.

	Control			Study		
	Before	After	Level of signi-ficance	Before	After	Level of signi-ficance
Number	29	29		11	11	
Hemoglobin g%	12.31	12.66	N.S	11.58	12.29	N.S.
Total Protein g%	6.19	6.27	N.S.	5.37	6.59	P<0.01
Albumin %	50.55	45.55	P<0.05	47.93	45.28	N.S.
α_1 Globulin %	3.55	2.82	P<0.01	3.69	3.00	P<0.01
α_2 Globulin %	14.73	13.91	N.S.	12.93	14.24	N.S.
β Globulin %	14.36	14.82	N.S.	14.66	14.38	N.S.
γ Globulin %	16.82	24.09	P<0.001	20.79	22.41	P<0.05

Table II_1

Changes in blood components in male subjects 12 to 26 years of age durin a two-month period consuming enriched bread and changes in the male controls.

	Control			Study		
	Before	After	Level of signi-ficance	Before	After	Level of signi-ficance
Number	5	5		7	7	
Hemoglobin g%	12.32	12.50	N.S.	11.65	12.42	N.S.
Total Protein g%	6.27	6.27	N.S.	5.35	6.35	N.S.
Albumin %	53.25	43.75	$P<0.05$	45.00	45.50	N.S.
α_1 Globulin %	3.50	2.27	N.S.	3.75	2.50	N.S.
α_2 Globulin %	15.00	14.25	N.S.	11.50	13.50	N.S.
β Globulin %	12.50	16.50	$P<0.05$	15.00	14.50	N.S.
γ Globulin %	15.75	26.00	$P<0.05$	24.75	24.00	N.S.

Table II_2

Changes in blood components in male subjects younger than 13 years of age during a two-month period consuming enriched bread and changes in the male controls.

	Control			Study		
	Before	After	Level of signi-ficance	Before	After	Level of signi-ficance
Number	6	6		16	16	
Hemoglobin g%	11.84	12.44	N.S.	10.79	11.27	N.S.
Total Protein g%	5.84	5.74	N.S.	5.17	6.02	P<0.01
Albumin %	49.00	44.40	N.S.	48.75	45.75	N.S.
α_1 Globulin %	3.60	2.60	P<0.05	3.63	2.94	P<0.05
α_2 Globulin %	14.4	14.20	N.S.	13.31	13.63	N.S.
β Globulin %	16.00	14.20	N.S.	15.50	14.06	N.S.
γ Globulin %	17.00	24.60	P<0.001	18.81	22.38	P<0.05

Table II_3

Changes in blood components in male subjects older than 25 years of age during a two-month period consuming enriched bread and changes in the male controls.

	Control			Study		
	Before	After	Level of signi-ficance	Before	After	Level of signi-ficance
Number	2	2		9	9	
Hemoglobin g%	13.45	13.55	N.S.	12.96	14.04	N.S.
Total Protein g%	6.90	7.00	N.S.	5.74	7.70	$P<0.001$
Albumin %	49.00	52.00	N.S.	47.78	44.33	N.S.
α_1 Globulin %	3.50	3.50	N.S.	3.78	3.33	N.S.
α_2 Globulin %	15.00	12.50	N.S.	12.89	15.67	$P<0.05$
β Globulin %	14.00	13.00	N.S.	13.00	14.89	$P<0.05$
γ Globulin %	18.50	19.00	N.S.	22.56	21.78	N.S.

Table III

Changes in blood components in female subjects during a two-month period consuming enriched bread and changes in the female controls.

	Control			Study		
	Before	After	Level of signi-ficance	Before	After	Level of signi-ficance
Number	20	20		37	37	
Hemoglobin g%	11.58	11.53	N.S.	11.04	11.68	N.S.
Total Protein g%	6.06	6.60	N.S.	5.96	6.70	$P<0.01$
Albumin %	43.75	40.95	$P<0.01$	46.57	43.35	$P<0.05$
α_1 Globulin %	3.25	3.00	N.S.	3.43	2.84	$P<0.01$
α_2 Globulin %	16.70	13.85	$P<0.001$	13.59	14.30	N.S.
β Globulin %	14.60	16.35	$P<0.05$	14.38	15.11	N.S.
γ Globulin %	21.70	25.35	$P<0.01$	22.03	24.59	$P<0.01$

Table III_1

Changes in blood components in female subjects 12 to 26 years of age during a two-month period consuming enriched bread and changes in the female controls.

	Control			Study		
	Before	After	Level of signi-ficance	Before	After	Level of signi-ficance
Number	7	7		10	10	
Hemoglobin g%	12.27	12.46	N.S.	10.68	11.92	$P<0.05$
Total Protein g%	5.43	6.71	$P<0.05$	6.13	6.57	N.S.
Albumin %	43.86	43.86	N.S.	48.33	44.00	N.S.
α_1 Globulin %	3.57	3.14	N.S.	2.50	3.00	N.S.
α_2 Globulin %	16.29	13.00	$P<0.05$	13.50	15.33	N.S.
β Globulin %	16.14	17.29	N.S.	14.67	14.17	N.S.
γ Globulin %	20.14	22.71	N.S.	21.00	25.17	$P<0.001$

Table III_2

Changes in blood components in female subjects younger than 13 years of age during a two-month period consuming enriched bread and changes in the female controls.

	Control			Study		
	Before	After	Level of significance	Before	After	Level of significance
Number	6	6		18	18	
Hemoglobin g%	11.26	11.27	N.S.	10.91	11.56	N.S.
Total Protein g%	6.21	5.70	N.S.	5.67	6.61	$P<0.01$
Albumin %	47.43	40.00	$P<0.05$	47.22	44.50	N.S.
α_1 Globulin %	2.86	3.14	N.S.	3.78	2.94	$P<0.001$
α_2 Globulin %	17.57	14.57	N.S.	14.06	14.50	N.S.
β Globulin %	12.57	15.57	N.S.	13.83	14.78	N.S.
γ Globulin %	19.57	25.29	$P<0.01$	21.11	23.11	N.S.

Table III_3

Changes in blood components in female subjects older than 25 years of age during a two-month period consuming enriched bread and changes in the female controls.

	Control			Study		
	Before	After	Level of signi-ficance	Before	After	Level of signi-ficance
Number	6	6		13	13	
Hemoglobin g%	11.17	10.17	N.S.	11.38	12.95	$P<0.05$
Total Protein g%	6.60	7.53	N.S.	6.28	6.80	N.S.
Albumin %	39.33	38.67	N.S.	44.85	41.46	N.S.
α_1 Globulin %	3.33	2.67	N.S.	3.38	2.62	$P<0.05$
α_2 Globulin %	16.17	14.00	N.S.	13.00	13.54	N.S.
β Globulin %	15.17	16.17	N.S.	15.00	16.00	N.S.
γ Globulin %	26.00	28.50	N.S.	23.77	26.38	N.S.

Table IV

Changes in blood components in all subjects during a two-month period consuming enriched bread and changes in controls.

	Control			Study		
	Before	After	Standard Deviation	Before	After	Standard Deviation
Number	31	31		66	66	
Hemoglobin g%	11.8	11.9	0.09	11.3	12.0	0.67
Total Protein g%	6.1	7.0	0.38	5.7	6.7	0.95
Albumin %	46.2	42.6	0.58	47.2	44.2	2.97
α_1 Globulin %	3.4	2.9	0.42	3.6	2.9	0.64
α_2 Globulin %	16.0	13.9	2.13	13.3	14.3	0.97
β Globulin %	14.5	15.8	1.29	14.5	14.8	0.29
γ Globulin %	20.0	24.9	2.15	21.5	23.6	4.97

Table IV_1

Changes in blood components in all subjects during a two-month period consuming enriched bread and changes in controls.

	Control			Study		
	Before	After	Level of signi-ficance	Before	After	Level of signi-ficance
Number	8	8		22	22	
Hemoglobin g%	11.74	11.46	N.S.	12.02	12.92	$P<0.5$
Total Protein g%	6.67	7.40	N.S.	6.06	7.21	$P<0.001$
Albumin %	41.75	42.00	N.S.	46.05	42.64	$P<0.5$
α_1 Globulin %	3.38	2.88	N.S.	3.55	2.91	$P<0.05$
α_2 Globulin %	15.88	13.63	N.S.	12.95	14.41	$P<0.5$
β Globulin %	14.88	15.38	N.S.	14.18	15.55	N.S.
γ Globulin %	24.13	26.13	N.S.	23.27	24.50	N.S.

Table IV_2

Changes in blood components in all subjects younger than 13 years of age during a two-month period consuming enriched bread and changes in controls.

	Control			Study		
	Before	After	Level of signi-ficance	Before	After	Level of signi-ficance
Number	12	12		34	34	
Hemoglobin g%	11.50	11.76	N.S.	10,86	11.43	N.S.
Total Protein g%	6.06	5.72	N.S.	5.44	6.34	P.<0.001
Albumin %	48.08	41.83	P<0.05	47.94	45.09	N.S.
α_1 Globulin %	3.17	2.92	N.S.	3.71	2.94	P<0.001
α_2 Globulin %	16.25	14.42	N.S.	13.71	14.09	N.S.
β Globulin %	14.00	15.00	N.S.	14.62	14.44	N.S.
γ Globulin %	18.50	25.00	P<0.001	20.03	22.76	P<0.05

Table IV$_3$

Changes in blood components in all subjects from 12 to 26 years of age during a two-month period consuming enriched bread and changes in controls.

	Control			Study		
	Before	After	Level of signi-ficance	Before	After	Level of signi-ficance
Number	12	12		17	17	
Hemoglobin g%	12.29	12.47	N.S.	11.07	11.58	N.S.
Total Protein g%	5.74	6.66	$P<0.05$	5.82	6.48	N.S.
Albumin %	47.27	43.82	N.S.	47.00	44.60	N.S..
α_1 Globulin %	3.55	3.00	N.S.	3.00	2.80	N.S.
α_2 Globulin %	15.82	13.45	$P<0.05$	12.70	14.60	N.S.
β Globulin %	14.82	17.00	N.S.	14.80	14.30	N.S.
γ Globulin %	18.55	23.91	$P<0.001$	22.50	24.70	N.S.

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