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Impact of daily consumption of iron fortified ready-to-eat cereal and pumpkin seed kernels (*Cucurbita pepo*) on serum iron in adult women

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Abstract. Iron deficiency, anemia, is the most prevalent nutritional problem in the world today.

The objective of this study was to consider the effectiveness of consumption of iron fortified ready-to-eat cereal and pumpkin seed kernels as two sources of dietary iron on status of iron nutrition and response of hematological characteristics of women at reproductive ages. Eight healthy female, single or non pregnant subjects, aged 20–37 y consumed 30 g of iron fortified ready-to-eat cereal (providing 7.1 mg iron/day) plus 30 g of pumpkin seed kernels (providing 4.0 mg iron/day) for four weeks. Blood samples collected on the day 20 of menstrual cycles before and after consumption and indices of iron status such as reticulocyte count, hemoglobin (Hb), hematocrit (Ht), serum ferritin, iron, total iron-binding capacity (TIBC), transferrin and transferrin saturation percent were determined. Better response for iron status was observed after consumption period. The statistical analysis showed a significant difference between the pre and post consumption phase for higher serum iron (60 ± 22 vs. 85 ± 23 ug/dl), higher transferrin saturation percent (16.8 ± 8.0 vs. $25.6 \pm 9.0\%$), and lower TIBC (367 ± 31 vs. 339 ± 31 ug/dl). All individuals had higher serum iron after consumption. A significant positive correlation ($r = 0.981$, $p = 0.000$) between the differences in serum iron levels and differences in transferrin saturation percentages and a significant negative correlation ($r = -0.916$, $p < 0.001$) between the differences in serum iron levels and differences in TIBC was found, as well.

Fortified foods contribute to maintaining optimal nutritional status and minimizing the likelihood of iron insufficiencies and use of fortified ready-to-eat cereals is a common strategy. The results showed that adding another food source of iron such as pumpkin seed kernels improves the iron status. Additional and longer studies using these two food products are recommended to further determine the effect of iron fortification on iron nutrition and status among the target population, and mainly in young children, adolescents, women of reproductive ages and pregnant women.

Keywords: Ready-to-eat cereal, pumpkin seed kernels, iron deficiency, anemia, women

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1. Introduction

Iron deficiency and anemia are among the most common nutritional problems in the world affecting billions of people through the world. These are frequently prevalent in both underdeveloped and developing countries [15] and mainly affect infants, young children, adolescents, women of reproductive age and pregnant women [21]. It impairs growth and development cognition and immune function, as well as school and work performance. Iron is an essential component of hemoglobin and myoglobin, the oxygen carrying proteins in blood and muscle cells. To fulfill its key functions, to transport oxygen, iron has to be absorbed and utilized. Thus, absorption, mobilization, transport and cell formation mechanisms have to be intact. This requires adequate levels of several other essential nutrients acting in concert. Only about half of anemia is thought to be related to iron deficiency; other micronutrient deficiencies such as vitamin A, vitamin C, B-vitamins, zinc and copper as well as infections and undernutrition also play a crucial role. Several strategies are used to prevent and treat iron deficiency: varying the diet, adding medical supplements and fortifying the iron content of some foods. Improvements in serum iron, transferrin saturation and hemoglobin occur only when vitamin A status is optimized [19]. Ascorbic acid seems to improve the bioavailability of elemental iron even when used at a low molar ratio. If this enhancing effect is confirmed, ascorbic acid could be used together with elemental iron as a fortificant [12].

Consumption of dietary sources of iron over supplemental sources is recommended when possible, because food has physiological factors that improve iron absorption and other factors beneficial to overall health. Low compliance with iron supplementations among women has been reported [18] and symptoms of abdominal discomfort. Bloating, nausea, heartburn, diarrhea, or constipations are among the causes responsible for discontinuation or rejection of the use of iron supplements. Enhancement or fortification consists of adding iron to the composition of some foods. There is no such thing as an ideal food. The enhancement strategy must take into account the diet of the target population, the cost of fortification, the shelf-life of the food, and its compatibility with the chemical form of iron used for the enhancement (taste, color, and bioavailability). Thus, companies began to enrich milled grain products since half a century ago and enriched their products with iron and other micronutrients as a mechanism to reduce diseases and conditions of nutrient deficiency. At present, a significant amount of research is being conducted to evaluate ways to increase iron intake, and iron fortification of basic foods is the most economical and more convenient approach and has the advantages that it does not require food habit modifications. It is reported that consumption of fortified ready-to-eat cereals would increase the intake of iron and vitamins and do not represent a risk of over consumption in adults whose requirements are lower [1]. The food vehicle is a key issue or an important step for the success of a fortification program. The food vehicle selected should reach the entire population and deliver most of the calories of the diet and has to be consumed daily. In underdeveloped countries cereal flours, especially wheat, rice, and corn are frequently used as fortification vehicles, because grain products are the staple foods for that population [9], and other common and similar fortified foods in industrialized countries could provide a significant amount of iron, as well [7]. In addition, these food vehicles or products are suitable to be added with different nuts and dry fruits and similar food items, to increase nutrition values of these products. Thus, it was decided to include the pumpkin seed kernels (*Cucurbita pepo*) as an additive and extra source of iron to a ready-to-eat cereal product fortified with iron, with the assumption to increasingly elevate the daily intake of iron. The general objective of this study was to assess the effectiveness of consumption of two natural food items or products as a source of dietary iron on improvement of the iron nutrition and status of serum iron profile in women at menstruating ages.

2. Subjects and methods

Eight female subjects, either single or non pregnant married women aged 20–37 y were recruited to participate in this randomized, cross sectional, and prospective study. At the time of recruitment, the volunteers were not consuming nutrient supplements containing iron or any kind of medicines and were free from gastrointestinal disorders. They all signed a consent form and were asked to stay on their habitual and regular diet throughout the study. The height and weight of each individual was determined for BMI calculation. In the test phase, purchased fortified ready-to-eat cereal and pumpkin seed kernels were provided as products rich in iron. They were asked to consume 30 gm of cereal (providing approximately 7.1 mg iron per day) with breakfast meal, plus 30 gm of pumpkin seed kernels (providing approximately 4.0 mg iron per day) [22] during the day as a snack for four weeks.

It was intended to add 10–11 mg iron to their regular daily intake which is nearly equal to the level of recommended daily intake (RDA). Subjects were asked to not drink tea when taking the food products, which could have inhibited iron absorption. Compliance for consumptions was supervised by recording on a form and by checking out the leftovers, if any exists. Blood samples were collected on the day 20 of menstrual cycle in an accredited medical diagnosis laboratory before the consumption trial began (Before study) and again after 4 weeks or the day 20 of the next menstrual cycle (After study). Determination of iron status such as reticulocyte count, hemoglobin, hematocrit, serum ferritin, serum iron, total iron binding capacity (TIBC), transferrin and transferrin saturation percent was undertaken. Reticulocyte counts were analyzed with Sysmex K-1000 and total serum iron, TIBC and transferrin were determined by Auto Analyzer RA 1000, and the levels of serum ferritin were determined by RIA.

Analytical statistics (mean determinations [paired t-test]) was used to compare the data obtained at two test periods. Data was processed and analyzed by using the SAS statistical package (SAS Institute Inc. Cary, NC). Pearson correlation was used to see the relation between serum iron with TIBC and transferrin saturation percent.

3. Results

The commercial cereal used as the experimental food product was Kellogg's Special K Breakfast Cereal (UK). This product contains a mix of rice and wheat flakes and a 30 g serving provides at least 50% of the recommended daily allowances (RDA) of selected vitamins and minerals, especially iron (Table 1), as reported by manufacturer.

Anthropometric and hematological parameters were determined for each of the subjects participated in the study. They were in apparent good health with a Mean \pm SD of age 29 ± 6 and BMI 24 ± 2 . Hematological profiles are shown in Table 2.

The statistical comparisons showed a significant difference between the pre and post consumption phase for serum iron, transferrin saturation percent, and TIBC. The subjects had significantly higher serum iron level ($p = 0.004$), and transferrin saturation percent level ($p = 0.008$), with decrease in TIBC ($p = 0.03$); which are considered as indicators of improvement in serum iron status. It is expected that upon increase in iron intake, transferrin saturation percentage increases and consequently TIBC decreases as a result of iron repletion.

Individual iron concentrations in the study group and changes overtime are shown in Fig. 1.

The increased differences in serum iron levels between the fortification condition and baseline phase correlated strongly with the increased differences in transferrin saturation percentages ($r = 0.981$, $p = 0.001$), (Fig. 2).

Table 1

Declared nutrient contents per 100 g (dry weight) portion of the cereal

Energy	370 (Kcal)	
Protein	16 g	
Carbohydrate:	74 g	
Sugars	17 g	
Starch	57 g	
Fat	1 g	
Fibre	3 g	
Sodium	0.85 g	
Vitamins:		(%RDA)
Vitamin D	8.3 ug	(165)
Vitamin C	100 mg	(165)
Thiamin (B1)	2.3 mg	(165)
Riboflavin	2.7 mg	(165)
Niacin	30 mg	(165)
Vitamin B6	3.3 mg	(165)
Folic Acid	333 mg	(165)
Vitamin B12	1.7 mg	(165)
Mineral:		
Iron	23.3 mg	(165)

Table 2

Hematological characteristics of the subjects consuming cereals and Kernels (mean \pm SD)

Hematological profile	Before	After	p-value
R.B.C (Mill/ μ l)	4.8 \pm 0.31	4.8 \pm 0.25	NS
Hb (mg/dl)	13.2 \pm 1.4	13.2 \pm 1.1	NS
H t (%)	40.7 \pm 4.0	40.4 \pm 3.3	NS
MCV (C μ μ)	85 \pm 5.2	85 \pm 5.1	NS
MCH (μ μ g)	27.6 \pm 2.3	27.5 \pm 2.2	NS
MCHC (%)	32.3 \pm 0.91	32.2 \pm 1.0	NS
Serum Fe (mg/dl)	60 \pm 22	85 \pm 23	0.004
Transferrin Saturation (%)	16.8 \pm 8.0	25.6 \pm 9.0	0.008
TIBC (mg/dl)	367 \pm 31	339 \pm 31	0.030
Transferrin (mg/dl)	307 \pm 26	301 \pm 30	NS
Ferritin (ng/ml)	21 \pm 31	16 \pm 20	NS

NS: Not Significant.

The increased differences in serum iron levels between the fortification condition and baseline phase correlated negatively with the reduced differences in TIBC levels ($r = -0.916$, $p = 0.001$), (Fig. 3).

4. Discussion

Iron deficiency anemia is the most common nutritional problems in the world affecting billions of people through the world and impairs growth and cognition.

Young children and women of reproductive age, especially pregnant and lactating women, are at greatest risk. Iron deficiency anemia impairs immunity and reduces the physical and mental capacities of people of all ages, and in young children, even mild anemia can impair intellectual development. Anemia in pregnancy is also an important cause of maternal mortality, increasing the risk of hemorrhage and sepsis during childbirth. Thus, achieving and maintaining a safe and nutritionally adequate food supply is

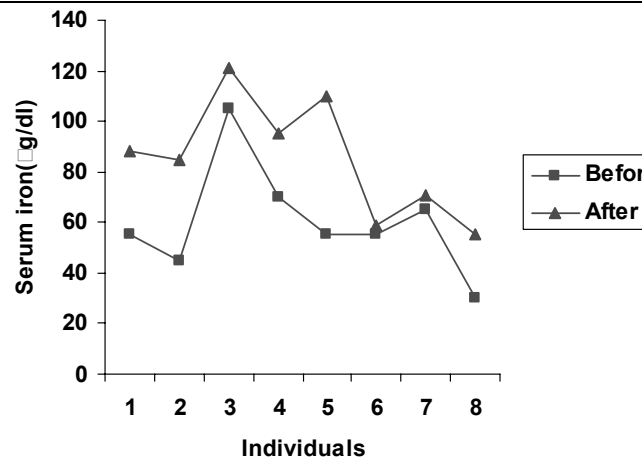


Fig. 1. Individual iron concentrations at baseline and after 4 weeks consumption.

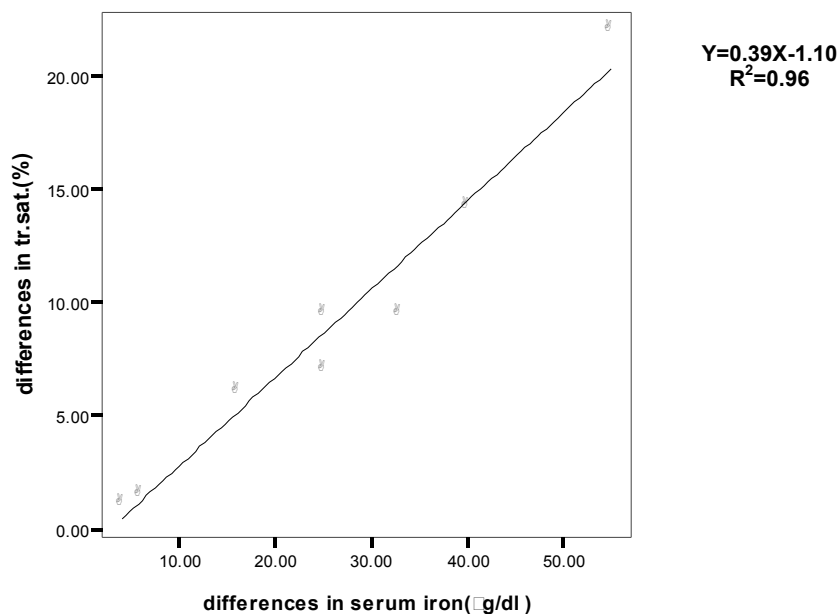


Fig. 2. Positive correlation between the differences in serum iron levels and the differences in transferrin saturation percentages.

an important public health goal. Addition of nutrients to specific foods has been considered an effective approach for obtaining this goal [23]. The most cost-effective intervention to increase iron intake is fortification. As iron deficiency and anemia are more prevalent in children, among other susceptible groups, and in women at reproductive ages, targeted fortification of certain food items consumed by these age groups, could help to reduce iron deficiency. Consumption of fortified ready-to-eat cereals would increase the intake of iron and vitamins and do not represent a risk of over consumption in adults [1,6].

The enhancement strategy must take into account the diet of the target population, the cost of fortification, the shelf-life of the food, and its compatibility with the chemical form of iron used for the enhancement. Foods can be fortified with nonheme or heme iron. The strategy can also be combined

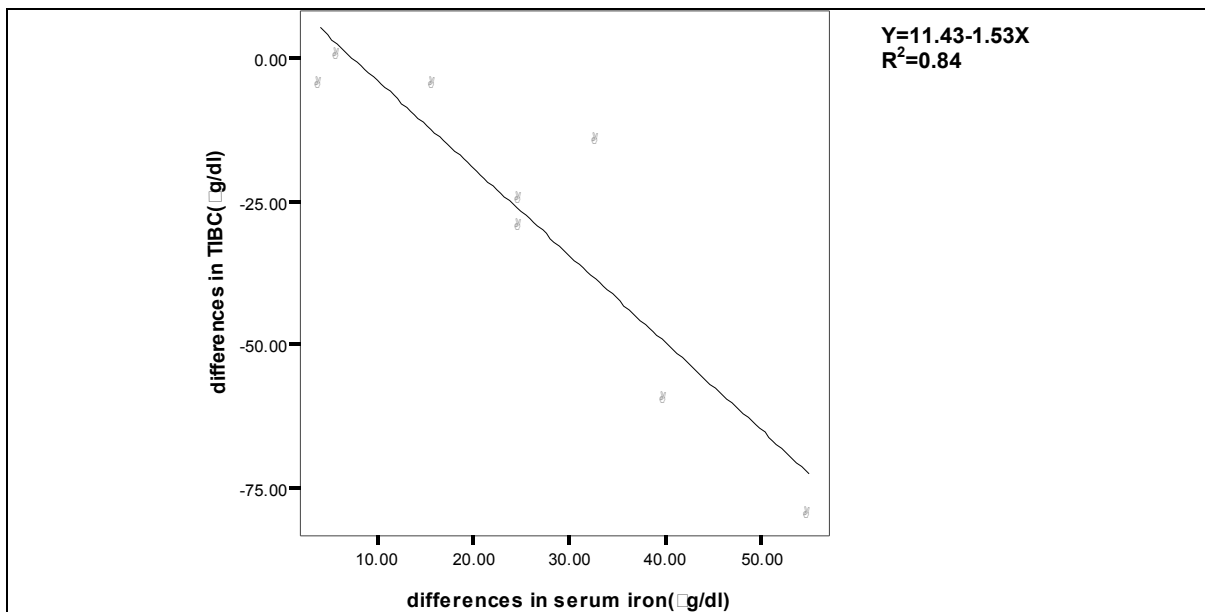


Fig. 3. Negative correlation between the differences in serum iron levels and the differences in TIBCs.

with the consumption of other fortified foods or foods rich in iron content [14]. Fortified foods can contribute to maintaining optimal nutritional status and minimizing the likelihood of iron insufficiencies [13, 16] and it is shown that fortified breakfast cereal contributes to overall nutrient intake and achievement of current dietary recommendations. In underdeveloped countries cereal flours, especially wheat and corn, are frequently used as fortification vehicles, because grain products are the staple foods for those populations. Other common fortified foods are ready-to-eat and infant cereals, which in industrialized countries could provide a significant amount of iron [2]. It is also reported that simultaneous addition of vitamin A and vitamin C to it may produce a 3.6-times increase on iron absorption compared to no vitamin addition and a 2-times increase compared to the absorption from the cereal fortified with only one of the vitamins [8].

To increase the daily amount of iron intake, pumpkin seed kernels was provided to increase daily iron consumption in addition to fortified cereal portion, as well. Like most nuts and seeds, pumpkin seeds are especially delicious roasted and for best nutritional value, it is best to eat them raw. They are great tossed in salads, mixed with grains, or ground up and added to salad dressing, casseroles, soups, and baked goods. Flour samples could be potentially added to food systems such as bakery products and ground meat formulations not only as a nutrient supplement but also as a functional agent in these formulations [4,5]. It represents a useful source of many nutrients essential to humans [10] and according to USDA- National Nutrient Database it contains 4.3 mg iron per one ounce [22]. Some health benefits and concerns associated with these seeds such as reduction of the risk of bladder stone disease [20] and in treating lower urinary tract symptoms and benign prostate hyperplasia [3] are claimed.

After consumption of these products most of the hematological parameters were still within normal range and showed no significant differences in their concentrations or levels. It is usually expected to see such a result with no changes within four weeks and it seems a longer period of times, possibly more than three to four months may be required to observe any change on most indices studied. But, it is noteworthy to indicate that four weeks consumption by this group of women resulted in a significant

improvement in their serum iron concentrations ($p \leq 0.004$), transferrin saturation percent ($p \leq 0.008$), and lower TIBC ($p \leq 0.030$), an indication of positive response to consumption and suggesting that both levels of foods were safe and that the regimen used did not cause any symptoms of discomforts or poor adherences and rejections. Serum iron concentration is a measure of the total amount of iron in the serum and is often provided with results from other routine tests and the concentration increases after intake of dietary iron sources [17]; TIBC is a measure of the iron-binding capacity within the serum and reflects the availability of iron-binding sites on transferrin. Thus, TIBC increases when serum iron concentration (and stored iron) is low and decreases when serum iron concentration (and stored iron) is high [11]. Transferrin saturation indicates the extent to which transferrin has vacant iron-binding site (e.g., a low transferrin saturation indicates a high proportion of vacant iron-binding sites). It is based on two laboratory measures, serum iron concentration and TIBC. Accordingly, a positive significant correlation ($r = 0.981$, $p = 0.000$) observed with differences in serum iron concentrations and transferrin saturation percentages and a negative significant correlation ($r = -0.916$, $p < 0.001$) with differences in serum iron concentrations and TIBCs. Improvement of the above indices is generally an indication of a better iron status in healthy women and possibility of the needs to explore further and justify the use of daily consumption of iron fortified ready-to-eat cereals and pumpkin seed kernels as a rich sources of iron in a 12–16 weeks study and longer to assess their effects on other indices and in particular the hemoglobin and red blood cell responses to iron fortification.

Fortification of foods, especially infant foods and cereal-based products, with iron has been successful in significantly reducing levels of iron-deficiency anemia and is one of the most cost-effective and sustainable approaches to controlling iron deficiency anemia. Since, pumpkin seed kernels are nutritionally known for their good content of essential amino acids, minerals and trace elements, and fatty acids, we would recommend the manufacturers to consider the addition of these seeds as a source of natural iron to their fortified cereal products, bakery products, and ground meat formulations, as well.

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