Iron-fortified flour: can it induce lipid peroxidation?

Mitra Abtahi1, Tirang Reza Neyestani1, Hamed Pouraram2, Fereydoun Siassi2, Ahmad Reza Dorosty2, Ibrahim Elmadfa3, and Aazam DoustMohammadian1

1Department of Nutrition Research, National Nutrition and Food Technology Research Institute and Faculty of Nutrition Sciences and Food Technology, Shahid Beheshti University of Medical Sciences, Tehran, Islamic Republic of Iran, 2Department of Community Nutrition, School of Nutritional Sciences and Dietetics, Tehran University of Medical Sciences, Tehran, Islamic Republic of Iran, and 3Department of Nutrition Sciences, University of Vienna, Vienna, Austria

Abstract

This community-based study was conducted to evaluate the effects of iron-fortified bread consumption on certain biomarkers of oxidative stress in an apparently healthy population. Evaluation of food intake, anthropometric and laboratory variables was performed in the beginning and after the 8-month intervention for all participants. There was no significant change in oxidative stress biomarkers in women following 8 months intervention. However, in men, final values of total antioxidant capacity, compared to the initial ones, showed a significant decrease in (p = 0.01) which was accompanied by a significant increase in superoxide dismutase (p = 0.002). It could be concluded that although the short-term period (8 months) of extra iron intake did not show severe effects of lipid peroxidation, significant changes of serum iron and some oxidative stress indices suggested that fortification of flour with iron among non-anemic adults in the long term was not without adverse effects.

Keywords

Flour, fortified feed, iron, oxidative stress

Introduction

Due to the high prevalence of iron-deficiency and its consequent anemia in different age groups of most provinces of Iran that has been shown in the latest report of the National Integrated Micronutrient Survey (NIMS; Sheikholeslam, 2002), flour fortification with iron and folic acid received high priority in the Iran’s Ministry of Health and Medical Education (Sadighi et al., 2008). Food fortification might be an inexpensive, simple, and effective approach for controlling and preventing iron deficiency and its related anemia in many countries (Allen et al., 2006). However, one of the major concerns regarding its implication is the potential adverse effects of increased iron intake in individuals without iron deficiency. Though iron is essential for metabolism, it has the potential of causing devastating adverse effects (Lasheras et al., 2003; Pouraram et al., 2010). Free iron is harmful to cells as it can convert anion superoxide and hydrogen peroxide to hydroxyl radicals (Dröge, 2002; Pouraram et al., 2010), hence increasing the formation of free oxygen radicals which are toxic to the cells (Dröge, 2002), a condition known as oxidative stress.

The potential risks of excess intake of iron in rats were first reported over 50 years ago (Burke et al., 2001; Richmond, 1959). Perhaps one of the most dramatic discoveries in recent years concerning mechanisms of oxidative damage in biological systems has been unraveling the potential of superoxide anions ($O_2^-$) to release iron from ferritin, which may in turn catalyze peroxidation of cell membranes (Keyer & Imlay, 1996). Previous animal studies on colonic response to high dietary iron intakes have shown that iron may act as a tumor promoter, whereas phytate could be protective, an effect presumed to be due to its iron-binding capacity (Soyars & Fischer, 1998). Though Soyars & Fischer reported no effect of iron on cell proliferation or development of aberrant crypt foci (ACF) in rats (Soyars & Fischer, 1998), Davis & Feng reported an increase in ACF with high intake of dietary iron (140 µg/g diet) (Davis & Feng, 1999). The amount of iron in Iranian flat breads varies depending on the flour type and is 1.9–3.32 mg/100 g which can increase to 4.9–6.32 mg/100 g after fortification (Tarsarkisian, 2010). In flour fortification process, the amount of iron which is usually added to flour is 3 mg/100 g; the amount of iron in fortified flour is actually higher than whole wheat grain. Since mean bread consumption per capita in Iran has been estimated to be 320 g (equal to 250 g flour), daily consumption of fortified bread provides about 7.5 mg/d excess iron (about 40% of daily need) (Kalantari et al., 2005). Despite the advantages of the flour fortification program, questions have been raised from the beginning of the intervention about the potential hazards of adding iron to flour for those without anemia or iron deficiency.

It is noteworthy that most, if not all, studies on the possible outcomes of iron-fortified food consumption have been performed on iron deficient and/or anemic individuals with increased need to iron intake (Sun et al., 2007). In such studies the possible effects of increased iron intake in subjects with sufficient body iron stores have been neglected. Even in iron deficient subjects, iron supplementation may not be beneficial in all aspects (Knutson et al., 2000). A clinical trial conducted in Iran showed that administration of iron supplements to anemic women resulted in increased lipid peroxidation, as judged by serum levels of...
malondialdehyde (MDA), and decreased total antioxidant capacity (TAC) (Amirkhizì et al., 2007).

Increased iron storage has been shown to augment lipid peroxidation and to decrease activity of some antioxidant enzymes (Amirkhizì, 2006). Some studies have found a significant positive correlation between serum iron and plasma MDA levels while in animal studies no significant association has been observed between plasma iron levels and the activity of antioxidative enzymes, superoxide dismutase (SOD) or glutathione peroxidase (GPX) (Knutson et al., 2000; Soyars & Fischer, 1998).

Despite the extensive literature on iron-induced lipid peroxidation (Davis & Feng, 1999; Lasheras et al., 2003; Sun et al., 2007), only a few studies have investigated the effects of iron-fortified food consumption on oxidative stress among non-anemic persons to date (Rehman et al., 1998). Therefore, this community-based study was conducted to evaluate the effects of iron-fortified bread consumption on certain biomarkers of oxidative stress in an apparently healthy population of adults from Semnan, central north of Iran.

Methods

Among 31 provinces of the Islamic Republic of Iran, Semnan province with a lower prevalence of anemia and iron deficiency (Sheikholeslam, 2002) was selected for this study. The sample size was calculated based on serum MDA variations which actually gave the largest sample size compare to other oxidative stress biomarkers including TAC, SOD and oxidized-low density lipoprotein (ox-LDL). Considering the possible lost to follow-up during the study and the previous reports of the prevalence of anemia in this province (Amirkhizì, 2006; Amirkhizì et al., 2007), a sample of 202 participants (99 men and 103 women) would provide 95% power to the study. The subjects were accessed based on a multi-stage stratified method by calling up their homes in a cluster manner. This survey was conducted in the city of Semnan, the center of Semnan province, which had the lowest prevalence of anemia and iron-deficiency among other cities in this province (Sheikholeslam, 2002) where the national fortification program (Sheikholeslam, 2002) was selected for this study. The sample size was calculated using the equation BMI (kg/m^2) = Weight (kg)/Height^2 (m).

During the first visit, before completing the informed consent form, the objectives of the study were fully described to the participants and then the general information questionnaire was filled out. Evaluation of food intake, anthropometric and laboratory variables was performed in the beginning and after the 8-months intervention for all participants. This study was approved by the Research Committee and the Ethics Committee of the institution. Laboratory investigations were conducted to evaluate the effects of iron-fortified bread consumption on certain biomarkers of oxidative stress in an apparently healthy population of adults from Semnan, central north of Iran.

Exclusion criteria were:
- Diet and lifestyle change (including physical activity and smoking habits) during the intervention period
- Surgery or pathological blood loss for any reason
- Intake of multivitamin and iron supplement during the intervention
- Unwilling to continue participation

Quality control of flour fortification program

Thirty ppm elemental iron as ferrous sulfate and 1.5 ppm folic acid were added to flour for fortification. Daily evaluations of flour samples (at least 3 samples per day) and a semi-quantitative spot test was used by authorities at the mills to determine the amount of iron (Nestel & Nalubola, 2000). Central Food and Nutrition Laboratory experts in the province also regularly visited flour mills and factories to take samples for the Food & Drug Organization (FDO) Laboratory of the province where the exact amounts of flour iron and folate were measured using quantitative tests (Nalubola & Nestel, 2000) and the results were compared with the readings at the mills. Feedback was provided if necessary. Moreover, the investigation team took bread samples from bakeries and assessed them in a private laboratory to check the reports of FDO. Therefore, besides the routine and daily monitoring of the fortification program, an additional controlling mechanism was applied.

Dietary assessment

Dietary intake was assessed by using a validated food frequency questionnaire (103 food items) and 24-h recall questionnaire for 3 d (Fazeltabar Malekshah et al., 2006).

Anthropometry

Height and weight were measured with a digital scale (Deteco, Webb City, MO) in light clothing without shoes with 100 g and 0.5 cm precisions, respectively. Body mass index (BMI) was calculated using the equation BMI (kg/m^2) = Weight (kg)/Height^2 (m).

Laboratory investigations

After 12–14 h fasting, 10 ml of venous blood was collected from each participant. Blood samples were kept for 30–45 min at room temperature (RT) followed by centrifugation at 800 g at RT for 15 min. Then, sera were separated and stored at −80°C. Biomarkers of oxidative stress in this study included serum TAC, MDA and ox-LDL concentrations as well as SOD activities.

Serum TAC was evaluated by calorimetric method as described elsewhere (Neyestani et al., 2007b). Circulating MDA, a marker of lipid peroxidation, was measured as originally described (Kei, 1978) with some minor modifications (Neyestani et al., 2007a). The activities of SOD were evaluated using the commercial kits (Cayman, Ann Arbor, MI). Enzyme immunoassay was used to measure ox-LDL (Mercodia, Uppsala, Sweden). Serum concentrations of iron and ferritin were determined using chemical and immuno turbidometric methods, respectively (both from Pars-Azmoon, Tehran, Iran).

Normal distribution of data was evaluated using the Kolmogrov–Smirnov method. Paired t test was applied to evaluate within-group changes. Level of significance was set at p < 0.05. Statistical Package for Social Sciences (SPSS, Chicago, IL), version 16 was employed for statistical analyses.

Results

Energy and nutrient intake showed no significant difference before and after the intervention with the exception for iron that increased due to the consumption of fortified bread (Table 1).
The mean age of the participants was 50.4 ± 6.4 years. Weight and BMI did not differ significantly after the intervention while serum iron level increased significantly in both genders after 8 months (Table 2).

Initially, the changes were not significant in the two sexes separately. Then the serum iron and ferritin level in both sexes were divided into three parts (tertile) and compared to each other, which not statistically significant as well. Therefore, changes in highest level of serum iron and ferritin were compared. The results of this comparison are shown in Tables 3 and 4.

There was no significant change in oxidative stress biomarkers in women following 8 months of intervention. However, in men the effects of iron administration on oxidative stress biomarkers were compared. The results of this comparison are shown in Table 4.

Discussion

Our research was the first community-based study that evaluated the effects of iron administration on oxidative stress biomarkers in healthy individuals in Iran. In the study population, extra iron intake increased serum iron level significantly in both genders.
but augmented oxidative stress only in men. Possible reasons for the observed differences between men and women are due to the higher energy intake in men than in women, which reflects the higher consumption of fortified bread as a staple food in men (Table 1). Another possibility except from iron intake is higher fat intake in men compared to women, which will be discussed later.

Oxidative stress is an unbalancing between free radicals, mainly reactive oxygen species (ROS) and protective radical scavenging antioxidants resulting from either an overproduction of ROS or a deficit in antioxidant protection (Terada, 2006). A huge body of evidence has suggested oxidative stress as a major contributing factor in pathogenesis of many human diseases such as neurodegenerative disease and type 2 diabetes (Robertson et al., 2007; Uttara et al., 2009).

The effects of dietary iron intake on oxidative stress were particularly evaluated in many studies (Amirkhizi, 2006; Amirkhizi et al., 2007; Isler et al., 2002; Kato et al., 1999; Kurtoglu et al., 2003; Wurzelmann et al., 1996). In a study, long-term alimentary iron overload resulted in a positive serum iron balance, which in turn leads to an increased OS (Rehema et al., 1998). In a cross-sectional study, plasma iron levels were positively associated with markers of lipid peroxidation (Amirkhizi, 2006). Also another study indicated that high MDA levels in breast cancer patients were related to high serum iron concentrations (Bae et al., 2009).

Some studies showed increased SOD activity in patients with iron deficiency anemia (Acharya et al., 1991; Jansson et al., 1985; Panchenko et al., 1978). A study suggested that increased SOD formation was a compensatory factor for increased oxidant stress (Jansson et al., 1985). Other research showed decreased activities of antioxidant enzyme such as SOD in patients with iron deficiency anemia (Cellerino et al., 1976; Kumerova et al., 1998). Isler et al. showed that SOD activity in anemic patients was lower than that of normal group which might be resulted from insufficient nutrition and oxidative stress under hypoxic condition. In this study SOD activity increased to normal levels in all anemic groups after the treatment (Isler et al., 2002). In the present study, mean of the activities of SOD and TAC showed a significant increase in the highest levels of serum iron and ferritin in Iranian men, most other oxidative stress indices increased insignificantly. So, it can be concluded that after 8 months extra iron intake, an unbalance has happened between ROS and antioxidant system only among men due to iron intake. Expression of SOD during inflammation and oxidative stress increases as a cellular defense mechanism (Markovskaya et al., 2003).

Also in several studies, the relation of the amount of iron stores and oxidative stress has been shown (Amirkhizi, 2006; Choi et al., 2008; Lasheras et al., 2003). These studies indicated that the amount of iron stores and levels of plasma iron has a positive and significant relation with the increase of lipid peroxidation and oxidative stress. In the most recent of these studies, Choi et al. showed in a cohort study that high iron intake can increase oxidative stresses in the body which in turn increases the risk of prostate cancer (Choi et al., 2008). In our study the only factor that could induce ROS was the iron intake. These changes suggest that they may become significant upon continued consumption of fortified bread. It was related to no significant changes in other related factors such as food and vitamin intake (Table 1). We found no significant change in the level of MDA (indicative of lipid peroxidation). Considering the fact that the MDA did not change in our study (despite its positive trend in both sexes), longer studies are required to evaluate whether lipid peroxidation is increased in individuals with high serum iron and ferritin levels.

To the best of our knowledge, no single component of the serum antioxidant complex could fully reflect the protective sufficiency of blood, probably because of interactions that occur in vivo among different antioxidant compounds. TAC considers the cumulative effect of all antioxidants present in blood and body fluids, thus providing an integrated parameter rather than the simple sum of measurable antioxidants (Suresh et al., 2009).

Many studies have suggested that ox-LDL is the most important contributing factors of atherosclerosis (Bononimini et al., 2008; Keaney et al., 2003) and the hypothesis of the role of oxidative stress in development and progression of atherosclerosis has been proposed for two decades (Vertechy et al., 1989). We observed that in the higher levels of serum ferritin and serum iron, despite the increase in mean oxidized LDL in both genders, the change was not significant. Absence of significant change in markers of oxidative stress among female subjects in our study can be related to their enhanced physiologic ability in facing oxidative stress (Borrás et al., 2006; Viña et al., 2005). It has been suggested that mitochondria of females have a specific ability for producing less amounts of ROS, so females produce half amounts of ROS compared to males (Borrás et al., 2003). In another study, this ability of females has been known as a specific genetic specification (Viña et al., 2005) and others related it to the specific role of estrogens as a strong antioxidant (Borrás et al., 2006; Ruiz-Larraea et al., 1997). Compared to men, women have been shown to exhibit improved triglycerides clearance following high-fat intake. It is believed that elevated triglycerides strongly correlated to increased oxidized macromolecules so related to exhibiting enhanced triglyceride removal from the blood circulation, women are less susceptible to oxidative stress than men (Bloomer et al., 2010; Bloomer & Fisher-Wellman, 2010).

The results of our study showed the early stages of developing oxidative stress, which can serve as an alarming sign for the decision-makers of the country to pay attention to the amount of added iron and manage the iron fortification program in different age groups in order to prevent the adverse effects of oxidative stress. It is also suggested that non-fortified flour should be available to individuals with an acceptable iron status.

Attempts were made to consider and minimize the limitations of the previous studies, which were evaluation of only the anemic patients (Isler et al., 2002; Khoshfetrat et al., 2013; Kurtoglu et al., 2003; Sun et al., 2007), small sample size (Acharya et al., 1991; Amirkhizi et al., 2007; Amirkhizi, 2006; Bae et al., 2009; Kurtoglu et al., 2003), short duration of the intervention (Sun et al., 2007), or using iron supplementation instead of iron-fortified flour (Binkoski et al., 2004; Khoshfetrat et al., 2013). However, this study has some limitations; convincing local authorities to cooperate in this national survey was a major problem in conducting this survey and the duration of intervention (8 months) was not enough to make sure of the long-term effects of consuming fortified bread such as iron overload. Other minerals, such as Cu, Zn, Mn, and Se, which mediated the activities of some enzymes, may also play important roles in the alteration of enzyme activity in oxidative stress condition, which were not evaluated in our study (Isler et al., 2002).

To make sure of the consumption of fortified bread, we fortified all bakeries’ flour of the cities while normally; all the flour that is used in bakeries is not fortified. We also recommend that not all kinds of flour in the country should be fortified (Pouraram et al., 2012).

Conclusions

It could be concluded that although the short-term period (8 months) of extra iron intake did not show severe effects of lipid peroxidation, significant changes of serum iron and some oxidative stress indices suggested that fortification of flour with iron among non-anemic adults in the long term was not without
adverse effects. However, the findings of this study can be used by health decision makers to reconsider the amount of added iron in the national fortification program, its constant monitoring and non-fortification of all bakers’ flour.

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Declaration of interest

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References


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