

Original Research

Iron and Folate in Fortified Cereals

Paul Whittaker, PhD, Paul R. Tufaro, and Jeanne I. Rader, PhD

Center for Food Safety and Applied Nutrition, Food and Drug Administration, Washington, DC

Key words: iron, folate, fortification, cereals

Background: Fortification of cereal-grain products was introduced in 1941 when iron and three vitamins were added to flour and bread. Ready-to-eat cereals were fortified at about the same time. These fortifications have contributed to increased dietary iron intake and reductions in iron deficiency anemia in the US. In 1996, FDA finalized rules for fortification of specific enriched cereal-grain products with folic acid. This measure was instituted to increase the folate intakes of women of child-bearing age and thereby reduce the risk of having a pregnancy affected with a neural tube birth defect. However, with recent increases in fortification, public health officials in the US are concerned that excess intake of specific nutrients such as iron and folic acid may result in toxic manifestations.

Objective: Our objective was to measure iron and total folate content in breakfast cereals and compare assay to label values for % Daily Value. We also determined by weight the amount of a ready-to-eat breakfast cereal adults would eat and compared this to the labeled serving size, for which the reference amount for this cereal per eating occasion was 1 cup or 30 g.

Design: Twenty-nine breakfast cereals were analyzed for iron content using the bathophenanthroline reaction. Twenty-eight cereals were analyzed for total folate, utilizing a microbiological assay with tri-enzyme digestion. Serving size quantities were estimated in seventy-two adults who regularly ate breakfast cereal and were asked to fill a 16 or 22 cm round bowl with the amount of cereal that they would consume for breakfast.

Results: When the labeled value was compared to the assayed value for iron content 21 of the 29 breakfast cereals were 120% or more of the label value and 8 cereals were 150% or more of the label value. Overall, analyzed values for iron ranged from 80% to 190% of label values. Analyzed values for folate ranged from 98% to 320% of label values. For 14 of 28 cereals, analyzed values exceeded label declarations by more than 150%. Bran-containing cereals contained the highest amounts of folate relative to their label declarations. The median analyzed serving size for the breakfast cereal was 47 g for females, 61 g for males with a combined median of 56 g as compared to the label value of 30 g.

Conclusions: Analyzed values of iron and folic acid in breakfast cereals were considerably higher than labeled values. For adults, the amount of cereal actually consumed was approximately 200% of the labeled serving size. When the quantity of cereal consumed is more than the labeled serving size and when the levels of iron and folate are higher than declared, the intake of both will be significantly greater than the labeled values. It will be important to continue monitoring serum ferritin and folate levels in NHANES IV, since daily consumption of breakfast cereals may contribute to excessive intakes of iron and folate.

INTRODUCTION

In the US, achieving and maintaining a safe and nutritionally adequate food supply is an important public health goal. Addition of nutrients to specific foods has been considered an effective approach for obtaining this goal. However, vigilance is needed to assure consumers that systematically high fortification of foods does not produce nutrient imbalances and/or

overages in the food supply. As part of its responsibility for the safety of the food supply, the US Food and Drug Administration (FDA) is interested in continually monitoring both the effectiveness and safety of fortification practices [1]. The Nutritional Labeling Education Act (NLEA) mandated nutrition-labeling on most foods marketed to American consumers and provided for a nationally uniform food labeling regulatory system. Iron and folic acid content are labeled as % Daily

Address reprint requests to: Paul Whittaker, PhD, Center for Food Safety and Applied Nutrition, Food and Drug Administration, 200 C Street SW, HFS-236, Washington, D.C. 20204. E-mail: paul.whittaker@cfsan.fda.gov

Journal of the American College of Nutrition, Vol. 20, No. 3, 247-254 (2001)
Published by the American College of Nutrition

Value (DV) per serving size. This is based on the Reference Daily Intake (RDI) which is 18 mg for iron and 400 μg for folic acid.

In 1941, iron and the vitamins, thiamin, riboflavin and niacin were added to enrich flours, so that cereal was restored to the 100% whole grain level [2]. In 1955, the first cereal fortified with iron and vitamins beyond the whole-grain restoration levels was introduced. Currently, manufacturers are fortifying ready-to-eat cereals with levels of iron ranging generally from 8% to 100% and folate from 4% to 100% of the DV. Recently, there has been an increase in the number of cereals with 100% DV of iron and folate, leaving very few breakfast cereals without fortification.

The iron used for food fortification must have either GRAS (generally recognized as safe) or food additive status. Fourteen iron salts and elemental iron have been affirmed by the FDA as GRAS with reduced iron, a form of elemental iron, and ferric phosphate currently being used in cereal fortification [1]. Selection of the form of iron to be used in a food as a fortifying agent requires consideration of the chemical and physical properties of both the iron compound and food to be fortified. Solubility, stability, bioavailability, organoleptic qualities and cost are all important factors.

Iron is a trace element that is essential to cell metabolism and life. Approximately 85% of total body iron can be classified as essential because it serves well-defined physiological functions. The recommended dietary allowance (RDA) of iron for women 15 to 50 years of age is 15 mg/day and for men 10 mg/day [3]. There are, however, two important concerns with respect to the dietary intake of iron. The first is iron deficiency, and the other is iron overload. Both of these problems have important public health consequences. Iron deficiency anemia is the most prevalent nutritional problem in the world today. 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.

Excess intake of iron can also result in toxic manifestations. Iron overload is a relatively common disorder of iron metabolism. A genetic form of iron overloading known as hereditary hemochromatosis affects one in 400 individuals of Northern European descent and has an estimated carrier frequency of one in ten [4,5]. The increased intestinal absorption of iron in hereditary hemochromatosis results in deposition of iron in parenchymal organs, eventually leading to cirrhosis, hepatocellular carcinoma, diabetes mellitus, congestive heart failure, hypogonadism and bronze skin pigmentation.

Folic acid, unlike iron, is a food additive rather than a GRAS substance [6]. Folate derivatives are essential for all cells as biochemical cofactors and serve as acceptors and donors of single-carbon units in a wide variety of reactions

involved in amino acid and nucleotide metabolism. Deficiency of the vitamin can cause reductions in serum and erythrocyte folate and megaloblastic changes in the bone marrow and anemia. Human requirements for folate are increased in a number of physiological conditions such as pregnancy, lactation and infancy, and megaloblastic anemia from folate insufficiency may occur during pregnancy. Foliates occur in foods mainly as reduced polyglutamate derivatives. The form of folate used as a food fortificant is the highly bioavailable, oxidized monoglutamate form, folic acid.

In 1996, FDA concluded that 1000 μg folate/day is the safe upper limit of folate intake for the general population [7]. The agency also determined that safe and effective delivery of folate to the target population was best achieved by limited addition of folic acid to enriched cereal-grain products. The fortification level was set at 140 μg folic acid/100 g for enriched cereal-grain products. For breads meeting reference amounts customarily consumed (RACC) per eating occasion, this provides approximately 10% of the DV/serving.

The ability of folate to mask the anemia of vitamin B₁₂ deficiency is the most widely recognized adverse effect of high intakes of the vitamin [8]. In the presence of excess folate and inadequate vitamin B₁₂, the megaloblastic anemia of vitamin B₁₂ deficiency may not develop, thus "masking" one of the early symptoms of a vitamin B₁₂ deficiency and delaying its diagnosis and treatment. However, other adverse effects of vitamin B₁₂ deficiency continue to progress and severe and irreversible neurologic damage may occur. Because the effects of high intakes of folic acid are not well known, but include complicating the diagnosis of vitamin B₁₂ deficiency, the US Public Health Service recommended that care should be taken to keep total folate consumption at less than 1000 μg /day except under the supervision of a physician.

In this study, breakfast cereals were analyzed for iron and total folate. Cereals were studied because the amounts of the two nutrients in cereals are significantly higher than amounts added to other foods.

SUBJECTS AND METHODS

Methods

Nutrition information from the food labels of 29 different cereals was compared with iron values obtained from an analysis of those cereals. The choice of cereal products was based on a review of market data. The top-ranked sellers were identified from 1998 Nielsen data [9]. Nutrition information from 28 cereals was compared with total folate values obtained through analysis. The 29 cereals used in the iron analysis consisted of three hot cereals and 26 ready-to-eat cereals. The cereals analyzed for iron were fortified with reduced iron, ferric phosphate or were unfortified. Standard reference materials were used in both the folate and iron assays and six cereal-grain

check samples from the American Association of Cereal Chemists International Check Sample Service (AACC, St. Paul, MN) were analyzed throughout the study and results obtained were within reported values. Cereals from single production codes were purchased locally between February, 1998 and July, 1999.

Iron Assay

Iron content of the cereals was determined by a modified bathophenanthroline method [10] and expressed as mg iron/g of cereal. The bathophenanthroline method is a sensitive colorimetric method for determining both the intrinsic and added iron. The bathophenanthroline method measures only the non-heme iron as compared to atomic absorption spectroscopy which determines both heme and nonheme iron. From each box of cereal, random samples of cereal were ground with a mortar and pestle. One or two g of the ground samples were weighed and placed in a 50-mL polypropylene centrifuge tube. Each cereal was analyzed three to four times in duplicate. Distilled water was added to bring the volume to 20 mL. The cereal was then homogenized for 30 seconds with a Polytron. A 3-mL sample of the homogenate was transferred to another 50-mL centrifuge tube, and 10 mL of acid reagent were added (6 M HCl and 1.2 M trichloroacetic acid, 1:1, v/v) and mixed well. The mixture was then heated in an oven at 65°C for 20 hours, cooled, and centrifuged at 3,600 X g for 20 minutes. Duplicate 0.2-mL aliquots of the supernatant fraction were pipetted into small polypropylene tubes, and 1.8 mL of freshly prepared color reagent was added, mixed and incubated for 10 minutes at room temperature. Absorbance was determined spectrophotometrically at 535 nm, and iron concentration ($\mu\text{g Fe/mL}$) was determined by reference to a standard curve. The bathophenanthroline color reagent, which was protected from light, was prepared by dissolving 62.5 mg bathophenanthroline-disulfonic acid (Sigma Chemical Co., St. Louis, MO) and 0.25 mL thioglycolic acid (Eastman Kodak, Rochester, NY) in distilled water and diluting to 25 mL. The final color reagent was a solution of the bathophenanthroline color reagent, saturated sodium acetate (4.5 M) and distilled water (1:20:20 by volume).

Assay for Total Folate

Whole packages of cereal, weighing one to two or more pounds, were ground in a Waring blender or coffee grinder. Preparation was carried out under subdued light, and care was taken to minimize contact with air. A minimum of three independent analyses were carried out for each cereal. The ground samples were stored at room temperature or frozen in tightly sealed glass bottles.

Total folates were determined by microbiological assay using a modification of AOAC official method 992.05 [11,12,13]. *L. casei* (ATCC#7469) was the assay microorganism (American Type Culture Collection, Rockville, MD). Test portions of the composites equal to about 0.25 to 1.0 g of dry

solids and containing about 1 μg folic acid were placed in 125 mL Erlenmeyer flasks containing 10 mL buffer (1.42% Na_2HPO_4 and 1% ascorbic acid, pH to 7.8 with 4 N NaOH) and treated as described previously [13]. Four mL of chicken pancreas conjugase (Difco Laboratories, Detroit, MI) preparation and 1 mL of α -amylase preparation (Sigma Chemical Co., St. Louis, MO) were then added to each flask. Flasks were covered and incubated for four hours at 37°C. After four hours, 1 mL of pronase E (Sigma) was added and the flasks were incubated overnight at 37°C. The enzymes were inactivated by autoclaving the sample for three minutes at 100°C, followed by cooling. An 8-point fourth degree polynomial regression plot and a computer program designed according to the official AOAC protocol were used to calculate ng folate/mL extract and μg folate/serving.

Estimation of Serving Size

Cereal-consuming adults were asked to pour the amount of a ready-to-eat cereal (whole wheat flaked cereal, weighing 30 g/cup) that they would consume for breakfast into a 16 or 22 cm round bowl. The cereal was then weighed on a balance to determine the weight of the reported serving size; this amount was then compared to the cereal's labeled serving size of 30 g. Seventy-two FDA employees, interns and summer students (32 females and 40 males) between the ages of 17 and 63 participated in the survey. The importance of this small survey in adults was to support the overall effect of increasing intake.

RESULTS

Twenty-one of the 29 breakfast cereals had iron levels of 120% or more of the labeled value, and eight cereals had values of 150% or more (Table 1). Overall, analyzed values for iron ranged from 80% to 190% of labeled values. In order to compare the percent of labeled values, cereals were grouped into labeled % Daily Value (DV) ranges of 8% to 10%, 20% to 30%, 45% to 50%, and 80% to 100%. Group 1 (8% to 10%, n=8) had analyzed values from 80% to 190% of label values, group 2 (20% to 30%, n=12) had analyzed values from 124% to 184% of label values, group 3 (45% to 50%, n=5) had analyzed values from 88% to 127% of label values, and group 4 (80% to 100%, n=4) had analyzed values from 87% to 136% of label values (Table 1). It should be noted that label DV values are rounded, i.e., 10% DV can include actual % DV values of 9.0% to 12.49%; 20% DV can include 17.5% to 22.49%, 30% can include 27.5% to 32.49%, 40% can include 38.5% to 42.49%, 50% can include 47.5% to 54.99%, 80% can include 75.0% to 84.99%, and 100% can include 95.0% to 104.99% [14]. The fiber content of the 29 cereals varied from 0 to 8 g/serving, and the serving size specified on the nutrition label varied from 21 to 61 g (Table 1).

Table 1. Labeled and Assayed Values for Iron in Breakfast Cereals

Cereal Grain	Type of Iron	Label Values			Assay Values		
		Serving Size (g)	Daily Value (%)	Iron ¹ (mg Fe/g)	Iron (mg Fe/g)	Daily Value ² (%)	Percent of Label Value ³ (%)
Whole wheat	None	49	8	0.029	0.036 ± 0.003 ³	10	125
Milled corn	Reduced Fe	28	8	0.051	0.072 ± 0.011	11	138
Whole wheat	None	41	10	0.044	0.055 ± 0.003	13	130
Whole grain oats	None	40	10	0.045	0.038 ± 0.003	8	80
Rice	Reduced Fe	33	10	0.055	0.067 ± 0.009	12	120
Milled corn	Reduced Fe	30	10	0.060	0.100 ± 0.017	17	170
Rice	Reduced Fe	39	10	0.046	0.078 ± 0.009	17	170
Wheat	Ferric phosphate	32	10	0.056	0.108 ± 0.015	19	190
Corn, wheat & oat flour	Reduced Fe	27	20	0.133	0.171 ± 0.023	26	130
Milled corn	Reduced Fe	32	20	0.113	0.203 ± 0.009	36	180
Wheat bran	Reduced Fe	61	25	0.074	0.136 ± 0.010	46	184
Whole grain oats	Reduced Fe	30	25	0.150	0.190 ± 0.021	32	128
Corn, wheat & oat flour	Reduced Fe	30	25	0.150	0.183 ± 0.006	31	124
Corn meal	Reduced Fe	31	25	0.145	0.246 ± 0.026	42	168
Whole grain oats	Reduced Fe	31	25	0.145	0.197 ± 0.014	34	136
Whole grain oats	Reduced Fe	33	25	0.136	0.186 ± 0.024	34	136
Corn meal	Reduced Fe	32	25	0.141	0.209 ± 0.019	37	148
Corn meal & whole wheat	Reduced Fe	36	30	0.150	0.243 ± 0.017	49	163
Whole wheat & rice	Reduced Fe	39	30	0.138	0.208 ± 0.027	45	150
Rice & wheat gluten	Reduced Fe	21	30	0.257	0.378 ± 0.080	44	147
Milled corn	Reduced Fe	28	45	0.289	0.367 ± 0.029	57	127
Whole grain oats	Reduced Fe	30	45	0.270	0.317 ± 0.033	53	118
Corn	Reduced Fe	30	45	0.270	0.310 ± 0.042	52	116
Wheat bran	Reduced Fe	29	45	0.279	0.301 ± 0.018	49	109
Farina	Ferric phosphate	33	50	0.273	0.240 ± 0.021	44	88
Whole wheat	Reduced Fe	51	80	0.282	0.384 ± 0.024	109	136
Multi-grain	Ferric phosphate	60	90	0.270	0.233 ± 0.013	78	87
Whole wheat	Reduced Fe	30	100	0.600	0.569 ± 0.070	95	95
Multi-grain	Reduced Fe	30	100	0.600	0.553 ± 0.032	92	92

¹ Iron (mg Fe/g) = % Daily value/100 × 18 mg Fe ÷ Serving size (g).

² Daily value (%) = mg Fe/g × Serving size (g) ÷ 18 mg Fe × 100.

³ Percent of label value (%) = $\frac{\text{Assayed value (\% Daily value)}}{\text{Labeled value (\% Daily value)}} \times 100$.

⁴ Mean ± SD.

Twenty-eight ready-to-eat breakfast cereals were analyzed for total folate (Table 2). The % DV/serving ranged from 16 to 400 µg/100 g. The labels of the majority of cereals stated that they contained folate at the level of 25% DV/serving.

Twenty-seven of the cereals had label statements of folate content. For 14 of these 27 products (52%), the analyzed values exceeded label declarations by more than 150%. For the remaining 13 products, the differences among the analyzed values and the labeled values were 98% to 144%. Bran-containing cereals had the greatest discrepancies between analyzed folate values and product labels. For four such products, the differences in folate content between label statements and analyzed values were 125% to 320%. Percent differences were compared by grouping cereals into labeled % DV of 25 and 100, group 1 (25%, n=17) had analyzed values from 98% to 320%, and

group 2 (100%, n=6) had analyzed values from 106% to 137% (Table 2).

Fig. 1 shows the serving size values as determined for adult males and females. The labeled serving size of the cereal was 30 g. In contrast, a mean value for the serving size of 75 ± 6 g (mean ± SEM) (median 61 g) was obtained for the adult males and 56 ± 4 g (median 47 g) was obtained for adult females. The overall mean was 66 ± 4 g (median 56 g).

DISCUSSION

Fortified foods can contribute to maintaining optimal nutritional status and minimizing the likelihood of iron and folate insufficiencies. Studies have suggested that fortified breakfast

Table 2. Total Folate in Ready-to-Eat Cereals

Cereals	Label Information			Analyzed Values of Folate ($\mu\text{g}/\text{serv}$)	Percent of Label Value ³ (%)
	DV ¹ (%)	Serving (g)	Folate ($\mu\text{g}/\text{serv}$)		
Multigrain, cereal	100	30	400	546 \pm 20 ²	137
Multigrain, rice, oats	100	50	400	508 \pm 58	127
Corn cereal	100	30	400	505 \pm 75	126
Raisin bran cereal	100	55	400	499 \pm 104	125
Corn flakes	100	30	400	466 \pm 74	117
Multigrain cereal	100	30	400	422 \pm 4	106
Whole grain oats	50	30	200	248 \pm 30	124
Natural wheat bran	25	29	100	320 \pm 48	320
Multi-bran cereal	25	58	100	292 \pm 59	292
Bran flakes cereal	25	30	100	250 \pm 37	250
Corn cereal	25	30	100	207 \pm 17	207
Corn cereal	25	30	100	204 \pm 17	204
Wheat & barley cereal	25	48	100	202 \pm 13	202
Corn cereal	25	30	100	197 \pm 14	197
Corn cereal	25	30	100	186 \pm 27	186
Whole grain oat cereal	25	32	100	183 \pm 42	183
Oat cereal	25	28	100	168 \pm 34	168
Wheat cereal	25	51	100	161 \pm 30	161
Corn & oat cereal	25	27	100	144 \pm 6	144
Corn & rice cereal	25	29	100	139 \pm 21	139
Multigrain cereal	25	55	100	135 \pm 15	135
Multigrain cereal	25	30	100	132 \pm 25	132
Corn cereal	25	30	100	114 \pm 16	114
Rice cereal	25	33	100	98 \pm 10	98
Instant oatmeal	20	28	80	122 \pm 17	153
Oat bran flake cereal	10	28	40	86 \pm 6	215
Shredded wheat	4	46	16	28 \pm 4	175
Granola (unfortified)	—	64	—	44 \pm 11	N/A

¹ DV, Daily Value for folate, 400 μg .

² Analyzed values are means \pm SD.

³ Percent of label value (%) = $\frac{\text{Analyzed value}}{\text{Labeled value}} \times 100$.

cereal contributes to overall nutrient intake and achievement of current dietary recommendations [15,16]. Fortification of foods, especially infant foods and cereal-based products, with iron has been successful in significantly reducing levels of iron-deficiency anemia [17] and is one of the most cost-effective and sustainable approaches to controlling iron deficiency anemia [18].

A study of 2,432 middle-class children, at a private pediatric clinic in the US, revealed that anemia as measured by hematocrit and erythrocyte protoporphyrin, decreased from 6.2% in 1969–1973, to 5.8% in 1974–1977, to 3.8% in 1978–1981, and to 2.7% in 1982–1986 [19]. It was concluded that the improvement in nutritional status related to iron may be the result of feeding practices that have included iron-fortified formulas and iron-fortified cereals. Two other studies have shown an improvement in iron status among low-income children [20,21]. These studies are consistent with the Centers for Disease Control's Pediatric Nutrition Surveillance System data that also demonstrated a significant decline in the prevalence of anemia among infants and preschool children [22].

Cook *et al.* [23], using data from the second National Health and Nutrition Examination Survey (NHANES II), evaluated the

iron status of US adults between 18 and 64 years of age. They found that the prevalence of iron deficiency anemia was surprisingly low, ranging from only 0.2% in adult men to 2.6% and 1.9% in pre- and postmenopausal women, respectively.

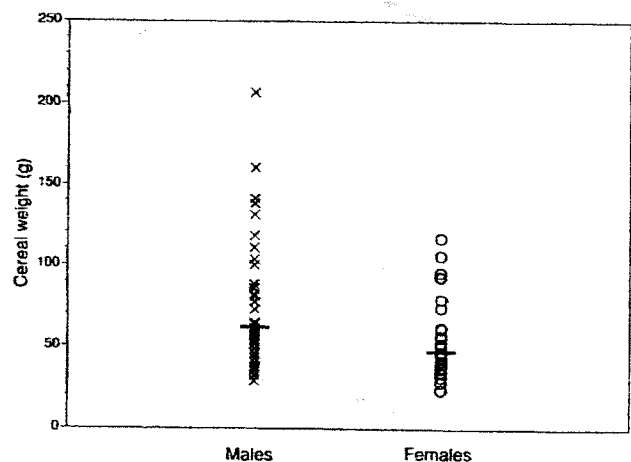


Fig. 1. Serving sizes and medians for males and females for a ready-to-eat cereal with a labeled serving size of 30 g.

They noted that iron deficiency anemia is less common in certain segments of the US population than the homozygous state for hereditary hemochromatosis.

It is possible that iron overload may outweigh iron deficiency and may be a more serious problem in adult males and non-pregnant females in the US [23]. A study of men in Finland revealed that high concentrations of serum ferritin or high iron intake increase the incidence of myocardial infarction [24]. Other studies have shown that serum ferritin was the strongest indicator of the presence and progression of carotid artery disease [25,26]. In a recent study of an elderly Dutch population, elevated serum ferritin concentrations were associated with increased risk of myocardial infarction [27]. The association was most evident in current or former smokers and in subjects with diabetes, raising the question of whether ferritin may adversely affect ischemic heart disease risk in the presence of other risk factors. It may be possible that these factors interact with elevated body iron stores and may accelerate atherogenesis by stimulating the oxidation of low density lipoproteins (LDLs). On the other hand, high iron status may be an indicator of high meat diets and, consequently, high saturated fat intakes, a known risk factor for vascular diseases.

Based on data from the NHANES I beginning in 1971 with more than 14,000 adults and a follow-up between 1981 and 1984, excess body iron stores have been reported to be associated with an increased risk of cancer [28]. Stevens *et al.* [29] reported a significant association between colon cancer and iron intake, as well as with transferrin iron saturation and serum iron. In an eight-year case-control study of diet and rectal cancer, there was an increase in rectal cancer in males, but not in females, with increasing intake of dietary iron [30]. There are at least three possible mechanisms for the role of iron in carcinogenesis: 1) production of free oxygen radicals, 2) promotion of the growth of transformed cells or 3) action as an essential cancer cell growth nutrient [31,32,33]. Identification of definitive relationships with increased iron intake, increased iron bioavailability, and high meat and high fat diets, will require more research.

Data from this study suggest on average that the amount of cereal reported as consumed by adult males and females is approximately twice the amount of the labeled serving size, resulting in an intake of at least twice the labeled % DV for iron. For example, from our data on reported serving size, an adult male (18 or above) consuming the mean observed quantity of 75 g of cereal, containing 100% DV for iron in a serving size of 30 g, would have an intake of 45 mg of iron from the cereal alone. Since the iron RDA for adult males is 10 mg, a single bowl of cereal would provide 4.5 times the daily allowance. In addition, 21 of the 29 breakfast cereals had iron levels of 120% or more when the labeled value was compared for iron content to the assayed value, and eight cereals had values of 150% or more. Roe and Fairweather-Tait [34] recently reported

a high bioavailability for reduced iron powder in female volunteers with low iron stores. The study showed that the bioavailability of reduced iron is comparable to that of ferrous sulfate. Reduced iron is commonly used in cereals in the US, and the higher bioavailability of this form of iron may be an important contributor to increases in iron status.

Because of the importance of serum ferritin levels in estimating iron status, this measurement is important for determining the changes in the iron status in the adult population. Serum ferritin in a US population was evaluated using data collected in NHANES III [35,36,37] and was compared to NHANES II data that were evaluated by Cook *et al.* [23]. There was an increase in serum ferritin for females (18 to 44 years of age) and males (18 to 64 years of age) when the NHANES III data were compared to NHANES II data. This increase in serum ferritin over an approximately ten-year period may be indicative of the increase in iron fortification, iron supplementation or a high meat intake. Because of the possible associations between heart disease and several types of cancer with increasing iron status, it may be important to reduce dietary iron, especially in adult males. For individuals with adequate iron nutrition (e.g., adult males and post-menopausal females) or patients with hereditary hemochromatosis, it would be helpful to have additional cereals available without iron fortification to allow individuals with an elevated iron status more choices.

Since January 1998, the FDA has required manufacturers of enriched cereal-grain foods to add folic acid at a concentration of 1.4 $\mu\text{g/g}$ of product. It was estimated that this level of fortification could increase the folic acid intake of the target population, that is, women of childbearing age, by 100 $\mu\text{g/day}$. It was also estimated that nontarget groups, such as the elderly and young men, who consume large amounts of grain products, would not be exposed to levels of folic acid over 1000 $\mu\text{g/day}$. This 1000 μg level of folic acid was the upper level set for adults by the Institute of Medicine, while it was adjusted to lower levels for children and adolescents on the basis of relative body weight; upper limit value ranges are 300, 400 and 600 $\mu\text{g/day}$ for children aged 1 to 3, 4 to 8 and 9 to 13, respectively [38].

Since the initiation of food fortification with folic acid, increases in plasma folate levels in various population groups have been reported. Jacques *et al.* [39] found that plasma folate levels in middle-aged and older adults who did not use folic acid supplements increased from a mean of 4.6 to 10.0 ng/mL . In an analysis of data from Kaiser Permanente for a heterogeneous population for the years from 1994 through 1998, serum folate levels increased from 12.6 to 18.7 ng/mL [40]. Recently, the Centers for Disease Control compared mean blood folate levels from NHANES III to NHANES 1999 for women of childbearing age (15 to 44 years) and reported an increase from 6.3 to 16.2 ng/mL [41]. In these studies food fortification was indicated as the main cause for the plasma folate increases.

Cereals contribute significantly to the total dietary intake in the US [38], and it has been reported that they provide 16.1% of total folate for men, 18.6% for women and an even greater amount, 30%, for children [42]. In this study we found that breakfast cereals are commonly labeled as containing at least 25% DV for folate and a significant number are labeled as containing 100% of the DV. Our data indicate that actual total values are higher. For 14 of the 27 cereals, the analyzed values exceeded label declarations by more than 150%, and, for the remaining 13 products, they ranged from 98% to 144%. It is not known whether some of the high values represent excesses added by the manufacturers or whether the tri-enzyme assay is measuring endogenous folates that are present at higher than expected levels. Bran-containing cereals had the greatest discrepancies between analyzed folate values and product labels (125% to 320%) possibly from the endogenous folates.

There are, however, some concerns about adverse side effects from consumption of high levels of folic acid. Folic acid is known to mask the anemia associated with vitamin B₁₂ deficiency and exacerbate progression of neurologic complications. The exact dose at which this occurs is not known, but it has been reported at doses of 400 µg/day as well as 1000 µg/day [43]. Based on our analyzed values and our survey on the amount of a breakfast cereal consumed, it is possible that the intake of folic acid in a single serving could be above the upper limit of 1000 µg/day level.

Flynn *et al.* [44] suggested that serum homocysteine level may be a marker for folate and/or vitamin B₁₂ (cobalamin) disorders because cobalamin is a cofactor in remethylation of homocysteine to methionine. Serum homocysteine showed an inverse relationship with red blood cell folate and serum total cobalamin. None of the elderly men and women (mean age 65 years) in this study had serum folate values below normal. However, it was found that 6% had abnormal total cobalamin (<200 pg/mL). Assessments of folate and vitamin B₁₂ status will be important to evaluate the impact of folate fortification. There is also concern about possible adverse effects of high-dose folic acid intake in individuals treated with anticonvulsants and methotrexate [38]. There have been some studies indicating that restriction of dietary folic acid inhibits growth and development of tumors. However, there is a growing body of evidence that relates folate deficiency to carcinogenesis in certain epithelial tissues. Folate deficiency is not a causal factor, but may act as a "co-carcinogen" with other risk factors [45].

As part of its general monitoring and safety evaluation responsibilities, FDA is concerned about the safety and effectiveness of fortification practices. In this study, we measured levels of iron and folate in breakfast cereals and report higher than expected levels of both in cereals. The deviations of both iron and folate from label values were highest in cereals labeled to contain between 10% and 30% DV.

CONCLUSION

Analyzed values for iron and folic acid in breakfast cereals were considerably higher than labeled values; values for folate ranged from 98% to 320% and for iron from 80% to 190%. For adults, the amount of cereal actually consumed was approximately 200% of the labeled serving size. When the quantity of cereal consumed is more than the labeled serving size and when the levels of iron and folate are higher than declared, adults may be approaching the safe upper limit of 1000 µg of folate/day and, for adult males, four to five times the recommended RDA level of iron. When folate from other foods as well as dietary supplements is combined with the folate of cereals, the total daily intake may be significantly above the safe upper limit. Because of a reported increase in serum ferritin and the possible associations between heart disease and several types of cancer with increasing iron status, it may be important to reduce dietary iron, especially in adult males. For individuals with adequate iron status or patients with hereditary hemochromatosis, it would be helpful to have additional cereals available without iron fortification to allow individuals more choices. Our results suggest that further general monitoring of serum ferritin and folate levels is needed to ascertain benefits as well as the potential for excesses of these two important nutrients.

REFERENCES

1. Code of Federal Regulations: Title 21, Parts 104, 170, 182, and 184. Washington, DC: US Government Printing Office, 2000.
2. Anderson RH: Breakfast cereals and dry milled corn products. In Clydesdale FM, Wiemer KL (eds): "Iron Fortification of Foods." Orlando: Academic Press, pp 111-120, 1985.
3. National Research Council: "Recommended Dietary Allowances." 10th ed. Washington, DC: National Academy Press, 1989.
4. Dadone MM, Kushner JP, Edwards CQ, Bishop DT, Skolnick MH: Hereditary hemochromatosis: analysis of laboratory expression of the disease by genotype in 18 pedigrees. *Am J Clin Pathol* 78:196-207, 1982.
5. Edwards CQ, Griffen LM, Goldgar D, Drummond C, Skolnick MH, Kushner JP: Prevalence of hemochromatosis among 11,065 presumably healthy blood donors. *N Engl J Med* 318:1355-1362, 1988.
6. Food and Drug Administration: Food additives; folic acid, potassium iodide and kelp. final rule. *Fed Regist* 38:20725, 1973.
7. Food and Drug Administration: Food additives permitted for direct addition to food for human consumption; folic acid (folacin), final rule. 61:8797-8807, 1996.
8. Lindenbaum J, Heaton EB, Savage DG, Brust JC, Garrett TJ, Podell ER, Marcell PD, Stabler SP, Allen RH: Neuropsychiatric disorders caused by cobalamin deficiency in the absence of anemia or macrocytosis. *N Engl J Med* 318:1720-1728, 1988.
9. Nielsen AC: "Information for Marketing Decisions." Schaumburg, IL: Nielsen Business Reports, June 15, 1998.
10. Whittaker P, Dunkel VC, Bucci TJ, Kusewitt DF, Thurman JD.

- Warbritton A, Wolff GL: Genome-linked toxic responses to dietary iron overload. *Toxicol Pathol* 25:556-564, 1997.
11. Association of Official Analytical Chemists: Folic acid (pteroylmonoglutamic acid). In *Infant formula, microbiological methods*, in "Official Methods of Analysis," 16th ed. Gaithersburg: Association of Official Analytical Chemists, 1995.
 12. Angyal G: "US Food and Drug Administration Methods for the Microbiological Analysis of Selected Nutrients." Gaithersburg: Association of Official Analytical Chemists International, pp 1-8, 21-28, 1996.
 13. Rader JJ, Weaver CM, Angyal G: Use of a microbiological assay with tri-enzyme extraction for measurement of pre-fortification levels of folates in enriched cereal-grain products. *Food Chem* 62:451-465, 1998.
 14. Code of Federal Regulations: Title 21, Part 101.9(c)(8)(iii). Washington, DC: US Government Printing Office, 2000.
 15. Darnton-Hill I, Mora JO, Weinstein H, Wilbur S, Nalubola PR: Iron and folate fortification in the Americas to prevent and control micronutrient malnutrition: an analysis. *Nutr Rev* 57:25-31, 1999.
 16. McNulty H, Eaton-Evans J, Cran G, Woulahan G, Boreham C, Savage JM, Fletcher R, Strain JJ: Nutrient intakes and impact of fortified breakfast cereals in schoolchildren. *Arch Dis Child* 75:474-481, 1996.
 17. Hurrell RF: Preventing iron deficiency through food fortification. *Nutr Rev* 55:210-222, 1997.
 18. World Bank: "Investing in Health: World Development Report 1993." New York: Oxford University Press, 1993.
 19. Yip R, Walsh KM, Goldfarb MG, Binkin NJ: Declining prevalence of anemia in childhood in a middle-class setting: a pediatric success story? *Pediatrics* 80:330-334, 1987.
 20. Miller V, Swaney S, Dainard AS: Impact of the WIC program on the iron status of infants. *Pediatrics* 75:100-105, 1985.
 21. Vazquez-Secane P, Windom R, Pearson HA: Disappearance of iron deficiency anemia in a high risk infant population given supplemental iron. *N Engl J Med* 313:1239-1240, 1985.
 22. Centers for Disease Control: Declining anemia prevalence among children enrolled in public nutrition and health programs, selected states, CDC pediatric nutrition surveillance system, 1975-1985. *MMWR* 35:565-566, 1986.
 23. Cook JD, Skikne BS, Lynch SR, Reusser ME: Estimates of iron sufficiency in the US population. *Blood* 68:726-731, 1986.
 24. Salonen JT, Nyssönen K, Korpela H, Tuomilehto J, Seppänen R, Salonen R: High stored iron levels are associated with excess risk of myocardial infarction in eastern Finnish men. *Circulation* 86:803-811, 1992.
 25. Kiechl S, Aichner F, Gerstenbrand F, Egger G, Mair A, Rungger G, Spogler F, Jarosch E, Oberhollenzer F, Willeit J: Body iron stores and presence of carotid atherosclerosis: results from the Bruneck study. *Arterioscler Thromb* 14:1625-1630, 1994.
 26. Kiechl S, Willeit J, Egger G, Poewe W, Oberhollenzer F: Body iron stores and the risk of carotid atherosclerosis: prospective results from the Bruneck study. *Circulation* 96:3300-3307, 1997.
 27. Klipstein-Grobusch K, Koster JF, Grobbee KE, Lindemans J, Boeing H, Hofman A, Witteman CM: Serum ferritin and risk of myocardial infarction in the elderly: the Rotterdam study. *Am J Clin Nutr* 69:1231-1236, 1999.
 28. Stevens RG, Jones DY, Micozzi MS, Taylor PR: Body iron stores and the risk of cancer. *N Engl J Med* 319:1047-1052, 1988.
 29. Stevens RG, Graubard BI, Micozzi MS, Neriishi K, Blumberg BS: Moderate elevation of body iron level and increased risk of cancer occurrence and death. *Int J Cancer* 56:364-369, 1994.
 30. Freudenheim JL, Graham S, Marshall JR, Haughey BP, Wilkinson G: A case-control study of diet and rectal cancer in western New York. *Am J Epidemiol* 131:612-624, 1990.
 31. Nelson RL: Dietary iron and colorectal cancer risk. *Free Rad Biol Med* 12:161-168, 1992.
 32. Siegers CP, Bumann D, Trepkau HD, Schadwinkel B, Baretton G: Influence of dietary iron overload on cell proliferation and intestinal tumorigenesis in mice. *Cancer Lett* 65:245-249, 1992.
 33. Weinberg ED: Association of iron with colorectal cancer. *BioMetals* 7:211-216, 1994.
 34. Roe MA, Fairweather-Tait SJ: High bioavailability of reduced iron added to UK flour. *Lancet* 353:1938-1939, 1999.
 35. Looker AC, Gunter EW, Johnson CL: Methods to assess iron status in various NHANES surveys. *Nutr Rev* 53:246-254, 1995.
 36. Looker A, Gunter E, Johnson C: Are body iron stores increasing in the US population? Serum ferritin data from NHANES II, HHANES, and NHANES III. Hyattsville: National Center for Health Statistics, 1995.
 37. U.S. Department of Health and Human Services (DHHS): "National Center for Health Statistics. Third National Health and Nutrition Examination Survey, 1988-1994, NHANES III Laboratory Data File (CD-ROM Series 11, No. 1A)." Hyattsville: Centers for Disease Control and Prevention, 1997.
 38. Institute of Medicine, National Research Council: "Dietary Reference Intakes: Folate, Other B Vitamins and Choline." Washington, DC: National Academy Press, 1998.
 39. Jacques PF, Selhub J, Bostom AG, Wilson PWF, Rosenberg IH: The effect of folic acid fortification on plasma folate and total homocysteine concentrations. *N Engl J Med* 340:1449-1454, 1999.
 40. Lawrence JM, Petitti DB, Watkins M, Umekubo MA: Trends in serum folate after food fortification. *Lancet* 354:915-916, 1999.
 41. Centers for Disease Control: Folate status in women of childbearing age—United States, 1999. *MMWR* 49:962-965, 2000.
 42. Life Sciences Research Office, Federation of American Societies for Experimental Biology: "Third Report on Nutrition Monitoring in the United States, Prepared for the Interagency Board for Nutrition Monitoring and Related Research." Washington, DC: US Government Printing Office, p 92, 1995.
 43. Savage DG, Lindenbaum J: Folate-cobalamin interactions. In: Bailey LB, ed. "Folate in health and disease." New York: Marcel Dekker, pp 237-285, 1995.
 44. Flynn MA, Herbert V, Nolph GB, Krause G: Atherogenesis and the homocysteine-folate-cobalamin triad: Do we need standardized analyses? *J Am Coll Nutr* 16:258-267, 1997.
 45. Kim Y-I: Folate and carcinogenesis: Evidence, mechanisms, and implications. *J Nutr Biochem* 10:66-88, 1999.

Received November 30, 2000; revision accepted February 18, 2001.