Evaluation of the potential effectiveness of wheat flour and salt fortification programs in five Central Asian countries and Mongolia, 2002–2007

Shamil Tazhibayev, Oksana Dolmatova, Galina Ganiyeva, Khotambek Khairov, Feruza Ospanova, Dalkhjav Oyunchimeg, Dilorom Suleimanova, and Nevin Scrimshaw

Abstract

Background. *A project for universal salt iodation with potassium iodate and wheat flour fortification with a vitamin-mineral premix was implemented in Azerbaijan, Kazakhstan, Kyrgyzstan, Mongolia, Tajikistan, and Uzbekistan between 2002 and 2007.*

Objective. *To determine the potential effectiveness of the food fortification programs in improving the micronutrient status of selected families in a sentinel population in each country.*

Methods. *An area was selected in each country in a sentinel population expected to have early access to iodated salt and fortified wheat flour. Within this area, an average of 40 families with a woman of reproductive age and two children between 2 and 15 years old were sampled at baseline. All the rounds of the study were carried out in women and children in the same households. Hemoglobin, serum ferritin, folic acid, and urinary iodine excretion were analyzed at baseline, one year, and three years later.*

Results. *In the third round in 2007 significant increases were observed in the average levels of blood hemoglobin, serum ferritin and folic acid, and urinary iodine. Corresponding decreases in the prevalence of anemia and increases in serum ferritin levels, folic acid, and iodine were found.*

Conclusions. *Salt and wheat flour fortification resulted in a significant improvement in the micronutrient status of children and women living in sentinel households in the countries participating in the Asian Development Bank project. Sentinel studies were a cost-effective way of determining potential national effectiveness.*

Key words: Azerbaijan, Kazakhstan, Kyrgyzstan, Mongolia, Tajikistan, Uzbekistan, wheat flour fortification, salt iodination, children, women of reproductive age, anemia, iron deficiency, folic acid deficiency, iodine deficiency, micronutrients

Introduction

When the Central Asian countries became independent following the breakup of the Soviet Union in 1991, salt iodation and wheat flour fortification programs were discontinued. As a result, some micronutrient deficiencies—in particular, iron deficiency and iodine deficiency disorders—reappeared along with deficiencies of some B vitamins. National surveys found a high prevalence of anemia among reproductive-age women, and children under five years old before implementation of the food fortification program: 69% and 41% in Azerbaijan [1], 61% [2] and 49% [3] in Uzbekistan, 41% and 62% in Tajikistan [4], 38% and 50% in Kyrgyzstan [5], 35.5% and 36.3% in Kazakhstan [6], and 28% and 20% in Mongolia [7]. The prevalence of iodine deficiency was high as well: 65% in Kazakhstan [6] and 57% in Tajikistan [4] among reproductive-age women; 64% among children under five years old in Tajikistan [4] and 46% among children 7 to 11 years old in Mongolia [8]. These micronutrient deficiencies contribute to high infant and maternal morbidity and mortality rates, retardation in physical and mental development of children, especially among the poorer parts of the population, as well as a deterioration of socioeconomic parameters and increase in poverty.

The Japan Fund for Poverty Reduction (JFPR)

Shamil Tazhibayev is affiliated with Kazakh Academy of Nutrition, Almaty, Kazakhstan. Oksana Dolmatova and Feruza Ospanova are affiliated with the Kazakh Academy of Nutrition in Kazakhstan. Galina Ganiyeva is affiliated with the Nutrition Center in Baku, Azerbaijan; Khotambek Khairov is affiliated with the Centre on Nutrition Problems, Dushanbe, Tajikistan. Dalkhjav Oyunchimeg is affiliated with Health Inspection Division SSIA, Ulanbaatar, Mongolia, and Dilorom Suleimanova is affiliated with the Institute of Haematology and Blood Transfusion, Tashkent, Uzbekistan. Nevin Scrimshaw is affiliated with the International Nutrition Foundation, Boston, Mass., USA.

Please direct queries to the corresponding author: Shamil Tazhibayev, Kazakh Academy of Nutrition, 66 Klochkov St., Almaty 480008, Kazakhstan; e-mail: stazhibayev@caffproject.net.

designed the JFPR-9005 Project "Improving Nutrition of Poor Mothers and Children in Asian Countries in Transition" for 2002–2004 and JFPR-9052 Project "Sustainable Food Fortification" for 2005–2007. The Projects have been implemented in mutual collaboration among the Asian Development Bank (ADB), the United Nations Children's Fund (UNICEF), the Kazakh Academy of Nutrition (KAN), and the country teams of Azerbaijan, Kazakhstan, Kyrgyzstan, Tajikistan, Uzbekistan, and Mongolia. These projects are expected to contribute to the elimination or reduction of iodine deficiency disorders (IDD), iron deficiency anemia (IDA), and a deficiency of some B vitamins, especially in the poor segments of the population in these countries. The project consisted of two interventions: salt fortification with potassium iodate and wheat flour fortified with a vitamin-mineral premix.

A high-level policy agreement of the Commonwealth of Independent States in Minsk, Byelorussia, 31 May 2001, established a uniform level of salt iodination at 40 parts per million (ppm) of potassium iodate. The vitamin-mineral premix KAP-complex #1, which consists of six micronutrients (electrolytic iron – 34.7%, zinc oxide – 18.7%, niacinamide – 6.7%, riboflavin – 2%, thiamin mononitrate – 1.3%, folic acid – 1%, and stuff [silicon dioxide 0.5 to 1.5% and calcium sulfate q.s. to make 100%] – 35.6%), was developed by the Kazakh Academy of Nutrition (KAN), and approved by all participating governments. The premium grade wheat flour (extraction rate 55–60%) was fortified with 150 g and the first grade wheat flour (extraction rate up to 72%) with 120 g of KAP Complex #1 per metric ton (MT) and supplied with the following ppm of micronutrients: 50 – iron, 22 – zinc, 10 – niacin, 3.0 – riboflavin, 2.0 – thiamin, and 1.5 – folic acid in premium grade; and 40 – iron, 17.6 – zinc, 8 – niacin, 2.4 – riboflavin, 1.6 – thiamin, and 1.2 – folic acid in first grade flour.

The Almaty 2001 Forum on the JFPR 9005 Project recommended "Sentinel Studies" in order to evaluate the effectiveness of the project implementation in pilot regions in all six countries involved in the Project, before investing in more costly and difficult national impact surveys. The specific plan of the sentinel studies was:

a) To obtain baseline data on blood hemoglobin (Hb), iron, folic acid, and iodine status of selected groups of families in which the population was expected to have access to iodinated salt and fortified wheat flour within 12 months of the initial survey, and in which improvement in fortified salt and flour distribution was expected.
b) To obtain data on the iron, folic acid, and iodine status in the same selected group of families in each of the six countries 12 and 24 months after beginning the fortification programs. It was hoped that the sentinel sites could be surveyed every one to two years until these deficiencies were no longer a problem.

Materials and methods

Study design

The study was designed to evaluate the effectiveness of the JFPR Program implementation in a favorable pilot region in each country. It was limited to 40 households and 120 individuals (80 children and 40 women) in each country. The baseline round of data collection was carried out from December 2002 to April 2003 before implementation of the food fortification programs. Within each area, 40 families with at least one woman of reproductive age and two children between 2 and 15 years of age were selected. A list of families meeting these criteria was prepared in a child polyclinic in a pilot area of each country. Forty families were randomly sampled from the list. In the second round, from May to August 2004, women of reproductive age living in these 40 households were also selected. This coincided with more extensive food fortification.

The third round was carried out in the second and third quarters of 2007 within the framework of the JFPR-9052 Project, with the exception of Kyrgyzstan, where the accessibility of fortified food in households was low. Therefore, sentinel data from Kyrgyzstan are not included in this paper. Azerbaijan did not participate in the second JFPR-9052 Project but the planned "third round" in this country was completed in May 2005.

Children were included in all three rounds, but women only in rounds 2 and 3. The same data were collected in all three rounds from the same individuals, ADB and UNICEF jointly supported the sentinel study among the children in the first and second rounds of the survey. The observations on women in rounds 2 and 3, and children in round 3 were financially supported exclusively by the Asian Development Bank.

Data collection and management

Data collection was carried out by teams consisting of a country project coordinator and a field assistant, both of whom were either physicians or biologists. They were responsible for carrying out the fieldwork, and interviewing women to determine their knowledge and use of iodated salt and fortified wheat flour. They were also responsible for the quality control for their studies. The country project coordinators managed all activities of the country team, including the field work, interviewing women, collecting blood, urine, salt and flour samples, testing salt for iodine and flour for iron, blood samples for hemoglobin and ferritin levels, urine samples for iodine at National Laboratories, and sending collected data, and blood and urine samples to KAN. They also collated, compared, and interpreted the combined results for their sample.

Analytical procedures

The prevalence of anemia was assessed by measurement of hemoglobin level in whole capillary blood. The cut-off points used to define the different degrees of anemia are shown in **table 1** [9]. In the assessment of the public health significance of this indicator, the World Health Organization (WHO), UNICEF, and the United Nations University (UNU) [10] indicated that a prevalence of mild-to-severe anemia of at least 40% should be considered 'high', a prevalence of 15% to 40% 'medium', and a prevalence of less than 15%, 'low'. Hb was measured by HemoCue during the fieldwork at the sentinel site by the country team. The HemoCue system [11] is a reliable quantitative method for determining hemoglobin concentrations in the field surveys based on the cyanmethemoglobin method [12]. The HemoCue system gives satisfactory accuracy and precision [13]. A field hemoglobin analyzer (HemoCue™) was used to assess hemoglobin to the nearest 0.1 g/dL. Hemoglobinometers were checked several times a day with a control cuvette. The instruments were used only if the reading was within ±0.3 g/dL of the factory value. (HemoCue Inc., Lake Forest CA)

Iron status was assessed by measuring serum ferritin, an important iron-binding protein whose main function is iron storage. Low serum ferritin indicates low iron stores, while iron overload conditions are characterized by elevated serum ferritin concentrations. Values lower than 12 µg/L for children under 5 years and less than 15 µg/L for children 5 years of age and older indicate depletion of body iron stores [14]. The ferritin level in blood samples in Uzbekistan was measured in a national laboratory using an immune enzyme method, and in KAN's laboratory in the other four countries. The radioisotope method was used for measurement of ferritin level in the first round, and the immune enzyme method in the second and third rounds in KAN's laboratory.

Urinary iodine excretion levels (UIE) to determine iodine deficiency (ID) were measured in 10 ml urine samples by the spectrophotometric method [15] in national laboratories in Mongolia, Tajikistan, and Uzbekistan, and in KAN's laboratory for Azerbaijan and Kazakhstan. The cut-off points to define the different levels of urinary iodine concentration were less than 20 µg/L to characterize severe ID, 20 to 49 µg/L – moderate ID, 50 to 99 µg/L – mild ID, 100 to 200 µg/L – normal UIE, 201 to 299 µg/L – higher than normal UIE, and 300 µg/L or more – iodine overload [16]. These cut-off points have been developed for school-aged children, but they have also been used for younger children and adult women in the absence of a clear recommendation for those age groups.

The assessment of folic acid (FA) status and folic acid deficiency (FAD) depended on measuring FA in blood plasma. The cut-off points to evaluate levels of FAD in blood plasma were less than 1.3 µg/L for severe FAD, 1.3–2.9 µg/L – mild FAD, 3 to 6 µg/L – borderline level of FA, and 6 µg/L or higher – normal level of FA [17, 18]. A 5 ml sample of venous blood was collected from the antecubital vein of all women and children in the study. Blood samples were centrifuged soon after collection. Plasma samples were transported in vaccine carriers at 4°C, frozen at –20°C within 12 hours after collection and kept frozen at this temperature until analysis. FA was analyzed by high-performance liquid chromatography using a fluorescent detector [19, 20] in KAN's laboratory. The analytical method for the measurement of 5-methyltetrahydrofolate (5-MTHF), the main metabolite of FA in blood, was based on the chromatographic separation of specially prepared serum samples with a C18 column, using mobile phase with acetonitrile-phosphate buffer containing hexanesulphonic acid as a coupling agent. The quantitative measurement of 5-MTHF was conducted in a concentration range of 0.5 to 40 ng/ml by using an external standard. The concentration of the external standard was controlled spectrophotometrically.

TABLE 1. Definitions of anemia according to blood hemoglobin concentration

Age group	Blood hemoglobin concentration (g/dL)			
	Severe anemia	Moderate anemia	Mild anemia	No anemia
Children 6–59 mo	< 7.0	7.0–9.9	10.0–10.9	≥ 11.0
Children 5–11 yr	< 7.0	7.0–9.9	10.0–11.4	≥ 11.5
Children ≥ 12 yr	< 7.0	7.0–9.9	10.0–11.9	≥ 12.0
Women 15–45 yr	< 7.0	7.0–9.9	10.0–11.9	≥ 12.0

Results

Levels of fortification achieved

Levels of fortified wheat flour production in percent of annual consumption achieved nationally at the time of round 3 were 35% to 40% in Uzbekistan, 25% to 30% in Mongolia, and 15% to 20% in both Kazakhstan and Tajikistan. According to Food and Agriculture Organization (FAO) data, the populations of Central Asia countries consume large amounts of wheat flour: 404 g/person/day in Azerbaijan, 284 in Uzbekistan, 278 in Kazakhstan, and 202 in Mongolia. About 90% is "first grade" wheat flour and the remainder is "supreme grade" (highest extraction). Wheat flour is used in the households for baking bread and preparation of noodles, pastry, dough sheet for preparation of traditional food (for example *besbarmak*—boiled meat with dough sheet, etc.). These products are consumed by all the members of the family.

The following quality control measures were put in place to ensure that the fortificant concentrations in fortified flour and iodine concentrations in salt were within the specified ranges. Fortified wheat flour samples from all the countries were analyzed for measurement of the levels of all micronutrients (iron and zinc by atomic absorption spectrometry; folic acid, thiamin, riboflavin and niacin by HPLC) in KAN's laboratory and in the laboratory of American Ingredients Company, in Kansas City, Missouri, USA. Salt samples were analyzed for measurement of iodine levels by titration. These quality control activities were integral to both the JFPR-9005 and JFPR-9052 Projects.

Levels of nationally iodized salt production when the round 3 samples were taken were 95% to 100% in Kazakhstan, 90% to 100% in Tajikistan, 55% to 60% in Uzbekistan, and 50% to 60% in Mongolia. According to the results of a national study in 2006, adequately iodized salt was available in 92% of households in Kazakhstan, and this country applied for international certification in 2007.

Women's awareness of iron deficiency anemia and use of fortified wheat flour

By round 3, women's awareness of wheat flour fortification was good in all countries; 100% of women in Kazakhstan, Mongolia, and Uzbekistan, 98% in Azerbaijan, and 60% in Tajikistan were aware of fortified wheat flour. The majority of women in Kazakhstan, Mongolia, and Uzbekistan (100%, 88% and 87%, respectively), 49% in Tajikistan, and 25% in Azerbaijan said that they used fortified wheat flour products in their homes. In round 3 the duration of using these varied in each of the participating countries: 11 months in Kazakhstan, 18 in Azerbaijan, 19 in Mongolia, 29 in Uzbekistan, and 49 months in Tajikistan.

The results of spot tests of wheat flour for iron in round 3 revealed that flour was fortified in most households (83%–100%) in all countries. The package of fortified flour contained appropriate information on fortification in most of the countries (65% in Mongolia, 86% in Kazakhstan, and 100% in Uzbekistan). A majority of women in all of the countries (54% in Tajikistan, 60% in Mongolia, 73% in Azerbaijan, and 100% in both Kazakhstan and Uzbekistan) were aware that fortified wheat flour prevents anemia, but most women did not know about the efficacy of fortified flour in preventing vitamin deficiencies.

Anemia rates: hemoglobin levels in whole capillary blood samples

Baseline data on anemia prevalence among children with significant differences in the countries were collected in the first round. Rates were 70% in Tajikistan, 50% in Kazakhstan, 31.4% in Uzbekistan, 20.9% in Azerbaijan, and 12.5% in Mongolia. Children in Tajikistan and Kazakhstan were at the highest risk for anemia. The prevalence of anemia among children in Uzbekistan and Azerbaijan placed these countries in the medium-to-high risk range and Mongolia at a mild-to-medium risk.

Statistically significant increases of hemoglobin in children's whole capillary blood samples as compared with baseline data were observed in Kazakhstan, Tajikistan, and Uzbekistan in round 3 (**table 2**). Statistically significant increases of hemoglobin levels between round 1 and round 3 were found in children 5 years or older but less than 12 years old in Kazakhstan and Tajikistan and in the children 12 years or older in Tajikistan and Uzbekistan. The prevalence of anemia among children significantly decreased in all countries between the first and third rounds of the study with the exception of Mongolia. Anemia among women of reproductive age was very high in Azerbaijan (69%), Kazakhstan (57.5%), and Uzbekistan (51.3%) at baseline. In Tajikistan, the initial levels of anemia prevalence among women (33.3%) placed them at medium-to-high risk; in Mongolia only 10% of women had anemia at baseline (**table 2**).

The prevalence of anemia among women decreased in Azerbaijan, Kazakhstan, and Uzbekistan, but not in Mongolia and Tajikistan, between round 2 and round 3. In all the countries, with the exception of Azerbaijan, the level of anemia dropped below 40%, but in Azerbaijan it was 50% among women in round 3 and remained a public health priority.

Ferritin level in blood serum and iron depletion

Statistically significant increases of serum ferritin levels in children were observed in Kazakhstan and Tajikistan by round 3 compared with baseline data (**table 3**). In Kazakhstan, the prevalence of low ferritin levels among children significantly decreased between the first and third rounds. No improvement in the serum ferritin levels in children between rounds 1 and 3 was found in the other countries. The lowest serum ferritin levels in round 3, indicating depleted iron stores, occurred among children in Kazakhstan (22.9%) and Uzbekistan (22.6%), followed by Mongolia (16.4%), Azerbaijan (16.3%), and Tajikistan (12.9%).

The average ferritin level for women in Azerbaijan and Tajikistan significantly increased in round 3 compared with round 2. Ferritin levels increased in all countries between rounds 2 and 3 with the exception of Uzbekistan (**table 3**). Low serum ferritin levels were still found in Uzbekistan (33.3%), followed by Kazakhstan (25.0%), Tajikistan (22.8%), Mongolia (20.9%), and Azerbaijan (20.0%).

TABLE 2. Hemoglobin measurements in whole capillary blood of children 2 to 15 years of age and women 15 to 49 years of age according to country

Variable	Year[a]	Azerbaijan[b]	Kazakhstan	Mongolia	Tajikistan	Uzbekistan
Children 2–15 yr: comparative data from baseline and round 3						
Mean ± SE hemoglobin (g/dL)	2003					
All children		12.0 ± 1.0	11.4 ±0 .2	12.6 ± 0.1	10.7 ± 0.2	11.9 ± 0.1
≥ 2 <5 yr		11.2 ± 0.7	11.1 ± 0.4	11.4 ± 0.4	9.4 ± 0.5	11.2 ± 0.4
≥ 5 <12 yr		12.0 ± 0.1	11.4 ± 0.2	12.7 ± 0.2	10.8 ± 0.2	12.0 ± 0.2
≥ 12 yr		12.3 ± 0.2	12.3 ± 0.4	13.2 ± 0.3	11.1 ± 0.5	12.3 ± 0.2
95% CI						
All children		11.9–12.2	11.2–11.7	12.3–12.9	10.3–11.1	11.7–12.2
≥ 2 < 5 yr		8.1–14.3	10.2–12.0	10.6–12.3	8.3–10.6	10.5–12.0
≥ 5 < 12 yr		11.8–12.2	11.1–11.7	12.4–13.0	10.4–11.3	11.7–12.3
≥ 12 yr		11.9–12.8	11.5–13.1	12.5–13.8	10.1–12.1	11.8–12.8
Mean ± SE hemoglobin (g/dL)	2007					
All children		12.3 ± 0.1*	12.3 ± 0.1*	12.9 ± 0.2	12.8 ± 0.2*	13.2 ± 0.2*
≥ 2 < 5 yr				12.6 ± 0.5		
≥ 5 < 12 yr		12.1 ± 0.1	12.4 ± 0.2*	12.7 ± 0.2	12.6 ± 0.2*	11.5 ± 0.1
≥ 12 yr		12.5 ± 0.1	12.2 ± 0.2	13.0 ± 0.2	12.9 ± 0.2*	13.6 ± 0.1*
95% CI						
All children		12.2–12.4	12.1–12.6	12.6–13.2	12.5–13.1	12.9–13.5
≥ 2 < 5 yr				10.7–14.5		
≥ 5 < 12 yr		12.0–12.3	12.1–12.7	12.2–13.1	12.2–13.1	11.2–11.7
≥ 12 yr		12.4–12.7	11.8–12.7	12.6–13.4	12.5–13.4	13.3–13.9
Anemia (%)						
Total	2003	20.9	50.0	12.5	70.0	31.4
	2007	6.3*	32.4*	20.3	20.3*	16.7*
Mild	2003	18.6	38.8	12.5	40.0	29.1
	2007	6.3*	25.7	19.0	17.4	16.7
Moderate	2003	2.3	11.2		27.5	2.3
	2007		6.8	1.3	2.9	
Severe	2003				2.5	
Women 15–49 yr: comparative data from round 2 and round 3						
Mean ± SE hemoglobin (g/dL)	2004	11.4 ± 0.2	11.5± 0.3	13.0 ± 0.2	12.7 ± 0.3	11.8 ± 0.2
95% CI		11.1–11.7	10.9–12.0	12.7–13.4	12.1–13.2	11.5–12.2
Mean ± SE hemoglobin (g/dL)	2007	11.7 ± 0.1	13.2 ± 0.3*	12.8 ± 0.2	12.4 ± 0.2	12.3 ± 0.2
95% CI		11.5–12.0	12.5–13.9	12.3–13.3	11.9–12.8	11.8–12.8
Anemia (%)						
Total	2004	69.2	57.5	10.0	33.3	51.3
	2007	50.0	27.8*	25.0	37.1	27.8
Mild	2004	64.1	45.0	7.5	28.2	43.6
	2007	47.5	19.4	22.5	37.1	16.7
Moderate	2004	5.1	10.0	2.5	5.1	7.7
	2007	2.5	8.3	2.5		11.1
Severe	2004		2.5			

*$p < .05$ in comparison with baseline data (for children) or with round 2 data (for women).

a. Women were not included in baseline data; round 1 in Kazakhstan was carried out in December 2002, and 2002 data for this country are in 2003 lines.

b. Round 3 in Azerbaijan was carried out in 2005, and 2005 data for this country are in 2007 lines.

TABLE 3. Ferritin measurement in blood serum samples of children 2 to 15 years of age and women 15 to 49 years of age according to country

Variable	Year[a]	Azerbaijan[b]	Kazakhstan	Mongolia	Tajikistan	Uzbekistan
Children 2–15 yr: comparative data from baseline and round 3						
Mean± SE ferritin (μg/L)	2003					
All children		29.4 ± 2.0	17.6 ± 1.5	38.0 ± 2.2	32.8 ± 2.0	31.2 ± 1.6
≥ 2 < 5 yr		24.2 ± 3.5	12.8 ± 2.3	30.8 ± 4.1	21.4 ± 3.0	26.7 ± 4.4
≥ 5 yr		29.6 ± 2.0	18.7 ± 1.7	39.1 ± 2.5	34.6 ± 2.2	32.1 ± 1.7
95% CI						
All children		25.5–33.3	14.7–20.4	33.5–42.4	28.8–36.7	28.0–34.4
≥ 2 < 5 yr		9.3–39.1	7.9–17.9	21.7–39.9	14.7–28.1	17.2–36.3
≥ 5 yr		25.6–33.7	15.4–22.1	34.2–44.1	30.3–38.9	28.7–35.5
Mean ± SE ferritin (μg/L)	2007					
All children		36.7 ± 2.6	32.5 ± 3.8*	41.8 ± 4.1	48.4 ± 4.8*	25.7 ± 1.6
≥ 2 < 5 yr				33.6 ± 14.9		
≥ 5 yr		36.7 ± 2.6	32.5 ± 3.8*	42.1 ± 4.2	48.4 ± 4.8*	25.7 ± 1.6
95% CI						
All children		31.5–41.9	24.8–40.1	33.7–49.9	38.8–58.0	22.5–28.9
≥ 2 < 5 yr				30.4–97.6		
≥ 5 yr		31.5–41.9	24.8–40.1	33.7–50.4	38.8–58.0	22.5–28.9
Low ferritin level (%)	2003	15.2	43.8	5.0	8.7	8.1
	2007	16.3	22.9*	16.4	12.9	22.6
Women 15–49 yr: comparative data from round 2 and round 3						
Mean ± SE ferritin (μg/L)	2004	16.0 ± 2.2	27.7 ± 4.9	36.2 ± 6.1	27.9 ± 4.9	26.3 ± 2.9
95% CI		11.5–20.5	17.8–37.5	23.9–48.5	18.0–37.8	20.5–32.1
Mean ± SE ferritin (μg/L)	2007	30.2 ± 3.1*	31.7 ± 4.1	61.4 ± 8.3	52.6 ± 7.6*	21.8 ± 1.8
95% CI		23.9–36.5	23.3–40.1	44.7–78.1	37.1–68.1	18.1–25.5
Low ferritin level (%)	2004	50.0	47.4	25.0	43.6	28.2
	2007	20.0*	25.0	20.0	22.8*	33.3

*$p < .05$ in comparison with baseline data (for children) or with round 2 data (for women).

a. Women were not included in baseline data; round 1 in Kazakhstan was carried out in December 2002, and 2002 data for this country are in 2003 lines.

b. Round 3 in Azerbaijan was carried out in 2005, and 2005 data for this country are in 2007 lines.

Folic acid level in blood plasma and folic acid deficiency

Folic acid is one of the six micronutrients in the composition of the premix "KAP Complex," that was used for wheat flour fortification in the JFPR 9005 and 9052 Projects. Very high prevalence rates among children for folic acid deficiency (FAD) were found in the baseline data in all of the countries except Uzbekistan, where the FAD rate was 16.2%. The prevalence of severe FAD in children exceeded that of mild FAD in three countries (Azerbaijan, Kazakhstan, and Tajikistan) (**table 4**). In all the countries, the levels of FAD were lower by round 3. This decrease was highly significant: approximately 13-fold in Tajikistan, 5-fold in Azerbaijan, 4-fold in Kazakhstan, and 2-fold in Mongolia.

By round 3 the average plasma folic acid level in children increased significantly in all countries, except Uzbekistan, where the average was higher in all three rounds as compared with the other countries. Children had the following mean plasma folic acid levels (in μg/L): 7.2 in Uzbekistan, 5.2 in Mongolia, 6.9 in Tajikistan, 5.1 in Azerbaijan, and 4.7 in Kazakhstan in round 3. By the third round the mean levels of folic acid were above the cut-off for folic acid deficiency (<3 μg/L) among children in all countries (**fig 1**).

The average level of folic acid in blood plasma of women increased significantly in Mongolia, but less in the other countries in round 3 (**table 4**). The mean level of folic acid was higher than the cut-off for folic acid deficiency (< 3 μg/L) among women in the third round. The prevalence of FAD among women was highest in Mongolia (75%), followed by Kazakhstan (65%), Uzbekistan (33.3%), Tajikistan (30.8%), and Azerbaijan (12.8%) in round 2. In all the countries, the levels of FAD were lower by round 3.

TABLE 4. Folic acid measurement in blood plasma of children 2 to 15 years of age and women 15 to 49 years of age according to country

Variable	Year[a]	Azerbaijan[b]	Kazakhstan	Mongolia	Tajikistan	Uzbekistan
Children 2–15 yr: comparative data from baseline and round 3						
Mean ± SE folic acid (μg/L)	2003	1.6 ± 0.2	1.6 ± 0.2	1.9 ± 0.2	3.1 ± 0.3	8.6 ± 0.6
95% CI		1.2–2.0	1.1–2.0	1.5–2.3	2.5–3.8	7.3–9.8
Mean ± SE folic acid (μg/L)	2007	5.1 ± 0.3*	4.7 ± 0.3*	5.2 ± 0.5*	6.9 ± 0.4*	7.2 ± 0.5
95% CI		4.5–5.6	4.0–5.3	4.2–6.1	6.1–7.6	6.3–8.1
Folic acid deficiency (%)						
Total	2003	85.0	83.3	85.0	57.5	16.2
	2007	16.3*	18.9*	41.8*	4.4*	12.0
Mild	2003	28.8	20.5	47.5	21.3	10.5
	2007	12.5	18.7	39.3	2.9	10.8
Severe	2003	56.3	62.8	37.5	36.3	5.8
	2007	3.8		2.5	1.5	1.2
Women 15–49 yr: comparative data from round 2 and round 3						
Mean ± SE folic acid (μg/L)	2004	5.0 ± 0.4	3.2 ± 0.4	2.4 ± 0.3	5.0 ± 0.5	4.3 ± 0.3
95% CI		4.3–5.7	2.4–4.0	1.9–3.0	3.9–6.1	3.6–4.9
Mean ± SE folic acid (μg/L)	2007	5.5 ± 0.3	3.6 ± 0.2	5.4 ± 0.8*	7.7 ± 0.9	6.0 ± 0.7
95% CI		4.9–6.1	3.2–4.0	3.8–6.9	5.8–9.6	4.5–7.5
Folic acid deficiency (%)						
Total	2004	12.8	65.0	75.0	30.8	33.3
	2007	10.0	33.3	37.5*	17.2	24.1
Mild	2004	12.8	42.5	60.0	12.8	30.8
	2007	10.0	33.3	32.5	14.3	24.1
Severe	2004	0.0	22.5	15.0	17.9	2.6
	2007			5.0	2.9	

*$p < .05$ in comparison with baseline data (for children) or with round 2 data (for women).

a. Women were not included in baseline data; round 1 in Kazakhstan was carried out in December 2002, and 2002 data for this country are in 2003 lines.

b. Round 3 in Azerbaijan was carried out in 2005, and 2005 data for this country are in 2007 lines.

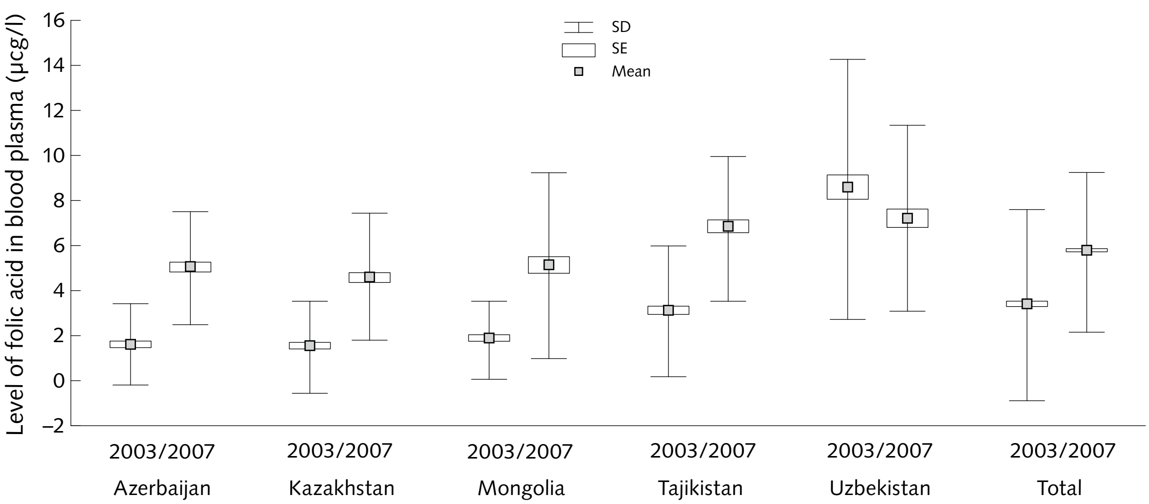

FIG. 1. The levels of folic acid in blood plasma in children, at baseline and round 3, by country

Awareness and knowledge of women about IDD and the role of iodated salt

By the third round, women's awareness of salt iodation was good in all countries. By this time, 94% of the women in Tajikistan and 100% of the respondents in other countries were aware of iodated salt, and 85% to 100% said that they had used iodated salt in their households for 24 to 84 months. Most of these women kept salt in a closed container (55%–97%), and knew that iodated salt prevents goiter (73%–97%). When household salt samples were tested, 100% in Azerbaijan and Mongolia, 84% in Kazakhstan, and 80% in Tajikistan and Uzbekistan were adequately iodated (> 15 ppm).

Urinary iodine excretion

Baseline values in 2002–2003 for median levels of iodine (µg/L) in urine samples of children were 154.7 in Azerbaijan, 109.3 in Uzbekistan, 104.5 in Kazakhstan, 68.8 in Mongolia, and 29.1 in Tajikistan (**table 5**). These data indicate that in Mongolia and especially in Tajikistan, the median levels of urinary iodine among children were below the cut-off for the definition of

TABLE 5. Urinary iodine measurements in children 2 to 15 years of age and women 15 to 49 years of age according to country

Variable	Year[a]	Azerbaijan[b]	Kazakhstan	Mongolia	Tajikistan	Uzbekistan
Children 2–15 yr: comparative data from baseline and round 3						
Median UIE (µg/L)	2003	154.7	104.5	68.8	29.1	109.3
	2007	323.5	187.9	118.6	119.8	136.8
Mean ± SE UIE (µg/L)	2003	152.4 ± 4.6	115.2 ± 8.1	86.3 ±7.7	43.5 ± 4.7	133.4 ± 1 1.6
95% CI		143.3–161.4	99.1–131.3	71.0–101.6	34.1–52.9	110.2–156.5
Mean ± SE UIE (µg/L)	2007	300.5 ± 9.0*	193.9 ± 10.5*	137.8 ± 9.8*	130.3 ± 5.6*	156.5 ± 11.7
95% CI		282.7–318.3	172.9–214.9	118.3–157.2	119.1–141.6	133.2–179.9
Iodine deficiency, total (%)	2003	10.0	48.8	63.8	88.8	47.7
	2007	1.25*	16.2*	43.0*	34.3*	28.6
Mild	2003	7.5	28.8	27.5	16.3	16.3
	2007	1.25	12.1	31.7	31.4	20.2
Moderate	2003	1.3	12.5	26.3	42.5	14.0
	2007		4.1	11.4	2.9	8.3
Severe	2003	1.3	7.5	10.0	30.0	17.4
	2007					
Women 15–49 yr: comparative data from round 2 and round 3						
Median UIE, total (µg/L)	2004	315.0	213.7	72.0	98.3	205.4
	2007	334.3	222.7	112.4	130.4	134.3
Mean ± SE UIE (µg/L)	2004	308.4 ± 7.8	191.3 ± 14.4	99.5 ± 14.0	90.9 ± 5.5	201.2 ± 13.7
95% CI		292.7–324.1	162.3–220.4	71.1–127.9	79.8–102.0	173.4–229.0
Mean ± SE UIE (µg/L)	2007	323.4 ± 12.7	231.1 ± 22.2	129.1 ± 12.7	138.4 ± 8.8*	151.9 ± 14.40
95% CI		297.8–349.1	186.0–286.2	103.4–154.8	120.4–156.3	122.6–181.2
Iodine deficiency (%)						
Total	2004	2.5	15.4	60.0	54.5	5.0
	2007	2.0	19.4	40.0	19.3*	25.7
Mild	2004	2.6	5.1	27.5	40.9	5.1
	2007	2.0	11.1	20.0	16.1	22.8
Moderate	2004		2.6	25.0	4.5	0.0
	2007		8.3	17.5	3.2	2.9
Severe	2004		7.7	7.5	9.1	
	2007			2.5		

*$p < .05$ in comparison with baseline data (for children) or with round 2 data (for women).

a. Women were not included in baseline data; round 1 in Kazakhstan was carried out in December 2002, and 2002 data for this country are in 2003 lines.

b. Round 3 in Azerbaijan was carried out in 2005, and 2005 data for this country are in 2007 lines.

iodine deficiency (< 100.0 μg/L). The median levels of iodine in urine samples of children in all the countries were more than 100 μg/L, and they increased 1.5 to 2.0 times in all the countries in round 3 when compared with baseline data.

The prevalence of low iodine excretion or actual deficiency among children was very high at baseline in all the countries except Azerbaijan, i.e., 88.8% in Tajikistan, 63.8% in Mongolia, 48.8% in Kazakhstan, 47.7% in Uzbekistan, and 10% in Azerbaijan. The average urinary iodine level in children in all countries, with the exception of Uzbekistan, increased significantly in round 3 as compared with the baseline data (**fig. 2**). Mild-to-moderate low urinary iodine excretion among some children persisted in round 3, but there was no severe ID. Some children in Azerbaijan exceeded the cut-off for iodine overload (≥ 300 μg/L).

In all of the countries, the median urinary iodine levels of women increased to more than 100 μg/L by round 3 (**table 5**). In women and children in Azerbaijan, it even exceeded the cut-off for defining an overload of iodine (≥ 300 μg/L). In round 2, more than half the women studied had low UIE (< 100 μg/L), in Mongolia (60.0%), and Tajikistan (54.5%); these percentages were much less in Kazakhstan (15.4%), Uzbekistan (5.0%), and Azerbaijan (2.5%). The prevalence of ID in women significantly decreased in round 3 as compared with round 2 data in Mongolia and Tajikistan (**table 5**). Mild-to-moderately low excretion of urinary iodine was prevalent among women in both rounds 2 and 3.

Discussion

Among women and children, significant decreases in iron deficiency anemia and in low levels of serum ferritin were found in all participating countries in the third round as the result of the implementation of the fortification programs. Sentinel studies of this type can reveal the potential national impact of an intervention. If no impact is observed, this may be due to ineffective implementation of the intervention or to an invalid hypothesis. In either case, this is detected by a sentinel site without the need for a logistically demanding and costly national survey. Sentinel studies should not be confused with effectiveness studies that are based on a national sample. However, when positive, sentinel studies such as this can indicate whether an intervention has the potential to be effective on a national scale if implemented similarly in all of a country. Thus, a process evaluation is required to determine this before proceeding with an effectiveness study.

When anemia rates are high, most cases are due to iron deficiency. However, anemia can be due to other nutritional causes (deficiencies of vitamins A, B_2, B_6, B_{12}, and folic acid), infections such as malaria, HIV/AIDS, hookworm, schistosomiasis, other causes of chronic blood loss, and genetic hemoglobinopathies such as thalassemia [21, 22]. With correction of iron deficiency in a population, there is usually a significant residual level of anemia due to other causes, as in the present study.

Iron bioavailability depends on the composition of the diet. Iron is present in food as either part of heme, as found in meat, poultry, and fish, or as non-heme iron, present in various forms in all foods and the only iron in foods of plant origin. Heme iron is always well absorbed and is only slightly influenced by dietary factors. The absorption of non-heme iron is strongly influenced by interaction with other meal components in the lumen of the upper small intestine. Meat, fish, and poultry improve iron nutrition both by providing highly bioavailable heme iron and by enhancing non-heme iron absorption. The mechanism of this enhanc-

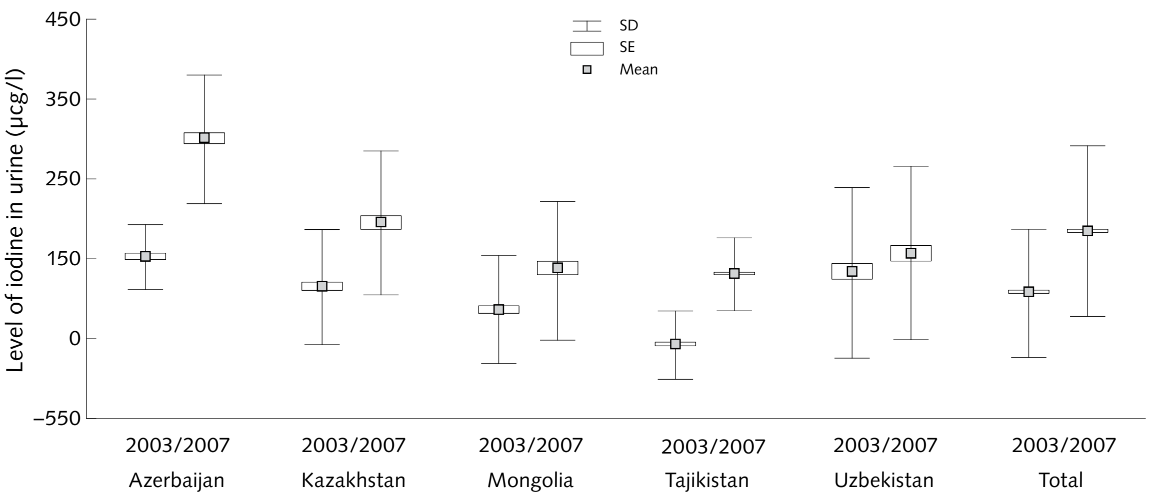

FIG. 2. The levels of iodine in urine in children, at baseline and round 3, by country

ing effect on non-heme iron absorption is believed to involve low molecular weight peptides that are released during digestion [23]. The low intake of meat and other animal products is one of the main reasons for the low bioavailability of iron and the high rate of iron deficiency anemia in the Central Asian region and Mongolia [24].

The absorption of added iron is inhibited by the high level of phytate in cereals and other foods of plant origin [25, 26]. Because phytate and micronutrients, including iron, are concentrated in the aleuronic layer and germ of grains, milling to white flour and white rice reduces the content of both phytate and iron. The populations of the participating countries consume large amounts of products prepared from first grade wheat flour. They are the potential source of many nutrients, but a large portion are lost during milling. This contributes to iron deficiency, and to the deficiency of some other micronutrients such as zinc and B vitamins (B_1, B_2, B_6, niacin, and folic acid). Serum ferritin level is a specific biochemical test that correlates with body iron stores. A low serum ferritin level reflects depleted iron stores and is a precondition for iron deficiency [14]. The high prevalence of low serum ferritin levels in children and women when sampled at baseline serves as evidence that iron deficiency is a main reason for anemia in Central Asia and Mongolia. However, serum apoferritin is an acute-phase reactant protein and is elevated in response to any infectious or inflammatory process. Consequently, serum ferritin in the normal range reflects iron sufficiency only in the absence of these conditions. The interpretation of serum ferritin requires allowance for this in populations in which the incidence of infection or inflammation is high [27].

The use of the fortified wheat flour improved folic acid status and decreased FAD more significantly and rapidly than iron status or anemia. Plasma folic acid levels reflect relatively recent dietary intake [18]. Hence, they are the most rapid and sensitive indicator of the efficacy of flour fortification.

There are some apparent anomalies in the findings. By round 3 in Tajikistan and Mongolia, hemoglobin levels in women decreased slightly instead of improving, and ferritin levels decreased slightly in Uzbekistan. These findings can be explained by the prior use of iron-folate supplements by women in these two countries. In the interviews in round 2, 74.4% of women in Tajikistan, 27.5% in Mongolia, and 20.5% in Uzbekistan reported that they took iron-folate supplements in the preceding 12 months. In round 3, the number of women who used supplements dropped to 28.6%, 22.5%, and 0% in these countries, respectively. In Uzbekistan, where wheat bread is the main staple, 28.9% of women answered that they bake bread at home, and 71.1% buy it in a shop.

The main potential confounding factor in comparing the results from the different countries is the variable sustainability of fortified wheat flour production nationally and for the sentinel sites. This can differentially influence the availability and consumption of fortified wheat flour in sentinel households. In addition, improvements in socioeconomic and health status of populations over the duration of the study could influence some of the results.

Legislation mandating salt iodation was adopted by all of the countries in the region. In a national micronutrient survey in 2006, 92% of households in Kazakhstan consumed adequately iodated salt, and this country applied for international certification in 2007, as a country achieving universal salt iodation. The production level of iodated salt in percentage of annual consumption achieved in 2007 was 95% to 100% in Kazakhstan, 90% to 100% in Tajikistan, 55% to 60% in Uzbekistan, and 50% to 60% in Mongolia.

The salt iodation program was effective in all participating countries as judged by the higher coverage of the households using iodated salt, an increase in the median level of urinary iodine excretion, and a decrease in low levels of urinary iodine in the third round compared to the baseline data.

The sentinel studies confirmed that effective implementation of food fortification programs can increase a population's knowledge and awareness of the importance of fortified food products, and identified their impact on iron, folic acid, and iodine status in a sample of women and children living where the program was implemented.

Sentinel studies of this kind are a valuable and cost-effective means of monitoring program knowledge and awareness of households, the availability and accessibility of fortified food, the quality of fortified food products, and the impact of the program at household and individual levels. They are a relatively inexpensive way to determine the potential effectiveness of a large scale intervention and to indicate when a more extensive survey is likely to be worthwhile.

Acknowledgments

We appreciate the hospitality and cooperation of the families, mothers and children in Azerbaijan, Kazakhstan, Mongolia, Tajikistan, and Uzbekistan who were interviewed and who allowed us to take food and biological samples for analysis. We are greatly indebted for the strong support received from the Director of KAN, Dr. Toregeldy Sharmanov. We are grateful to the Ministries of Health, local health authorities, research institutes and country teams in Azerbaijan, Kazakhstan, Mongolia, Tajikistan, and Uzbekistan for their collaboration with the sentinel study. We acknowledge the personnel in the regional coordination and administration office and country project offices in the participating countries for their technical and logistic support. Special thanks go to the country teams for collecting the sentinel study information and the personnel of the laboratories of the Kazakh Academy of Nutrition who analyzed blood and urine samples. Funding for this study was provided by the Japan Fund for Poverty Reduction through the Asian Development Bank. The financial and technical support for the first and second rounds of the survey was shared by UNICEF.

References

1. Ministry of Health. Nutrition survey in Azerbaijan—1995. Baku, Azerbaijan: Ministry of Health: (WHO-EURO/CDC),1996.
2. Institute of Obstetrics and Gynaecology, Ministry of Health of the Republic of Uzbekistan, Tashkent City, Uzbekistan. Demographic and Health Survey—1996. Calverton, MD: Macro International Inc., September 1997.
3. Analytical and Information Centre, Ministry of Health, State Department of Statistics, Ministry of Macroeconomics and Statistics, Republic of Uzbekistan. Uzbekistan health examination survey—2002. Calverton, MD: ORC Macro International. April 2004.
4. Nutrition status of mothers and children in Tajikistan—2003. Ministry of Health of the Republic of Tajikistan, UNICEF, Asian Development Bank, WHO, National Institute for Research on Food and Nutrition, Italy, and Kazakh Academy of Nutrition, Almaty, Kazakhstan. April 2004.
5. Research Institute of Obstetrics and Paediatrics, Ministry of Health of the Kyrgyz Republic, Bishkek City, Kyrgyz Republic. Demographic and health survey—1997. Calverton, MD: Macro International Inc., 1998.
6. Academy of Preventive Medicine, Almaty, Kazakhstan. Demographic and health survey—1999. Calverton, MD: Macro International Inc., 2000.
7. Bolormaa N, Byambatogtoch B, Bates J, Serdula MK, Kaufmann R, Woodruff BA, Khan LK, Gillespie C. Final report of a survey assessing the nutritional consequences of the Dzud in Mongolia. Ulaan Baatar: Mongolian Ministry of Health, 2003.
8. Kachondham Y. Report on the 2nd national child and nutrition survey, Mongolia 1999. Bangkok: Thailand, Mahidol University, Institute of Nutrition, 2000.
9. WHO, UNICEF, UNU. Methods of assessing iron status. In: Iron deficiency anaemia. assessment, prevention and control. A guide for programme managers. Geneva: WHO, 2001:33–46.
10. WHO, UNICEF,UNU. Indicators for assessing iron deficiency and strategies for its prevention. Draft based on a WHO, UNICEF, UNU consultation 6–10 December 1993, WHO: Geneva, 1996.
11. Bull BS, Houwen B, Koepke JA, Simson E, van Assendelf T. Reference and selected procedures for the quantitative determination of hemoglobin in blood: approved standards. 2nd ed. Villanova PA: National Committee for Clinical Laboratory Standards, 1994.
12. Van Schenck H, Falkensson M, Lundberg B. Evaluation of "HemoCue", a new device for determining hemoglobin. Clin Chem 1986;32:526–9.
13. Johns WL, Lewis SM. Primary health screening haemoglobinometry in a tropical community. Bull WHO 1989;67:627–63.
14. WHO. Iron deficiency anaemia: assessment, prevention and control. A guide for programme managers. Geneva: WHO, 2001.
15. Dunn JT, Crutchfield HE, Gutekunst R, Dunn A. Methods for measuring iodine in urine. Wageningen, The Netherlands: WHO/UNICEF/ ICCIDD, 1993.
16. WHO/UNICEF/ICCIDD. Urinary iodine. In: Assessment of iodine deficiency disorders and monitoring their elimination. A guide for programme managers. Geneva: WHO, 2001:31–6.
17. Institute of Medicine. Dietary reference intakes: thiamin, riboflavin, niacin, vitamin B_6, folate, vitamin B_{12}, pantothenic acid, biotin and choline: Washington DC: Institute of Medicine of the National Academy of Sciences, 2000.
18. Brody T, Shane B. Folic acid. In: Rucker RB, Suttie JW, McCormick DB, Machlin LJ, eds. Handbook of vitamins, 3rd ed. Marcel Dekker: New York, 2001:427–62.
19. Snow CF. Laboratory diagnosis of vitamin B12 and folate deficiency: A guide for the primary care physician. Arch Int Med 1999;159(28 June):1289–98.
20. Chladek J, Sispera L., Martinkova J., High-performance liquid chromatographic assay for the determination of 5-methyltetrahydrofolate in human plasma. J Chromatography, 2000;B 744 (2) (21 July):307–13.
21. Allen LH, Casterline-Sabel JE. Prevalence and causes of nutritional anemia. In: Ramakrishnan U, ed. Nutritional anemia. Boca Raton, FL: CRC Press, 2000:17–21.
22. Kraemer K, Zimmermann MB, eds. Nutritional anemia. Waldkirch, Germany: Sight and Life Press, 2007.
23. Taylor PG, Martinez-Torres C, Romano EL, Layrisse M. The effect of cysteine containing peptides released during meat digestion on iron absorption in humans. Am J Clin Nutr 1986;43:68–71.
24. KAN, UNDP, UNICEF. National nutrition survey in Kazakhstan. Almaty, Kazakhstan: Kazakh Academy of Nutrition, 1996.
25. Cook JD, Reddy MB, Burri J, Juillerat MA, Hurrell RF. The influence of different cereal grains on iron absorption from infant cereal foods. Am J Clin Nutr 1997;65:964–9.
26. Sandberg AS. 1991. The effect of food processing on phytate hydrolysis and availability of iron and zinc. Adv Exp Med Biol 289:499–508.
27. Asobayire FS, Adou P, Davidsson L, Cook JD, Hurrell RE. Prevalence of iron deficiency with and without concurrent anemia in population groups with high prevalences of malaria and other infections: a study in Cote d'Ivoire. Am J Clin Nutr 2001; 74:776–82.