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Authors: **MM Root***, J Hu, LS Stephenson, RS Parker and TC Campbell

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Objective: To determine the relationships of dietary iron sources, other dietary factors, and lifestyle to iron status among premenopausal and recently postmenopausal Chinese women with widely varying regional dietary patterns.

Design: Cross-sectional. Subjects were interviewed, blood samples were drawn, and dietary intakes were measured by a 3-day dietary survey for subjects in the five survey counties.

Setting: Rural China

Subjects: About 80 randomly selected subjects per county among women aged 32 - 66 y.

Main outcome measures: Blood hemoglobin, plasma ferritin, and plasma iron.

Results: Total iron intake was relatively high (15 - 29 mg/d) compared to developed counties. Heme iron intake was negligible in two of the study counties. Overall levels of iron deficiency anemia were relatively low in these generally iron-stressed women. There was no clear statistical relationship between iron intake and physiological iron status. Although several measures of dietary intake (heme iron, dietary calcium, animal protein) were correlated with several measures of iron status before adjusting for survey county, only dietary animal protein was significantly positively correlated with plasma ferritin after adjusting for the possibly confounding factor of the survey county ($r = 0.15$, $P = 0.009$). Intakes of potential inhibitors of iron absorption, such as tea, even in very high amounts, were not correlated to iron status. Plasma ferritin was positively correlated with plasma retinol ($P = 0.024$) and cholesterol ($P = 0.007$). Systemic inflammatory response, as indicated by high plasma C-reactive protein levels, was shown to be raised in a group of subjects with apparently contradictory high levels of ferritin and low levels of hemoglobin ($P = 0.03$).

Conclusions: Iron nutriture in these areas of rural China seemed more related to physiological factors such as inflammatory response, menses, plasma vitamin A and cholesterol, than to dietary factors.

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Descriptors: China; C-reactive protein; dietary iron; ferritin; hemoglobin; menopause

Introduction

China is a country with a wide diversity of ethnic groups, dietary habits, living environments and climates. Staple grains vary from rice to wheat to corn. Sources of protein range from almost entirely vegetable and grain based in poorer farming areas to a high proportion of meat and dairy products in the semi-nomadic areas of the north and west. In a large survey of 65 rural counties in China, these patterns were confirmed and were found to be associated with the mortality patterns in these areas (Chen *et al,* 1991).

Rural China has proved to be an invaluable environment in which to conduct studies of diet and health. Evidence mounts that a nearly vegan diet such as is largely consumed in rural China is not only nutritionally adequate but is effective in preventing the onset of the two main scourges of Western societies: cardiovascular and neoplastic diseases. Results from the 65 county ecological study generally, though not entirely, support these conclusions (Campbell *et al,* 1990; Wenxun *et al,* 1990; Chen *et al,* 1992; Marshall *et al,* 1992; Campbell and Chen, 1994).

The differing benefits of vegan and omnivorous diets have long been a topic for nutrition research. Numerous investigations have reported a lower iron status among vegetarians and vegans (D'Souza *et al,* 1987; Helman & Darnton-Hill, 1987; Alexander *et al,* 1994; Shaw *et al,* 1995). The populations studied varied considerably in their intake of dietary enhancers of iron absorption such as heme iron and vitamin C, and of dietary inhibitors such as tea, calcium, and dietary fiber. The net effect in a given study group would depend on the balance of these affectors.

This report evaluated iron status of middle-aged women in five rural Chinese counties in relation to diet and lifestyle factors. These women were previously chosen for studies of diet and osteoporosis (Hu *et al,* 1993a,b, 1994) and of diet and vitamin A status (Root *et al,* 1997). The specific aims were to examine the relationship between dietary enhancers and inhibitors, menopausal status, and inflammatory status on several measures of iron status, and to evaluate the overall iron nutriture of rural Chinese premenopausal women.

Subjects and methods

Sampling procedures

A field survey was conducted in rural China in the summer and autumn of 1989. Details of the survey, including subject selection, dietary survey, health and lifestyle questionnaire, and blood collection techniques, have been described previously (Hu *et al,* 1993a,b, 1994a,b). In summary, five counties were chosen from among the original 65 that participated in our 1983 ecological survey in rural China (Chen *et al,* 1991). These sites were chosen to represent a wide variety of dietary patterns that have been described in detail previously (Hu *et al,* 1993a,b; Root *et al,* 1997) and are summarized briefly in Table 1. In each of the five counties about 80 female subjects between the ages of 32 and 66 years were chosen randomly. The survey protocol was approved by the Human Subjects Committee of Cornell University and the Chinese Academy of Preventive Medicine. The experimental protocol was fully explained to the subjects and their signed approval was obtained at the beginning of the survey.

A fasting heparinized blood sample was drawn from each subject before breakfast and plasma was prepared by centrifugation. Frozen plasma was shipped to Beijing and then to Cornell University for analysis. A questionnaire on dietary and reproductive factors and work habits was administered. Menopausal status and parity were determined. Daily physical activity was determined by the investigators through questions on usual daily activity and was classified according to the method used in the Chinese RDA (Chinese Nutrition Society, 1989; Hu *et al,* 1994a) using a 5-point scale. The values of the scale were 1 for very light sedentary work (office work), 2 for light work (sales clerk), 3 for medium work (driving), 4 for heavy work (mechanical farming), and 5 for very heavy physical work (manual labor). Most of the subjects were classified as 2 or 3 based on occupation and intensity of work load. Postmenopausal women were 1 - 2 y postmenopausal. Forty eight subjects with undeclared menopausal status were also included.

Dietary assessment

The intake of foods and food ingredients was determined in the 3-day weighed food dietary survey (Hu *et al,* 1993a, 1994b). Before each meal, investigators first separated and weighed selected foods for each subject. After the meal, the investigators returned and weighed the remaining food for each subject. Tea consumption was determined in only 3 of the 5 counties. Although not directly measured, the pattern of tea consumption in Changle and Cangxi counties was observed to be rather low and similar in scale to that for Jiexiu county. Black brick tea was consumed in large amounts in Tuoli and Xianghuangqi counties, while smaller amounts of green tea were consumed in the other counties. No subject was observed in Jiexiu, nor assumed in Changle or Cangxi, to consume more than 10 g (dry weight) of tea per day. Tea leaf was weighed in the dry form before brewing.

Calculation of the major dietary sources of iron was performed by comparing the food intakes with the Chinese Food Composition Table (Institute of Nutrition and Food Hygiene, 1991). Since the amount of heme iron in meat is about 40% (Tseng *et al,* 1997), heme iron was calculated from all meat and seafoods as 40% of the total iron. Non-heme iron sources included all plant products, eggs and dairy products, and 60% of meat iron. A number of dietary constituents are known to inhibit or promote the absorption of dietary iron. In addition to considering the individual relationships of these constituents with iron status, their aggregate relationship was considered by way of an index of inhibition based on the consumption of four known affectors of iron absorption. Each subject was assigned a score of 0 - 8 based on the level of dietary tea, fiber, vitamin C, and heme. The score for each subject was increased by one for each of the following eight groups for which they qualified: vitamin C < 30 mg/d, vitamin C < 5 mg/d, fiber > 30 g/d, fiber > 50 g/d, tea > 10 g/d, tea > 30 g/d, heme iron < 0.4 mg/d, and heme iron < 0.04 mg/d.

Blood analyses

Hemoglobin was measured immediately after blood was drawn using the cyanomethemoglobin method of Drabkin and Austin (1932). Plasma samples were stored frozen at -80°C (4 - 6 y) before analysis. Plasma retinol was measured by modifications of the method of Driskall *et al* (1982) as described previously (Root *et al,* 1997). Plasma cholesterol was measured using Sigma kit 352 (St Louis, MO, USA) by the cholesterol oxidase peroxidase method. Ceruloplasmin, transferrin, and C-reactive protein (CRP) were measured by automated nephelometry using a model BN100 instrument (Behring Diagnostics, Somerville, NJ, USA). Plasma iron was measured with Sigma kit 565 using the ferrozine method. Transferrin saturation was calculated from plasma iron and transferrin data. Ferritin was quantified with a Micromedic® monoclonal immunoradiometric assay kit (ICN Biomedicals, Costa Mesa, CA, USA).

Statistical analysis

The data were analyzed using Systat 5.03 statistical software (Evanston, IL, USA). County means were compared by the Tukey - Kramer post-hoc multiple comparisons test. General linear models with county of residence as an independent variable were used to determine partial correlations of a number of dependent variables with plasma ferritin, plasma iron, and blood hemoglobin levels. Logarithm or square root transformations were used for variables with highly skewed distributions.

In statistical models with menopausal status as a variable, subjects with no indicated menopausal status but who were over age 55 y were classified as postmenopausal. The mean age of menopause in the counties studied was 47 y. Women under age 40 y but with no indicated menopausal status were classified as premenopausal. Four women over age 55 y reported themselves to be premenopausal. These subjects and subjects with no indicated menopausal status between the ages of 40 - 55 y were excluded from statistics involving menopausal status.

Results

The lifestyles and diets of the subjects in the survey counties varied considerably (Table 1). Jiexiu and Cangxi were relatively impoverished areas with minimal meat consumption. Changle, in wealthier Fujian province, was characterized by a more varied diet with more animal products, including eggs, poultry and fish. The survey subjects in Tuoli and Xianghuangqi counties were largely

Table 1 Location, dietary patterns, and selected characteristics of women in five rural counties in China

	County (Province)				
	Jiexiu (*Shanxi*)	*Changle* (*Fujian*)	*Cangxi* (*Sichuan*)	*Tuoli* (*Xinjiang*)	*Xianghuangqi* (*Neimongol*)
Terrain	Plains	Coastal	Mountains	Plateau	High plateau
Diets[1]	Wheat noodles Vegetables Millet	Rice Vegetables Fish and pork	Rice Corn flour Sweet potatoes	'Nan' bread Mutton	Wheat flour Dairy products
			Major sources of iron		
Heme iron	none	Pork Dried fish	none	Mutton	Mutton Blood sausage
Non-heme iron	Wheat flour	Rice	Rice Wheat flour	Wheat flour	Cheese Wheat flour
Characteristics[2]					
Number of subjects	70	81	89	91	74
Age (y)	43 ± 9	43 ± 6	44 ± 7	43 ± 6	43 ± 6
Parity	5.1 ± 2.2^{a}	4.5 ± 1.8^{a}	4.6 ± 2.2^{a}	7.1 ± 3.0^{b}	5.2 ± 2.3^{a}
Postmenopausal (%)	14	21	24	22	15
Physical activity (scale of 1 - 5)	2.3 ± 0.5^{b}	2.0 ± 0.2^{a}	2.8 ± 0.4^{c}	2.8 ± 0.4^{c}	2.9 ± 0.3^{c}

[1]Reproduced in part from previously published tables (Hu ***et al,*** 1993a; Root ***et al,*** 1997).
[2]Values within rows (mean ± s.d.) with different superscript letters are statistically significantly different at $P < 0.05$ using the Tukey-Kramer post-hoc test.

ethnic minorities and were semi-nomadic herders. Their diets consisted of minimal vegetables and fruits but they consumed large amounts of dairy and meat products. A major food in Xianghuangqi was hot milk-tea which was consumed year round in great quantities. Dietary iron in rural China was derived from a limited number of sources. The levels of heme iron in the diet were very low in the poorer areas, but in Tuoli and Xianghuangqi the diets included mutton and sheep's blood sausage, both rich sources of heme iron. Fruits and vegetables contributed only minimally to dietary non-heme iron. The various staple grains were the major sources of dietary iron in all of the counties.

Child bearing practices varied among the survey counties (Table 1). Average parity varied from 4.5 to 7.1. According to the Chinese RDA (Chinese Nutrition Society, 1989) a physical activity score of 2 represented light physical work such as standing and walking and a score of 3 represented medium activity such as household work or dairy milking.

Dietary sources of macronutrients and micronutrients varied across the counties (Table 2) and reflected the dietary patterns. Subjects in Jiexiu and Cangxi counties were virtually vegans, with little meat or dairy intake and with most of their calories and protein coming from plant (principally grain) sources. The consumption of vitamin C and fiber was noticeable higher in those counties than the other counties. In contrast, subjects in Xianghuangqi county consumed relatively high levels of dairy and meat products. The low intake of vitamin C and very high

Table 2 Selected daily nutrient and food intakes of women in five rural counties in China[1]

	County (Province)				
	Jiexiu (*Shanxi*)	*Changle* (*Fujian*)	*Cangxi* (*Sichuan*)	*Tuoli* (*Xinjiang*)	*Xianghuangqi* (*Neimongol*)
Non-dairy animal protein[2] (g)	2 ± 5^{a}	12 ± 9^{b}	1 ± 2^{a}	14 ± 13^{b}	12 ± 11^{b}
Dairy protein (g)	0 ± 3^{a}	0	0	5 ± 4^{a}	38 ± 23^{b}
Plant protein (g)	59 ± 13^{b}	38 ± 8^{a}	59 ± 18^{b}	35 ± 9^{a}	35 ± 10^{a}
Soy protein (g)	1.3 ± 3.9	0.8 ± 2.8	1.5 ± 4.2	0	0
Tea[3] (g dry wt)	2 ± 2^{a}	-[4]	-[4]	9 ± 6^{b}	38 ± 23^{a}
Vitamin C (mg)	154 ± 94^{c}	61 ± 40^{b}	147 ± 91^{c}	38 ± 40^{ab}	21 ± 29^{a}
Dietary Fiber (g)	15 ± 5^{c}	7 ± 2^{b}	18 ± 7^{d}	4 ± 2^{a}	6 ± 2^{b}
Inhibitor index	1.9 ± 0.8^{bc}	1.3 ± 1.0^{a}	2.3 ± 1.0^{c}	1.5 ± 1.2^{ab}	2.8 ± 1.0^{d}
Heme iron (mg)	0.1 ± 0.2^{a}	0.5 ± 0.4^{b}	0.1 ± 0.1^{a}	1.0 ± 1.0^{c}	1.3 ± 1.1^{c}
Non-heme iron[5] (mg)	21 ± 5^{b}	14 ± 3^{a}	22 ± 6^{b}	27 ± 7^{c}	21 ± 8^{b}
Total iron (mg)	21 ± 5^{b}	15 ± 4^{a}	22 ± 6^{b}	28 ± 7^{c}	22 ± 9^{b}

[1]Values (mean ± s.d.) within rows with different superscript letters are statistically significantly different at $P < 0.05$ using the Tukey-Kramer post-hoc test.
[2]Includes meat, fish, and eggs.
[3]Green tea was consumed in Jiexiu county and black brick tea made with milk was consumed in Tuoli and Xianghuangqi counties.
[4]Data not collected.
[5]Includes plant, egg, and dairy sources of iron and 60% of iron from meats.

Table 3 Selected indicators of iron status of women in five rural counties in China[1]

	County (Province)				
	Jiexiu (*Shanxi*)	*Changle* (*Fujian*)	*Cangxi* (*Sichuan*)	*Tuoli* (*Xinjiang*)	*Xianghuangqi* (*Neimongol*)
Hemoglobin (g/l)	146 ± 12^{c}	155 ± 11^{d}	128 ± 9^{a}	129 ± 17^{a}	137 ± 12^{b}
Iron[2] (μmol/l)	10.7^{a}	13.3^{ab}	11.5^{a}	11.0^{a}	15.4^{b}
	(5.0–22.8)	(7.5–23.7)	(5.9–22.3)	(5.1–23.8)	(8.2–28.8)
Transferrin (g/l)	3.2 ± 0.5^{ab}	3.1 ± 0.6^{a}	3.1 ± 0.6^{a}	3.4 ± 0.9^{b}	3.1 ± 0.6^{a}
Transferrin saturation[2]	0.15^{a}	0.20^{ab}	0.16^{a}	0.15^{a}	0.22^{b}
	(0.07–0.34)	(0.11–0.37)	(0.08–0.34)	(0.06–0.33)	(0.11–0.45)
Ferritin[2] (μg/l)	41^{a}	55^{ab}	36^{a}	48^{a}	85^{b}
	(15–111)	(19–160)	(15–82)	(14–169)	(29–246)

[1]All analytes were in plasma except hemoglobin which was measured in whole blood. Values (mean ± s.d.) within rows with different superscript letters are statistically significantly different at $P < 0.05$ using the Tukey–Kramer post-hoc test.
[2]Geometric mean. Values in parentheses are geometric mean ± 1 s.d.

intakes of tea were reflected in the high index of iron-absorption inhibitors in Xianghuangqi. The low average inhibitor index in Changle county reflected the high intakes of vitamin C and heme iron and the low intakes of fiber and tea. The intake of total dietary iron was generally above the RDA (both Chinese and American) of 18 mg/d for middle-aged women.

Iron status across the study counties is described in Table 3 and Figures 1 - 3. Blood hemoglobin concentrations were generally in the normal range. Altitude was not likely to significantly elevate hemoglobin levels, since the highest county was only 1000 m above sea level. The county with the highest hemoglobin levels, Cangxi, was at sea level. Iron levels below about 11 mmol/l (60 μg/dl) have been reported to reflect iron-deficient erythropoiesis (Gibson, 1990). County average levels near that value were found in three of the five sites. Average values for trans ferrin saturation were similarly suggestive of widespread transport-level iron deficiency in these same counties. Average ferritin levels suggest generally adequate though somewhat low levels of liver iron stores in those same areas. There were also several subjects with either iron, transferrin saturation, or ferritin levels that were suggestive of iron overload. These subjects had levels above 30 μmol/l iron, 0.6 transferrin saturation, or 300 μg/l ferritin (Gibson, 1990).

Correlates of plasma ferritin, iron, and blood hemoglobin levels were examined (Table 4). These blood measures of iron status were positively correlated with each other and negatively correlated with transferrin levels. Although many dietary factors were correlated with ferritin, iron, or hemoglobin levels, some of these correlations were greatly reduced in significance after controlling for the survey county. Ferritin was correlated with menopausal status, being 59% higher in postmenopausal women, and with CRP, an indicative marker of recent or chronic inflamma tion or infection. Ferritin was also positively correlated with dietary animal protein and with plasma retinol and cholesterol concentrations. After controlling for county, plasma iron was not correlated with any dietary factors. Hemoglobin was positively correlated with plasma retinol and negatively correlated with CRP.

Heme iron intake levels were correlated with plasma ferritin and iron concentrations only before adjusting for county. Non-heme dietary iron intake was negatively cor related with hemoglobin concentrations only before adjust ing for county. Dietary animal protein, including meat, fish, dairy and eggs, was positively correlated to plasma ferritin

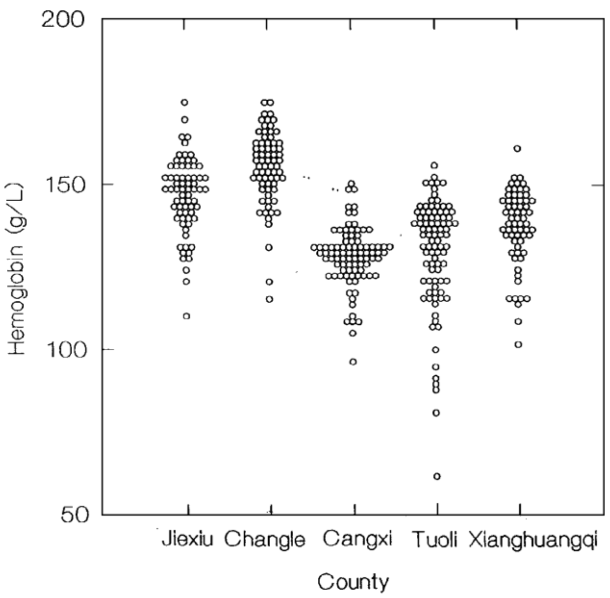

Figure 1 Distribution of blood hemoglobin values among women in five rural counties in China.

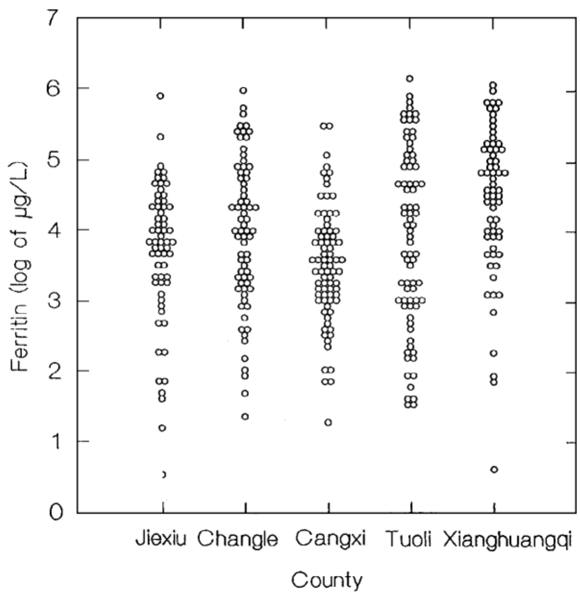

Figure 2 Distribution of plasma ferritin values among women in five rural counties in China.

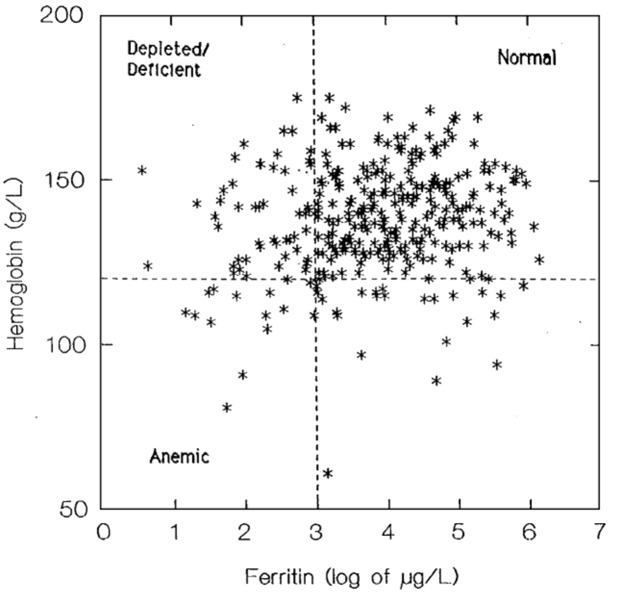

Figure 3 Individual blood hemoglobin and plasma ferritin values among women in five rural counties in China.

and iron. The levels of four dietary inhibitors of iron absorption were combined in a single index of inhibitor intake. The levels of intake of tea and dietary fiber were not correlated with any measure of iron status (data not shown) and the composite index was not significantly correlated after adjusting for county. In a separate analysis considering only the pastoral counties with high tea and heme consumption and low vitamin C intake, there was also no correlation between iron status and the inhibitor index. Transferrin saturation was similar to plasma iron in its pattern of correlations with dietary and biochemical markers though slightly less statistically significant (data not shown).

Figures 1 and 2 show the distribution of hemoglobin values and the log of ferritin values in the survey counties. Only a few subjects in Tuoli county were more than slightly anemic. In Figure 3 the upper right quadrant represents a healthy iron status, with hemoglobin levels above 120 g/l and ferritin levels above 20 μg/l. The upper left quadrant represents depletion of liver iron stores or plasma iron transport-level deficiency. The lower left quadrant represents iron-deficiency anemia. The lower right quadrant represents apparently conflicting measures of iron status with high ferritin and low hemoglobin levels. A comparison of features of the subjects in the four quadrants highlights some physiological differences (Table 5). Plasma iron and transferrin followed expected patterns for iron deficiency in the first three quadrants. In the final quadrant iron and transferrin values approximated those of the depleted/deficient stage. CRP was significantly higher in the lower right quadrant than in the other quadrants combined, suggesting an acute-phase response from an increased level of infection or inflammation among these subjects. The levels of ceruloplasmin, another acute-phase reactant, were also increased in the fourth quadrant. Plasma retinol levels were lower in both the anemic quadrant and the lower right quadrant than in the first two quadrants.

Discussion

The counties in this study were chosen for their diversity of diet between animal and plant food sources and provide an opportunity to compare a variety of interacting nutrients

Table 5 Plasma measurements and stages of iron deficiency of women in five rural counties in China[1]

Hemoglobin: *Ferritin:* *Stage:*	*Normal* *Normal* *Normal*	*Normal* *Low* *Depleted/ deficient*	*Low* *Low* *Anemic*	*Low* *Normal* *Undefined*
Number of subjects	253	51	14	23
Iron (μmol/l)[2]	13.4[c]	9.6[ab]	5.7[a]	11.1[bc]
Transferrin (g/l)	3.0 ± 0.5[a]	3.7 ± 0.5[b]	3.9 ± 0.9[b]	3.6 ± 1.2[b]
C-reactive protein (mg/l)[3]	4.5[ab]	1.4[a]	7.7[ab]	12.1[b]
Ceruloplasmin (mg/L)	357 ± 108[a]	341 ± 101[a]	384 ± 89[ab]	441 ± 191[b]
Retinol (μmol/l)[2]	1.32[b]	1.28[ab]	1.05[a]	1.12[a]

[1]Hemoglobin values below 120 g/l are defined as low. Ferritin values below 20 μg/l are defined as low. Values (mean ± s.d.) within rows with different superscript letters are statistically significantly different at $P < 0.05$ using the Tukey-Kramer post-hoc test.
[2]Geometric mean.
[3]Back-transformed square root transformation.

Table 4 Correlations (%) between plasma ferritin, iron, and blood hemoglobin and other measures of interest in the iron nutrition of women in five rural counties in China[1]

	Ferritin (log)		*Iron (log)*		*Hemoglobin*	
Variables	*Unadjusted*	*Adjusted by county*	*Unadjusted*	*Adjusted by county*	*Unadjusted*	*Adjusted by county*
Ferritin (log)	-	-	20***	17**	16**	20***
Plasma iron (log)	20***	17**	-	-	11*	9
Hemoglobin	16**	20***	11*	9	-	-
Transferrin	−44***	−46***	−16**	−14**	−18**	−18***
Menopause[2]	21***	-	4	-	0	-
Dietary heme iron	22***	8	12*	6	−6	3
Dietary non-heme iron	6	9	−4	0	−32***	0
Dietary animal protein	30***	15**	15**	5	−4	3
Plasma cholesterol	23***	14**	9	2	10	2
Plasma retinol (log)	14**	12*	11*	7	30***	16**
Dietary inhibitors	3	−6	2	−4	−19***	0
C-reactive protein (square root)	15**	17**	−4	1	−11*	−7

[1]Unadjusted correlation coefficients between the logarithm of plasma ferritin, the logarithm of plasma iron, or blood hemoglobin concentrations and row variables are Pearson univariate coefficients. Values adjusted by county are partial coefficients.
Significance: *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.
[2]Dichotomous variable with 1 = premenopausal and 2 = 1-2 y postmenopausal.

with their physiological outcomes. The average total iron intake (22 mg/d) was relatively high compared to that of adult women in economically developed countries such as Taiwan (13 mg/d) (Shaw *et al,* 1995), New Zealand (14 mg/d) (Alexander *et al,* 1994), Japan (11 mg/d) (Satoh 1991), and France (10 mg/d) (Preziosi *et al,* 1994). There was no consistent pattern between dietary intakes across counties (Table 2) and average indices of iron status in those counties (Table 3). After adjusting for county, there were no correlations between individual intakes and measures of iron status with the exception of dietary animal protein and plasma ferritin. Some authors have reported correlations between iron intake, usually as heme iron, and iron status (Preziosi *et al,* 1994; Roebothan & Chandra, 1996), while others have failed to demonstrate an association between iron status and intake of dietary iron due to variability in iron absorption and blood losses and inaccuracies and errors in diet reporting (Heitmann *et al,* 1996). While it remains axiomatic that iron status is dependent on iron intake, a number of modifying factors must be considered. One of these is the difference in bioavailability of heme and non-heme iron. Since the more bioavailable heme iron is present only in meat products and is therefore not present in vegetarian diets, it is consistent that vegetarians should have lower iron status. It has been shown that vegetarians have lower serum ferritin (Helman & Darnton-Hill, 1987; Alexander *et al.,* 1994; Shaw *et al,* 1995) and that meat intake specifically increases serum ferritin (Reddy & Cook, 1991; Yokoi *et al,* 1994). In the present study a strong ($P < 0.001$) positive correlation between dietary heme iron intake and plasma ferritin largely disappears after adjustment for the survey county. This may be due to the decreased range of intakes and plasma values and to the increased role of inhibitors of absorption at the individual level.

The quality of the iron status data in this study was variable. The cyanomethemoglobin assay for hemoglobin is normally a very reliable assay although the diagnostic range is rather narrow (Looker *et al,* 1990; Borel *et al,* 1991; Ahluwalia *et al,* 1993). The average values for Jiexiu and Changle counties, 146 and 155 g/l, were rather high compared to Western values of about 130 - 140 g/l (Looker *et al,* 1990; Borel *et al,* 1991). The hemoglobin measurements were made in the field and by different technicians and with different batches of reagents in the different counties. It may be that the differences between counties are, in part, due to these methodological differences. However, these measurements do correlate well with other measures of iron status (Table 4) and in the expected manner with plasma retinol, and CRP before adjusting for county. Since transferrin levels are generally raised in iron deficiency, the negative correlation with other iron biomarkers is as expected. Serum iron generally has a rather high coefficient of variation compared to other measures of iron status and to other blood chemistry measurements (Eckfeldt & Witte, 1994; Tietz, 1994). This variability comes from the method of analysis as well as from large day-to-day variation and diurnal variation among indivi dual subjects (Beaton *et al,* 1989; Looker *et al,* 1990; Borel *et al,* 1991; Ahluwalia *et al,* 1993). In this study, plasma iron also was correlated only marginally with other iron status measurements and other determinants of iron status (Table 4). Ferritin appears to be an effective measure of iron deficiency, when compared to bone marrow iron levels (Guyatt *et al,* 1990). Although only one measurement is necessary for an accurate assessment of ferritin levels, compared to seven for serum iron (Ahluwalia *et al,* 1993), there has been variation in ferritin values reported for physiological and environmental reasons including an association with the menstrual cycle (Kim *et al,* 1993). In this study ferritin values showed a wide variation both between counties and between individuals within counties. Iron intake from the diet was calculated from a detailed interview and food weighing method of food intake assessment that was shown to be very effective and reliable (Hu *et al,* 1993a, 1994b).

A number of affectors of iron absorption are known and were examined in this study. These included low vitamin C intake (Hunt *et al,* 1990; Reddy & Cook, 1991; Siegenberg *et al,* 1991), high fiber intake (D'Souza *et al,* 1987; Reddy & Cook, 1991), high calcium intakes (Preziosi *et al,* 1994; Whiting, 1995), and high tea or polyphenol consumption (Reddy & Cook, 1991; Siegenberg *et al,* 1991). Tea and dietary fiber intakes were not associated with iron status among the subjects in this study either before or after adjusting for survey county. Dietary calcium was positively correlated with iron status before adjustment for survey county. This may reflect the higher level of heme iron intake in those counties with high dairy intake. With the very high levels of fiber and tea consumption in those counties, a negative correlation would have been expected based on earlier work of others. A high intake of soy products has been suggested as an inhibitor of iron absorption (Reddy & Cook, 1991), but this was not testable in this population with its very low consumption of soy protein (Table 2).

The compiled index of inhibitor intake was developed for potential inhibitors and inhibitor intake levels charac teristic of the survey population. In developing this method the data were examined to determine appropriate classifica tion of low, medium, and high intakes in this population. Cutoffs used by other investigators were also considered (Cook, 1990; Cook *et al,* 1991). Several models were considered with different cutoffs and more cutoffs per inhibitor. We settled on the index presented here for its simplicity and applicability in China. Even with the wide range of the index, no compounding effects of inhibitors was evident.

Plasma ferritin, as a reflection of iron stores, increased significantly from an average of 46 μg/l to 79 μg/l ($P = 0.011$) within 2 years of the cessation of menses. Others have observed a doubling of serum ferritin and a reduction in iron deficiency anemia among postmenopausal women (Cook *et al,* 1986; Satoh, 1991; Itoh *et al,* 1992; Bartfay *et al,* 1995). Menopause was not correlated with changes in plasma iron or blood hemoglobin (Table 4).

The progression of iron deficiency may be followed by the changing pattern of blood markers (Gibson, 1990). As liver iron stores are depleted, serum ferritin decreases. Below serum levels of about 20 μg/l, liver stores are considered depleted (Gibson, 1990; Guyatt *et al,* 1990; Heitmann *et al,* 1996). If deficiency continues to progress, then eventually hemoglobin levels start to fall. A generally accepted hemoglobin cutoff for iron deficiency anemia is 120 g/l. In Figure 3, three of the four quadrants reflect this deficiency progression. The remaining quadrant represents those subjects with ferritin values above 20 μg/l and hemoglobin values below 120 g/l. We suggest that these subjects do not follow the usual deficiency progress owing to complicating infection or inflammation states. Table 5 describes the characteristics of the subjects in these four quadrants and highlights the increased acute-phase response indicators in the unusual quadrant with the con flicting measures of iron status. C-reactive protein, ceruloplasmin, and retinol are acute-phase respondents and were significantly altered from the normal or depleted quadrants. These subjects may have sustained an inflammatory response during the previous 4 - 6 weeks from a vaccination (Olivares *et al,* 1993), an acute infection (Brown *et al,* 1993) such as acute pneumonia (Baynes *et al,* 1986), or they may have had a chronic inflammatory condition such as rheumatoid arthritis (Ahluwalia *et al,* 1995).

As reported previously, plasma retinol levels among these subjects did not reflect dietary intakes but were correlated with inflammation state as measured by CRP (Root *et al,* 1997). This finding was confirmed by the generally reduced retinol level among the acute-phase reaction quadrant subjects. Vitamin A levels also have a well known direct interaction with iron status. Among subjects at risk for iron-deficiency anemia, blood hemoglobin and serum retinol are highly correlated (Suharno *et al,* 1992; Wolde-Gebriel *et al,* 1993) as seen in Table 4. Short-term dosing with vitamin A among anemic subjects causes a rise in serum iron and blood hemoglobin levels while not affecting serum ferritin (Bloem *et al,* 1990; Mejia & Chew, 1988). In the present study, vitamin A intake was not correlated with iron status but plasma retinol was correlated with several markers of iron status both before and after adjusting for the survey county. The positive correlation with plasma ferritin was particularly interesting since both markers are acute-phase respondents, though in opposite directions. This correlation may reflect a general dietary effect since both plasma cholesterol and animal protein intake were also positively correlated with ferritin.

The results of this study suggest that iron status of middle-aged women in rural China was generally satisfactory, with a low incidence of overt iron deficiency anemia. Dietary sources of iron appeared to be both high and adequate despite very low intakes of heme iron in many locations. Statistically significant correlates of iron status found in this analysis did not include the forms of dietary iron, the intake of inhibitors of iron absorption (including very high consumption of tea and fiber), altitude, or physical activity. Possible determinants included menopausal status. Inflammation due to possible acute or chronic disease states appeared to complicate the interpretation of iron status in this study but did not have a strong statistical influence on iron status. The positive correlation between iron status and plasma retinol and cholesterol suggested a slight positive role for a richer diet in maintaining iron stores.

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