Zinc Intake and Dietary Pattern in Jiangsu Province, China: Consequences of Nutrition Transition

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ABSTRACT

Background: Jiangsu Province is an economically booming area in East China, where soil zinc concentrations are low. Nutrition transition to a dietary pattern with more animal source foods may have improved zinc intake in this area. However, such a transition may also have increased the burden of non-communicable diseases (NCDs), such as hypertension and obesity. Investigation of dietary patterns in relation to undernutrition and overnutrition could help to better address both problems.

Objectives: The first aim of this thesis was to assess zinc status in Jiangsu Province using dietary zinc intake, serum zinc and stunting as indicators, as well to investigate the potential of biofortified rice to improve zinc intake. The second aim was to investigate the association between dietary patterns and high blood pressure, taking obesity into account.

Methods: Data from the 2002 National Nutrition and Health Survey in Jiangsu Province were used to assess zinc intake in the population aged 4-89 years (n=3,867). Primary school children (n=2,268) were selected from three counties in the Province with relatively low soil zinc for assessment of stunting. Serum zinc was measured among children in the county where stunting was highest (n=297). Thirteen women were recruited in the same county for three test rounds with rice meals (zinc biofortified rice, zinc extruded fortified rice and control rice). Fractional zinc absorption (FAZ) was measured with the use of the double isotope tracer ratio method. Effect of biofortified rice with zinc, at a level of 2.7 and 3.8 mg/100g, on zinc intake was simulated in adults (n=2,819). For adults, four distinct dietary patterns were identified, named “traditional”, “Macho”, “sweet tooth” and “healthy” pattern. Associations were assessed between the four dietary patterns and blood pressure in adults (n=2,518) by using Poisson regression analysis.

Results: The overall prevalence of insufficient intake of zinc was 22.9%, with a higher prevalence in children (64.6%) and adolescents (64.9%), and in those with low socio-economic status (27.3%). Around 4% of the primary school children were stunted, and the prevalence of zinc deficiency measured by serum and hair zinc was 0.7%, and 15.2%, respectively. Biofortified ^{70}Zn enriched rice with an intrinsic label was found to have higher fractional zinc absorption (FAZ) than extrinsically labeled fortified extruded rice. However, FAZ could not be accurately quantified because we could not determine the exact amount of isotope infused to subjects due to adhesion of zinc to the vial. When simulating zinc intake by replacing normal rice with zinc biofortified rice with either 2.7 and 3.8 mg/100g of zinc, the prevalence of insufficient zinc intake decreased from 15.4% to 6.5% and 4.4%, respectively. The “traditional” dietary pattern in Jiangsu Province was most strongly associated with high blood pressure ($P_{\text{for trend}} = 0.005$). This pattern is characterized primarily by consumption of rice and fresh vegetable; secondary of pork and fish; and lastly of root vegetable and wheat flour, but also by high salt intake. Subjects with overweight and obesity were more likely to have high blood pressure than those with normal weight.

Conclusion: Children and adolescents had low dietary zinc intake, in Jiangsu Province, where the soil is also deficient in zinc. However, these findings did not match with the low prevalence of stunting and zinc deficiency based on serum zinc concentrations in primary school children from three rural areas of the Province. Zinc appears to be better absorbed from biofortified rice than from control rice or from extruded fortified rice, which needs further investigation. Simulated zinc intake from biofortified rice with zinc at a level of 2.7 mg/kg has the potential to significantly improve zinc intake, especially in the “traditional” dietary pattern. However, this pattern is also related to high blood pressure, which may be due to high salt intake. High blood pressure is also positively and independently related to obesity. Nutrition education is required to improve knowledge and awareness of healthy diets in Jiangsu Province.

CONTENTS

Chapter 1

Introduction

Zinc is an essential mineral for growth and immune function[1, 2]. Cereals, such as rice, wheat and maize, are important sources of zinc in the diet, but zinc absorption is usually poor from these foods[3]. Biofortification of rice with zinc may contribute to higher zinc intake in rice consuming countries such as China[4]. Moreover, zinc intake and absorption is expected to be higher in dietary patterns that include more animal products[3]. However, shifts towards more animal and high fat foods has also induced an increase in the prevalence of overweight and obesity in China, which adds to the burden of non-communicable diseases (NCDs)[5].

In this thesis, we have assessed dietary zinc intake, zinc status, anemia and stunting prevalence among the population of Jiangsu Province, China. We have thereby identified risk groups for insufficient zinc intake according to four distinct dietary patterns. Moreover, we have looked into the potential of biofortified rice to improve zinc intake. At the same time, we have investigated the prevalence of hypertension in association with obesity, two of the top risk factors for NCDs, in relation to the same dietary patterns.

Nutrition transition in China

China is currently undergoing a rapid transition in dietary pattern and disease[6]. The intake of cereals decreased, whereas intakes of meat, poultry, eggs and other animal products increased[7]. The proportion of dietary energy derived from fat in the adult diet increased from 19% to 28%, mainly due to replacement of calories from carbohydrate[7]. About one-half of dietary fat is derived from edible oil, such as soybean oil, while the consumption of solid animal fat for cooking decreased[8]. The average percentage of energy provided by saturated, monounsaturated and polyunsaturated fatty acids were 6.1%, 9.8% and 6.3% in the population[9]. Despite this favorable fatty acid composition sources, the high total intake of fat resulting in high calorie intake contributes to an increased risk of NCDs, such as obesity and cardiovascular disease[10]. In 2002, NCDs contributed around 83% to all deaths in China, compared with 73.8% in 1991[5]. Hypertension, as the primary risk factor for mortality, increased from 13.6% in adults in 1991 to 18% in 2002[11, 12]. The prevalence of overweight and obesity was 17.6% and 5.6% in 2002, whereas it was 12.8% and 3.3% in 1992, respectively[13] (Figure 1).

Increased intake of animal foods has improved micronutrient intake and related nutritional diseases[14]. Iodine deficiency and anemia have obviously decreased, and vitamin A deficiency and zinc intake inadequacy do not seem serious public health problems anymore[7, 15] (Figure 1). However, anemia still affects 245 million people in China, with children, elderly, pregnant and lactating women being most vulnerable[16]. Inadequacy of zinc intake remains high in children and women, especially in rural areas[15].

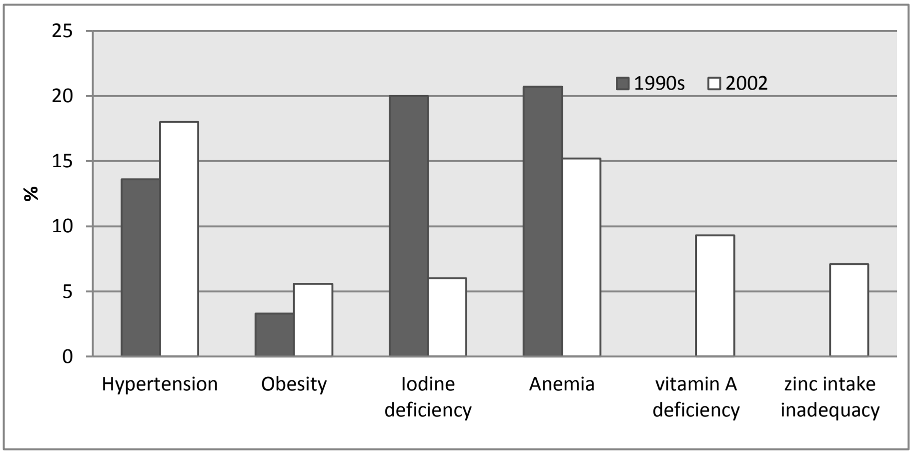

Figure 1 Selected NCDs and micronutrient deficiencies in China in 1990s and 2002

Dietary zinc intake and zinc deficiency

Zinc is an important component of more than 100 specific enzymes, which are involved in all major biochemical pathways and play multiple roles in a large number of metabolic processes[2, 17, 18]. Zinc deficiency is usually less recognized because accurate indicators for assessment of zinc status are lacking[19]. Dietary zinc intake is recommended and usually measured in population-based nutrition studies to identify subpopulations with an elevated risk of zinc deficiency[20-24]. In addition, measurement of serum zinc has been frequently used as a biochemical indicator of zinc status[25]. Although growth of children and teenagers has improved steadily in China, stunting is still a public health problem with a prevalence of 14.3% among preschool children and 16.0% among school children (6-18 years)[7]. It has been recommended as the best functional indicator to assess the risk of zinc deficiency[26]. Anemia has been suggested to reflect potential risk for zinc deficiency in the first technical document of IZiNCG[27]. It is usually considered that iron and zinc have a similar distribution in the food supply, and some food components affect the absorption of both minerals simultaneously[28].

In 2002, a National Nutrition Survey was conducted in a nationally representative population sample. The proportion of inadequate zinc intake was found to range from 2.8% in urban adult women to 29.4% in rural lactating women using the normative intake requirements of the World Health Organization[15]. However, there is hardly any national data on serum zinc. This includes Jiangsu Province (Figure 2), which is one of the most prosperous provinces, but has lower soil zinc concentrations (68.5 mg/kg) than the national average level (100 mg/kg)[29]. Since low soil zinc likely results in a low content of zinc in crops grown on such soils, it is necessary to evaluate zinc intake and status in the population of Jiangsu Province. Especially rural school-age children and adolescent are shown to have low zinc intake[15], and stunting is still prevalent among them[7].

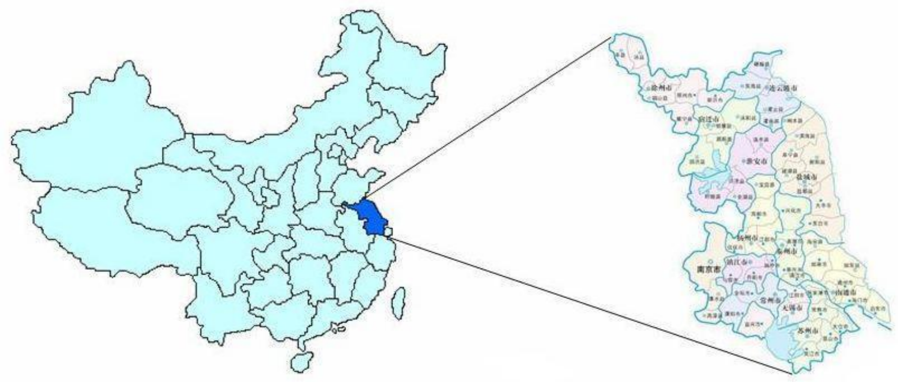

Figure 2 Jiangsu Province in China

Zinc bioavailability

Inadequate absorbable zinc intake is likely to be the most common cause for zinc deficiency[30]. A phytate : zinc molar ratio in meals < 5 (refined diets low in cereal fiber and where animal foods provide the principal source of protein), 5–15 (mixed/refined vegetarian diets that are not based on unrefined cereal grains or high extraction rat flours), and > 15 (cereal-based diets, with 50% of energy intake from unrefined cereal grains of legumes and negligible intake of animal protein) represent relatively high (50%), moderate (30%), and low (15%) absorption levels of zinc intake, respectively[27]. Fractional absorption of zinc (the percentage of dietary zinc intake that is absorbed) has shown to be over 30% in toddlers and women consuming a representative Chinese diet[31, 32].

Biofortification of rice with zinc has been suggested as a possible strategy to reduce zinc deficiency at the population level in rice consuming countries[4]. However, the availability of zinc-biofortified staple crops is still limited, and no randomized, controlled efficacy trials have been conducted to evaluate their impact on zinc status[33]. Absorption studies can form a first step in building the evidence that biofortification is efficacious, usually requiring only limited amounts of the biofortified product. In the end, larger randomized controlled studies are also required to demonstrate efficacy. While waiting for biofortified crops to become available in larger amounts, simulation modeling forms a cost-effective means to estimate the potential impact of biofortification on improved nutrient intakes. This has already been demonstrated for conventional fortification with some micronutrients, such as folic acid, and calcium[34-36]. For biofortification, this kind of modeling is still limited, and only two studies have focused on zinc[37, 38].

Health consequences of nutrition transition

With the shift to higher intake of animal food and fat, overnutrition-related health consequences increase rapidly, whereas undernutrition can persist simultaneously[14]. Because of the highly correlated nature of many nutrients in foods, it is often difficult to identify the effect of a single nutrient on health outcomes[39]. Dietary patterns combining nutrients and foods form an alternative and complementary approach to single-nutrient analysis in relation to nutrients intake profiles in populations with different dietary habits and may thus be more predictive of disease risk[39, 40].

The Chinese traditional dietary pattern includes cereals and vegetables with a few animal foods[41]. This kind of dietary pattern is considered as extremely healthy when adequate levels of intake are achieved[8]. However, the traditional dietary pattern is now shifting towards energy-dense, high-fat and low-fiber diets, particularly among low-income residents[41]. It has been shown that adherence to the Mediterranean diet reduced inadequate intakes of micronutrients including zinc[42]. Transition in dietary patterns has demonstrated to decrease the prevalence of anemia in China[7]. Whether the Chinese traditional or other dietary patterns also contributes to adequate zinc intakes and status should be addressed.

Hypertension has been reported in relation to western and south-east Asian dietary patterns[43-45]. A cohort study from Shanghai showed that high consumption of fruit and milk lowered the prevalence of both pre-hypertension and hypertension[46]. A Chinese traditional southern pattern, characterized by high intakes of fruit, pork, poultry, rice, and vegetables was inversely related with hypertension independent of body mass index[47]. Overweight/obesity and high salt intake are well-known risk factors of hypertension[48]. The average daily salt intake from Chinese diets has been estimated at 12 g/d[5], more than double the amount of 5 g/d recommended by World Health Organization[49]. Since hypertension is clearly on the rise in China, it is important to determine the prevalence in Jiangsu Province, to unravel its determinants, to identify risk groups, and to characterize dietary habits related to blood pressure in order to be able to better formulate and target dietary advice for prevention.

As already mentioned, due to nutrition transition in China, the prevalence of obesity is increasing, while at the same time, the prevalence of anemia is decreasing[7, 13]. However, several studies have demonstrated an inverse association between overweight/obesity and iron status[50-53]. As a chronic inflammatory condition, obesity would up-regulate hepcidin expression[54]. Since hepcidin is the main regulator of iron absorption, intestinal absorption of iron could be inhibited in overweight people, and diminish the amount of bioavailable body iron[55, 56]. However, results from studies remain inconsistent[50-53], and therefore additional studies in populations where both overweight and iron deficiency are present can help to unravel this further.

Rationale and outline of the thesis

Jiangsu Province has the lowest soil zinc concentrations in China, which may predispose its population to zinc deficiency and stunting. At the same time, Jiangsu Province is an economically booming Province undergoing rapid nutrition transition, where obesity and hypertension increasingly add to the disease burden. In this thesis, both ends of this nutritional intertwine have been addressed with the following research questions:

1. What is the zinc intake in the Jiangsu population, and which population groups can be identified to be at increased risk of inadequate zinc intake (Chapter 2)? What is the prevalence of low serum zinc status and stunting in the most at-risk population group, i.e. school children (Chapter 3)?
2. What is the zinc absorption from biofortified rice, the main staple food in China, and can zinc biofortified rice improve (absorbed) zinc intake (Chapters 4 and 5)?
3. Which dietary patterns are associated with either zinc inadequacy, anemia, obesity or hypertension, and is there any overlap between these associations (Chapter 5 and 6)?
4. Is there an association between obesity and anemia, as has been reported for several other populations (Chapter 7)?

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Chapter 2

Dietary intake of zinc in the population of Jiangsu Province, China

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Abstract

Objectives: To evaluate dietary zinc and other divalent minerals intake among the population of Jiangsu Province.

Methods: 3,867 subjects aged 4-89 years were representatively sampled in two urban and six rural areas of Jiangsu Province. Dietary intake was assessed using 24-hour recalls on three consecutive days. Insufficient zinc intake was calculated based on the Chinese Dietary Reference Intakes (DRIs).

Results: Overall, the percentage of subjects with insufficient intake of zinc was 22.9%, with a declining trend with age. Except for the group ≥50 years, mean zinc intake of all other groups was below the age- and sex- specific Recommended Nutrition Intakes (RNI). Approximately 2/3rds of the subjects ≤17 years of age had insufficient zinc intakes. Compared with the age group below 11 years, risk of insufficient zinc intake increased in the adolescents aged 11-17 years with a prevalence ratio (PR) of 2.10 (95% CI: 1.86-2.36), but decreased in adults aged 18-49 years and ≥50 years (PR: 0.76, 95% CI: 0.66-0.8; PR: 0.55, 95%CI: 0.47-0.64). Mean intake of iron, copper, magnesium and selenium met the Chinese DRIs respectively, except for selenium in females. The prevalence of insufficient intake of copper, magnesium and selenium was 37.2%, 22.8% and 29.3%, respectively, while the overall prevalence of insufficient iron intake was only 3.4%.

Conclusion: Dietary zinc intake of the Jiangsu Province population does not generally meet the Chinese DRIs. Children and adolescents in particular have a higher risk of insufficient zinc intake.

Introduction

Zinc is an essential trace element and is required for the activity of approximately 300 enzymes involved in most major metabolic pathways. Consequently, zinc is necessary for a wide range of biochemical, immunological and clinical functions[1, 2]. Adverse health consequences of Zn deficiency are: growth retardation, delayed sexual and bone maturation, skin lesions, diarrhea, impaired appetite, increased susceptibility to infections mediated via defects in the immune system, and the appearance of behavioral change, especially in infants, toddlers and children, pregnant women, and elders[3-6].

Insufficient dietary zinc intake is one of the causes of zinc deficiency, which is in isolation or in combination with other factors, such as increased requirements, malabsorption, increased losses, and impaired utilization[1]. Dietary factors can influence zinc absorption. Phytate and dietary calcium inhibit zinc absorption, whereas protein and amino acids may have a positive effect on zinc bioavailability[7]. In addition, the fractional absorption of zinc deceases with increasing amount of zinc intake[1]. Moreover, other divalent minerals, such as copper, iron, magnesium and selenium, may compete with zinc for absorption. Dietary reference intakes (DRIs) are developed as nutrient reference standards which provide guidance for maintaining and enhancing adequate nutritional status[8]. The Chinese DRIs has been developed by the Chinese Nutrition Association in 2000[9]. Average phytate intake in China is higher than that in western countries, which negatively affects the absorption of zinc, particularly when the molar ratio of phytate to zinc is above 15[10-12]. *Ma et al*[13] showed higher proportions of zinc inadequacy in a Chinese population consuming a diet with a higher phytate content, but they assessed the prevalence of zinc intake in accordance with the normative requirement of the WHO[4].

In Jiangsu Province, the average soil zinc concentration is lower than the national average level[14]. Consumption of plants growing on such soils might result in low zinc content of the diet[15]. The 2002 China National Nutrition and Health Survey was implemented in 2002, and Jiangsu Province was involved as a part of the survey. Based on the survey, our study aimed (1) to evaluate dietary zinc intake in a representative sample in Jiangsu Province; (2) to investigate demographic factors related to zinc intake; (3) to assess the intake of other nutrient intakes, including iron, copper, magnesium and selenium among the population.

Materials and methods

Sample

In 2002, China launched a national representative cross-sectional study on nutrition and health. A multistage cluster sampling method was used for subject selection. The data presented in this article are based on a sub-sample from Jiangsu Province, one of the economically booming areas in East-China, with a population of 73.6 million. Two urban cities (Nanjing, Xuzhou) and 6 rural areas (Jiangyin, Taicang, Suining, Jurong, Sihong, Haimen) were randomly selected based on geographic characteristics and economic development, and then three townships/sub-districts were randomly selected from each city or rural area. Two villages/neighborhood committees were randomly selected from the selected townships or sub-districts. Thirty households were randomly selected from each of the selected villages or neighborhoods for dietary intake assessment. The six counties and two cities represented a geographically and economically diverse population with a gross domestic product ranging from 3,221 Yuan/capita/year (US$403) to 35,169 Yuan/capita/year (US$4,396; mean US$1,993, SD 1,510; Jiangsu Bureau of Statistics, 2002). Nanjing, Jurong, Taicang and Jiangyin are in the South. The south has a higher gross domestic product than the north (24,702 vs. 7,183 Yuan). All members in the households were invited to take part in the study. Written consent was obtained from all the participants or their parents/guardians. Because of the small sample of toddlers below 4 years, we just included subjects above 4 years and altogether 3,867 subjects have been included in the present analysis.

Dietary intake assessment

Information on food intake was collected using the 24-hour dietary recall method for three consecutive days (two weekdays and one following weekend day) during September and November. Participants were interviewed in their homes by trained health workers from the local Center for Disease Control and Prevention using a precoded questionnaire. Interviews took approximately two hours to complete. Interviews for subjects below 18 years were completed by their parents/guardians. Energy and nutrient intake was calculated using the data from dietary recall in conjunction with the Chinese Food Composition Table published in 2002[16].

Other information

Other socio-demographic information was included in the questionnaire. Socio-economic status (SES) was assessed by the question “What was your family’s income per person in 2001?” The response categories for the question were less than 800, 800–1999, 2000–4999, 5000–9999, 10 000–19 999 and more than 20 000 Yuan. SES was constructed from income, “low” being less than 1999 Yuan, “medium” being 2000–4999 Yuan and “high” being more than 5000 Yuan. Age groups were categorized as < 11years, 11-17 years, 18-49 years and ≥50 years.

Statistical analysis

Values of dietary energy or nutrient intake are expressed as mean and standard deviation (SD) mg/day. Using age and gender- specific values from the Chinese DRIs

(Table 1), insufficient and adequate nutrient intake in specific population groups were determined as below 66% and 77% of the RNI or AI respectively[9, 17]. T-test, ANOVA with Tukey's post hoc comparisons and chi-squared test was used to compare the differences in nutrient intake and categorical variables between different gender and age groups, respectively. Poisson regression was used to determine the association between socio-demographic factors including residence, age, socioeconomic status, region and education and insufficient zinc intake. Poisson regression models were fitted by using SAS software (SAS Institute, Inc., Cary, North Carolina). Other analyses were performed using SPSS 12.0 (SPSS Inc., Chicago, IL, USA). Statistical significance was set at α = 0.05.

Table 1 Chinese Dietary Recommendations for Chinese people†

Age (yrs)	RNI		AI	
	Male	Female	Male	female
Zinc (mg/d)				
4-6	12			
7-10	13.5			
11-13	18	15		
14-18	19	15.5		
18-49	15	11.5		
50+	11.5			
Iron (mg/d)				
4-6			12	
7-10			12	
11-13			16	18
14-18			20	25
18-49			15	20
50+			15	
Copper (mg/d)				
4-6			1.0	
7-10			1.2	
11-13			1.8	
14+			2.0	
Selenium (mg/d)				
4-6	25			
7-10	35			
11-13	45			
14+	50			
Megnesium (mg/d)				
4-6			150	
7-10			250	
11+			350	

† Data is original from reference 9.

Results

Table 2 shows the characteristics of the sample. A total of 3867 subjects (1834 male and 2043 female), with balanced distribution in gender, age, region and socio-economic conditions, were involved in the study. Males had a higher education than females. As expect, energy and nutrients intakes in males were significantly higher than those in females.

Table2 Descriptive characteristics

		Male	Female
Total		1824	2043
Age group(years)	<11	154	174
	11-17	194	191
	18-49	884	1033
	≥50	592	645
Residence	Urban	458	495
	Rural	1366	1548
Region	South	919	1001
	North	905	1042
Education*	Primary	527	947
	Junior school	638	508
	High school	243	188
	University	68	33
Socio-economic status	Low	620	697
	Medium	562	670
	High	624	657

Data is presented by number (%) or mean (SD). For education, we just include adults with age above 18 yrs.

Differences of energy and nutrition intake between genders were analysed by t-test. Values in the same row with difference superscript letters are significantly different.

* P<0.001, analysed by chi-square test.

The average mean of zinc intake was 8.1 ± 2.7, 10.3 ± 3.1, 12.3 ± 3.7, and 11.6 ± 3.7 mg/d for age groups of 4-11 years, 11-17 years, 18-29 years, and above 50 years, respectively. Except for the group above 50 years, zinc intake in other groups was below the age- and sex- specific recommended RNI values (Table 1). Energy and zinc intake increased with age, for both males and females. The average mean intake of iron, copper and magnesium met the recommended daily intake for corresponding nutrients and age group, while selenium was below the recommended values in females. There were no differences in intake in terms of iron, copper, magnesium and selenium among different age groups, except for copper and selenium in males (Table 3).

Table 3 Dietary intake of energy and selected nutrients by gender and age groups

		Age group (yrs)			
		<11	11-17	18-49	50+
Male	N	154	194	884	592
	Energy (1000kcal/d)	1.7(0.5)[a]	2.3(0.6)[b]	2.7(0.7)[c]	2.5(0.7)[d]
	Zinc (mg/d)	8.5(2.8)[a]	11.2(3.1)[b]	13.7(3.8)[c]	12.7(3.8)[d]
	Iron (mg/d)	17.7(6.3)[a]	24.2(8.5)[b]	28.6(10.6)[c]	26.9(13.1)[d]
	Copper (mg/d)	1.9(2.7)[a]	2.2(0.8)[a]	2.5(1.0)[b]	2.4(1.1)[c]
	Magnesium (mg/d)	239.7(105.4)[a]	232.3(124.7)[b]	369.9(148.8)[c]	345.6(144.8)[b]
	Selenium (mg/d)	36.5(17.6)[a]	44.0(19.2)[b]	50.7(19.9)[c]	46.5(21.0)[b]
Female	N	174	191	1033	645
	Energy (1000kcal/d)	1.6(0.5)[a]	1.9(0.5)[b]	2.1(0.6)[c]	2.1(0.6)[d]
	Zinc(mg/d)	7.7(2.6)[a]	9.5(2.8)[b]	11.2(3.2)[c]	10.5(3.3)[d]
	Iron(mg/d)	16.3(5.7)[a]	20.6(7.4)[b]	23.9(9.5)[c]	22.4(9.8)[b]
	Copper (mg/d)	1.6(1.2)[a]	1.8(0.8)[a]	2.2(0.9)[b]	2.1(1.0)[b]
	Magnesium (mg/d)	222.9(80.2)[a]	264.2(121.8)[b]	311.1(130.7)[c]	292.3(127.5)[d]
	Selenium (mg/d)	31.6(15.5)[a]	34.7(13.8)[a]	41.5(17.6)[b]	38.9(17.9)[c]

Data is presented as mean (SD).

Differences in intake of energy and selected nutrients between age groups by gender were analysed by ANOVA and Tukey's post hoc comparisons. Values in the same row with difference superscript letters are significantly different.

The percentage of insufficient intake of zinc was 22.9%, with a declining trend with age. Subjects below 18 years old had a higher prevalence of insufficient zinc intake, while subjects above 18 years had a higher prevalence of adequate zinc intake. The prevalence of insufficient intake of copper, magnesium and selenium was 37.2%, 22.8% and 29.3%, respectively. In contrast to zinc insufficiency, the prevalence of copper, magnesium and selenium insufficiency tended to increase, while the prevalence of adequate intake decreased with age. The prevalence of insufficient iron intake was the lowest (3.4%) as compared to the other nutrients, and all age groups had adequate iron intakes except ages 11-17 years (Table 4).

The prevalence of insufficient intake of zinc, iron, copper and magnesium was higher in urban residents than in rural residents ($P<0.001$, respectively). Residents from the North had a higher prevalence of insufficient zinc intake than residents from the South, while insufficient intake of copper and magnesium was higher in the South ($P<0.001$, respectively). There were no differences in selenium intake between urban and rural areas, or between the South and the North (Table 5).

Table 4 Prevalence of insufficient intake of selected nutrients among people by gender and age according to Chinese dietary recommendations[†‡]

	Age group (yrs)				
	<11	11-17	18-49	50+	P value
Insufficient intake					
Zinc (%)	64.6	64.9	13.9	12.6	<0.001
Iron (%)	2.1	8.8	3.7	1.5	<0.001
Copper (%)	2.7	24.7	40.7	44.9	<0.001
Magnesium (%)	11.0	34.5	20.9	25.2	0.006
Selenium (%)	5.2	36.9	27.1	36.6	<0.001
Adequate intake					
Zinc (%)	19.2	16.9	73.4	76.5	<0.001
Iron (%)	94.2	83.6	92.5	96.5	<0.001
Copper (%)	94.5	75.1	82.2	77.8	<0.001
Magnesium (%)	78.6	50.6	64.2	57.1	<0.001
Selenium (%)	92.4	48.6	58.6	50.5	<0.001

Data is presented as prevalence. Insufficiency and adequacy of nutrient intake in specific population groups was determined as below 2/3 and above 77% of the 2001 RNI or AI of the age- and gender- specific values, respectively.

Differences in prevalence of insufficient nutrient intake among age groups by gender were analysed by chi-square test.

The prevalence of insufficient intake of zinc and selenium was lower in the high socio-economic status group compared with the lower socio-economic groups (P<0.001, respectively). In contrast, the prevalence of insufficient intake of iron, copper and magnesium increased with socio-economic status (P<0.01, respectively). Similarly, the prevalence of insufficient intake of zinc and selenium decreased with education, while insufficient intake of iron, copper and magnesium had a negative trend (P<0.01, respectively) (Table 5).

A Poisson regression was undertaken to assess the association of insufficient intake of zinc with demographic characteristics. Males have a higher risk for zinc insufficiency, with a prevalence ratio (PR) of 1.45 (95% CI: 1.31-1.60) compared to females (Table 6). There was a higher risk of insufficient intake of zinc in the age group of 11-17 years (PR: 2.10, 95% CI: 1.86-2.36), but a decreased risk in the age group of 18-49 years and older than 50 years (PR: 0.76, 95% CI: 0.66-0.8; 95%; PR: 0.55, 95%CI: 0.47-0.64), in comparison with that in the age group of children below 11 years. The risk was higher in the North than in the South, with the highest odds ratio in the Northern urban residents (PR: 1.52, 95% CI: 1.26-1.84). Subjects with a high socio-economic status showed a decreased risk for insufficient intake of zinc, as compared to subjects with a low socio-economic status.

Table 5 Prevalence of insufficient intake of selected nutrients by socioeconomic factors†‡

	Residents			Region			SES				Education			
	Urban	Rural	P	South	North	P	Low	Medium	High	P	Low	Medium	High	P
Zinc insufficiency	35.2	18.9	<0.001	17.0	28.7	<0.001	27.3	23.1	18.2	<0.001	26.1	20.4	17.6	<0.001
Iron insufficiency	6.6	2.3	<0.001	3.6	3.2	0.482	1.8	4.7	3.7	0.008	2.4	4.3	4.8	0.001
Copper insufficiency	56.8	30.9	<0.001	49.3	25.4	<0.001	25.7	39.4	47.2	<0.001	31.2	40.5	50.7	<0.001
Magnesium insufficiency	39.8	17.2	<0.001	30.7	14.9	<0.001	14.4	25.0	29.4	<0.001	21.4	21.6	30.4	<0.001
Selenium insufficiency	27.0	30.0	0.72	30.6	27.9	0.067	35.9	29.2	22.6	<0.001	33.9	26.4	19.9	<0.001

Data is presented as prevalence. Insufficiency of nutrient intake in specific population groups was determined as below 2/3 of the 2001 RNI or AI of the age- and gender- specific values.

Prevalence of nutrients insufficient intake were analysed by chi-square test.

Low, medium and high education is defined as primary school, junior high school, and high school and above, respectively.

Table 6 Poisson regression model for predictors of insufficient zinc intake

Characteristics	Prevalence ratio (95%CI)	P value
Gender		
Female	1	
Male	1.45(1.31-1.60)	< 0.001
Age group (yrs)		
< 11	1	
11-17	2.10 (1.86-2.36)	< 0.001
18-49	0.76 (0.66-0.86)	< 0.001
50^+	0.55 (0.47-0.64)	< 0.001
Residence		
South urban	1	
South rural	0.89 (0.73-1.07)	0.21
North urban	1.52 (1.26-1.84)	< 0.001
North rural	1.15 (0.94-1.39)	0.16
Socio-economic status		
Low	1	
Medium	0.91 (0.82-1.03)	0.15
High	0.78 (0.68-0.90)	< 0.001

Adjusted by daily energy intake.

Insufficiency of nutrient intake in specific population groups was determined as below 2/3 of the 2001 RNI or AI of the age- and gender- specific values.

Discussion

Our study showed that daily dietary zinc intake was low according to the Chinese DRIs in the study population from Jiangsu Province, especially in children and adolescents. Males were more likely to suffer from dietary zinc insufficiency than females.

The Chinese DRIs includes four concepts: EAR, RNI, AI and UL. The EAR (estimated average requirement) estimates an intake that is to prevent deficiency in 50% of a population. The RNI (recommended dietary intake) is approximately 2 standard deviations above the EAR and should provide adequate intake for 97.5% of the population for a given micronutrient. The UL (upper safe level) provides recommendations on values to avoid the sequels of excessive intake[9, 18, 19]. In our study, we used a cut-off of 2/3 of the RNI to estimate insufficiency of zinc intake and intake of other micronutrients among the population of Jiangsu Province.

As a subgroup analysis of the Chinese Nutrition and Health Survey, the study has its strengths and limitations. Our sample included an economically and geographically diverse population from Jiangsu Province, and therefore can be considered to be representative for the Province as a whole[20]. For the dietary assessment, we used a 3-day 24 h dietary recall in conjunction with the updated Chinese Food Composition Table, which can be sufficient to describe the subject's usual intake of nutrients[16, 21]. For assessment of dietary zinc insufficiency, we used the new Chinese DRIs, which has been specifically developed and updated for studies in China[9]. Prevalence ratios were calculated using the Poisson regression model, which provides a better estimation than odds ratio by logistic regression in cross-sectional studies[22]. A limitation is that we covered a few days within a short time frame for dietary intake assessment, and zinc insufficiency might have been either overestimated or underestimated in our study[23]. The recommended zinc intake by life stage from the Chinese DRIs is higher than the recommendations from IZiNCG and the WHO[1, 4]. This difference may partly explain why the prevalence of insufficient zinc intake in our study was higher than that previously reported at the national level using the other recommendations. Moreover, since soil zinc is low in Jiangsu Province, the actual zinc content of foods may have been lower than that reported in the Food Composition Table, which may underestimate insufficient intake of zinc in the population.

In our study, the mean zinc intake in age- and gender specific population groups was lower than the recommended values for each specific group, except in males above 50 yrs; this may be due to a higher energy intake and/or altered zinc metabolism[24]. About 58%-64% of children aged 4-18 yrs had low dietary zinc intake, although this was less than 20% among adults. Insufficient intake of zinc has also been reported in other developing and emerging countries, but also in some affluent countries. A National Food Consumption Survey in South Africa showed inadequate intake of zinc in all age groups, and 50-73% of children had an intake less than two-third of their RDA[25]. In a survey from the UK, the prevalence of inadequate zinc intake was reported to be 13% in young people aged 4-18 yrs, and higher prevalence was reported in girls aged 4-6 yrs (26.2%) and aged 11-14 yrs (34.5%), respectively. The UK reference nutrient intakes are lower than the Chinese recommendations[26, 27]. From the ZENITH study in European countries, the percentage of subjects who had an intake below 2/3 of the European RDA for people older than 55 yrs was lower than 4%[24]. In Mexican-American- and Anglo-American preschool children, 33% and 38% of zinc insufficiency were reported according to the 1989 US recommended dietary allowance. In the US, young children aged 1-3 yrs (81.1%, adolescent females aged 12-19 yrs (56.8%) and persons aged above 71 yrs (57.5%) were at the greatest risk of inadequate zinc intake, based on a total intakes of > 77% of the 1989 recommended dietary allowance.17 However, Arsenault *et al*[28] reported only 1% of inadequate zinc intake among US preschool children based on the 2001 recommended dietary allowance. The new recommended dietary allowance has lower age- and gender- specific values than the previous one. Differences in the prevalence of insufficient zinc intake among different countries and studies is attributed to differences in sampling methods and different target populations, but also for an important part the differences between the dietary recommended values used[29]. Therefore, it is not clear to what extent the differences in dietary habits and zinc content of foods consumed in different countries may explain the variation.

Although zinc intake was low in our study, caution is warranted in predicting zinc status. The absorption of zinc depend not only on the amount but also on the quality of zinc ingested with the diet, and can be modified by chemical compositions of the zinc sources and the nutrient matrix[7, 30]. Moreover, zinc absorption is increased in subjects with lower zinc status[31].

We found that Northern and urban residents had a higher risk for dietary zinc insufficiency, compared to Southern and rural residents, respectively. This may be due to differences in socio-economic status and different food choices[32]. People from the North had a lower socioeconomic status than those from the South. Nevertheless, it is surprising that city residents are more likely to have insufficient zinc intake than rural residents. This might partly be explained because rural residents consumed more energy than city residents. Moreover, although the quantity of zinc intake may be less in the cities, its bioavailability may be higher due to the higher quality of the diet. Our study also showed decreased risk for dietary zinc insufficiency with increased income, which was consistent with other reports[27, 32, 33]. The same trend was also seen with increasing education. Residents with a low socio-economic status or a low education level may spend more money for larger quantities of food that may have lower nutrient density at the expense of dietary quality[34]. A study by Thane *et al*[27] did not support the association between low zinc intake and poor socio-economic conditions.

We also studied the intake of other divalent minerals besides zinc, and showed high risk for insufficient intakes of copper, magnesium and selenium, with low risks for insufficient intake of iron. However, Shi *et al*[35] reported that 18.3% of men and 32.6% of woman above 20 yrs suffered from anemia in Jiangsu Province. Magnesium intake was shown to be related to anemia in this study. Dietary intakes of iron, copper, magnesium and selenium had different distributions in age and gender specific groups, when compared to zinc intake. Differences in dietary sources may account for these differences[29].

In conclusion, dietary zinc intake does not meet the Chinese DRIs, particularly in children and adolescents of Jiangsu Province. In addition, risk for insufficient zinc intake is more likely in the northern part of the Province, in city residents, and in residents with low socioeconomic status. Moreover, intakes of copper, magnesium and selenium are low among specific age groups of this population. Relationships between dietary patterns and nutrients intakes should be further investigated, and dietary intervention strategies should be adapted in accordance with different areas and age groups.

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Chapter 3

Stunting and zinc deficiency among primary school children in rural areas with low soil zinc concentrations in Jiangsu Province, China

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Abstract

Objective: To assess stunting and zinc deficiency among primary school children in north rural area of Jiangsu Province with low soil zinc concentrations, eastern part of China.

Methods: Two data collection rounds were conducted. In the first data collection round, 2268 primary school children aged 6-9 years were included by cluster sampling from three counties with low soil zinc concentrations. Anthropometric measures were assessed and stunting was defined as z-score of height-for-age (HAZ) below –2 according to the WHO new Growth Standards in 2006. For the second data collection round, the county with the highest prevalence of stunting was selected. From this county, 297 children aged 6-9 years were recruited by cluster sampling. Anthropometric measures, serum and hair zinc, and hemoglobin were measured at this stage.

Results: The total prevalence of stunting (HAZ <–2) and mild stunting (–2=<HAZ<–1) was 4.2% and 22.2% respectively, and Huai án had the highest prevalence of stunting (7.6%) among the three counties. In Huai án County the prevalence of zinc deficiency based on serum zinc concentration, hair zinc concentration, and both was 0.7%, 15.2% and 15.3% respectively， and 32.3% of subjects were anaemic. Boys had a higher prevalence of zinc deficiency than girls (19.1 vs. 10.5 %, P < 0.05), whereas the prevalence of anemia in boys was lower than that in girls (28.7 vs. 37.3%, P = 0.07).

Conclusion: Stunting and zinc deficiency were not highly prevalent among primary school children in rural counties with low soil zinc concentrations of Jiangsu Province.

Introduction

Almost one-third of the agricultural soils of China are considered to be zinc deficient[1]. On such soils, plants grow insufficiently resulting in low yields and low zinc content of crops[2]. In addition to this, the application of fertilizer with nitrogen is widely used in China, which may inhibit zinc uptake by crops[3]. Soil-to-plant transfer, as a food chain pathway, is one of the key components for zinc intake by animals and then humans, and therefore affect zinc status[2].

Rice is the staple food in the traditional Chinese diet and is usually consumed with vegetables and a small amount of animal-derived food. The dietary pattern changed quickly from 1992 to 2002 according to the National Nutrition and Health Survey, but the change was unbalanced between urban and rural inhabitants[4]. Low intake of animal source food leads to a low intake of important micronutrients, such as zinc, iron, vitamin A, and calcium[5]. Simultaneously, cereal foods contain a high amount of phytate and fiber, which has been shown to inhibit the absorption of zinc and iron. Zinc deficiency is known to be related to retarded growth, higher morbidity of infectious diseases, and higher mortality in children[6-10]. Especially the health of children is sensitive to zinc deficiency, since they have relatively higher requirements[11]. Ma et al[12]. reported phytate is higher in plant-based foods, and zinc deficiency may be prevalent in some rural areas in China, both as a result of low soil zinc and dietary habits that reduce zinc absorption.

From the Chinese Nutrition and Health Survey in 2002 it has been estimated that 14.0% of rural children aged 7-10 yrs were at risk of inadequate zinc intake[13]. However, no accurate index for zinc deficiency is available to assess zinc status adequately up till now. WHO/UNICEF/IAEA/IZiNCG has recently developed recommendations on evaluation of zinc status[14]. Their conclusion was that serum concentrations are usually used as indicators for zinc status in population studies[14-16]. Therefore, we have undertaken a cross-sectional study with two data collection rounds in 2002-2003, aiming to assess stunting and zinc status among primary school children living in rural areas with relatively low soil zinc content in Jiangsu Province, eastern China, using serum zinc as a biomarker.

Materials and methods

Study site

The first round of data collection was conducted in Dafeng, Taixing and Huai án, three counties of Jiangsu Province, in 2003. The sites were selected because soil zinc content in each of these counties (lowest in Taixing County with 58.2 mg/kg and highest in Huai án County with 71.7 mg/kg) was lower than the national average level (100 mg/kg)[17]. In addition, other considered criteria were: rice as staple food (rice is consumed at least twice per day); low average income (per capita income was 1502US in Dafeng, 1116 US in

Taixing and 1004US in Huai án, respectively); average 81.6% consumption of food from plant sources; main consumption of local produced food; supportive local health leaders; and experienced health workers. The three counties were all located north of Nanjing, which is the capital of Jiangsu Province. In the first data collection round, anthropometric measurements were done in all three counties. In the second data collection round, biochemical measurements were performed only in Huai án County, which had the highest prevalence of stunting.

Ethical approval

The study was approved by the ethical board of the Jiangsu Province Centre for Disease Control and Prevention. The nature of the study was also fully explained to the local education bureau, the school's principal and teachers, and to the parents of subjects.

Subjects

All school children in the three counties aged 6-9 years in grades 1-3 of primary schools that were apparently healthy were eligible for the study. In the first data collection round, a total of 16 schools and 50 students from each school in each county (total n= 2,400) were selected by cluster sampling, assuming a design effect for cluster sampling of 1.75[18], 10% non-response, and an expected prevalence of stunting of 31.4% based on the Chinese National Survey in 1992[19]. A year later, , a total of 320 subjects from 16 primary schools with 20 students from each school in Huai án County were selected by cluster sampling for the second data collection round, assuming a design effect of 2, 10% non-response, and an expected prevalence of zinc deficiency of 19%[20, 21]. Demographic information, such as name, gender, birth date of subjects, school, class and grade, was then collected after sampling.

Anthropometry

Anthropometry was measured in both data collection rounds. Height of the subjects was measured to the nearest 0.1 cm using the All Plastic Height Measure (Leicester model), with subjects standing erect without shoes on the floorboard. Weight of the subjects was weighed to the nearest 100 g on an electronic scale (Tanita Field work Scale, BWB-800), with subjects dressed in light clothes and without shoes. The instruments were provided by Chasmors Limited, London, UK. All measurements were done in the morning, according to the standardized procedures as described by WHO[22].

Blood and hair collection

Non-fasting morning blood samples were taken from the vein in the antecubital fossa between 8am and 10am. Before collection of blood samples, the tubes were soaked in acid for 24 hrs, washed three times with deionised water, and dried in an incubator to avoid environmental zinc contamination. Drops of blood were kept for assessment of hemoglobin before separation of serum from blood. After clotting for 30-60 minutes, blood samples were centrifuged at 1500g for 10 minutes to separate serum, and aliquots for zinc analysis and other biochemical measurements were frozen at –20 °C and analyzed within one month.

Hair samples were cut with stainless steel scissors from the occipitonuchal region of the head, adjacent to the scalp. The proximal 3 cm of hair close to the scalp was taken for analysis, weighing approximately 2 g. Blood and hair samples for zinc analysis were collected according to the procedures as suggested by Brown *et al*[3]. to avoid contamination.

Laboratory analysis

Serum and hair zinc was analyzed by flame atomic absorption spectrometry. All glassware used for analysis was soaked with acid for 24 hrs and rinsed with deionised water. Certified control sera were used for quality control. Hemoglobin was measured by the cyanmethemoglobin method, according to WHO recommendations[23]. Serum albumin was determined by an automated dye-binding method with bromocresol green[24]. C-reactive protein (CRP) was measured by immunoturbidmetric methods[25]. All measurements had inter- and intra-assay CVs < 10%.

Statistical analysis

Z-scores of height-for-age (HAZ), weight-for-age (WAZ) and BMI-for-height (BMIZ) were calculated using SPSS Macro provided by WHO website according to the WHO new Growth Standards[26]. HAZ, WAZ and BMIZ below –2 were defined as stunting, underweight and wasting respectively, and mild stunting was defined as height-for-age (HAZ) above –2 and below –1.

Overweight and obesity were calculated according to WHO-NCHS BMI criteria for children below 7 years old. For children above 7 years old, overweight was defined as a BMI between the 85th and 95th percentile, whereas obesity was defined as the 95th percentile or higher according to Chinese age-sex-specific BMI criteria for children[27].

Serum CRP concentration ≥10 mg/L was used as an indicator for inflammation. Subjects with inflammation were excluded from the data analysis. Zinc deficiency was defined in three ways[28]: 1) based on serum zinc concentration as recommended by WHO/UNICEF/IAEA/IZiNCG, with a cut-off of 65 μg/dL for morning non-fasting blood among children below 10 yrs; 2) based on hair zinc concentration with a cut-off of 70 μg/g[3]; 3) combined serum and hair zinc cut-offs. Anemia was defined as hemoglobin below 115 g/L, according to the WHO criteria[23].

Data were checked for normal distribution by using the Kolmogorov-Smirnov test of normality. Log transformation was done, if data were not normally distributed. Descriptive indices were expressed as mean (SD) or median for variables with normal or non-normal distribution, respectively. Differences in anthropometric and biochemical indices were analyzed by t-test, ANOVA or chi-square test. Linear regression model was fitted for serum zinc and hair zinc, hemoglobin, HAZ, by adjustment for age, gender, CRP and albumin. A *p*-value <0.05 was considered statistically significant. All analyses were done with SPSS version 11.5 (SPSS Inc., Chicago, IL, USA).

Results

Prevalence of stunting in the first data collection round

Table 1 General characteristic of school children in three Chinese counties

	Dafeng	Taixing	Huai án	Total
Subjects	759	756	753	2268
Age (yrs)	7.8 ± 0.9[a]	8.2 ± 0.9[b]	8.1 ± 1.0[b]	8.0 ± 0.1
Height (cm)	122.7 ±6.8[a]	126.4 ±7.1[b]	124.0 ±7.0[c]	124.3 ± 7.1
Weight (kg)	22.7 ±4.0[a]	24.2 ± 4.5[b]	23.3 ± 4.0[c]	23.4 ± 4.2
HAZ	-0.41 ±0.90[a]	-0.24 ±0.90[b]	-0.63 ±1.02[c]	-0.43 ±0.95
WAZ	-0.62 ±0.96[a]	-0.54 ±0.95[a]	-0.76 ±1.04[b]	-0.64 ±0.99
BMIZ	-0.56 ±0.94	-0.62 ±0.92	-0.56 ±0.94	-0.58 ±0.94

HAZ, WAZ, and BMIZ are z-scores of height-for-age, weight-for-age, BMI-for-age, respectively, calculated according to the WHO new Growth Standards. Data are mean ± SD.

Age, height, weight among three counties was compared by ANOVA, with adjustment for age and sex, respectively. Values in the same row with difference superscript letters are significantly different.

A total of 2268 eligible subjects from the three counties were included in the first data collection round (Table 1). The response rates for Dafeng, Taixing and Huai án were 94.9%, 94.5% and 94.1%, respectively. The overall prevalence of stunting and mild stunting was 4.7% and 22.8% respectively, and no difference was shown in stunting or mild stunting between boys and girls. Stunting increased with age in both boys (P = 0.024) and girls (P = 0.003). The total prevalence of underweight and wasting was 7.1% and 5.5%, respectively. The overall prevalence of overweight and obesity was 3.7% and 1.4%, respectively. Boys showed significantly higher prevalence of overweight and obesity than girls (4.6% and 1.7%, vs. 2.5% and 1.1%, P < 0.05), but no difference was found among age groups and areas (Table 2). Huai án County had the highest prevalence of stunting, underweight and wasting respectively among the three counties (Table 2).

Table 2 Prevalence of stunting, underweight, and wasting among primary school children in three Chinese counties

	Dafeng	Taixing	Huai'an	Total
Stunting (%)	3.6[a]	2.4[a]	8.1[b]	4.7
Mild stunting (%)	22.1[a]	18.5[a]	27.8[b]	22.8
Underweight (%)	6.3[a]	5.2[a]	9.8[b]	7.1
Wasting (%)	5.5	5.7	5.3	5.5
Overweight	4.1	3.2	3.9	3.7
Obesity	1.7	1.2	1.3	1.4

Data are presented in prevalence (%).

Stunting, underweight, wasting were defined as z-scores of height-for-age (HAZ), weight-for-age (WAZ) and BMI-for-age (BMIZ) below -2, and mild stunting was defined as height-for-age (HAZ) above -2 and below -1, according to the WHO new Growth Standards. Overweight and obesity were calculated according to WHO-NCHS BMI criteria for children below 7 years old. For children above 7 years old, overweight was defined as a BMI between the 85th and 95th percentile, whereas obesity was defined as the 95th percentile or higher according to Chinese age-sex-specific BMI criteria for children.

Significant differences among study sites were shown in different superscripts in the same row, measured by chi-square test.

Zinc deficiency in the second data collection round

Three hundred and twenty subjects were recruited from the 16 schools in Huai'an County, and 297 subjects completed the survey. The general information is shown in Table 3.

The prevalence of zinc deficiency based on serum zinc concentrations, hair zinc concentrations, and a combination of the two biomarkers was 0.7%, 15.2% and 15.3%, respectively, after exclusion of subjects with inflammation. C-reactive protein concentrations were low; only 2% had CRP concentrations higher than 10 mg/L. (Table 4). The prevalence of anemia was 32.3% in these primary school children. Boys had significantly lower concentrations of serum and hair zinc than girls ($P < 0.05$), while girls had lower hemoglobin concentrations than boys ($P < 0.05$), as presented in Table 3. Boys had a higher prevalence of zinc deficiency than girls ($P < 0.05$), whereas the prevalence of anemia in boys was lower than that in girls ($P = 0.07$) (Table 4).

Table 3 Zinc status of Chinese primary school children in Huai án County

Characteristics	Total	Boys	Girls
Age (yes)	8.2 ±1.0	8.2 ±1.0	8.2 ±0.9
Serum zinc (μg/dL) (n=294)	103.7	96.5[a]	108.9[b]
Hair zinc (μg/g) (n=286)	118.7 ±42.0	103.2 ±34.2[a]	138.2 ±42.9[b]
Hemoglobin (g/L) (n=297)	119.9 ±11.6	121.1 ±11.2[a]	118.3 ±12.0[b]
Albumin(n=294)	46.8 ±7.5	46.6 ±7.5	46.7 ±7.5
CRP(n=289)	1.1	1.1	1.1
HAZ	-0.49 ±1.27	-0.49 ±1.36	-0.50 ±1.17
HAZ<-2, %	11.1	12.3	9.7

Data are presented as Mean ± SD or median. HAZ was height-for-age z-score calculated according to the WHO new Growth Standards. Significant differences among genders were shown in different superscripts in the same row, using t-test, Mann-Whitney test or chi-square test.

Table 4 Prevalence of inflammation, zinc deficiency and anemia among Chinese school children in Huai án County

Indices	Prevalence (%)		
	Total	Boys	girls
Inflammation (n=289)	2.0	1.9	2.3
Zinc deficiency			
Based on Serum zinc < 65μg/dL (n=281)	0.7	0.6	0.8
Based on Hair zinc < 70μg/g (n=286)	15.2	19.0[a]	10.5[b]
Based on combination of serum and hair Zinc (n=271)	15.3	19.1[a]	10.5[b]
Anemia	32.3	28.2	37.3

Data are presented with prevalence (%). Significant differences among genders were shown in different superscripts in the same row, measured by chi-square test.

Inflammation was defined as CRP > 10mg/L. Zinc deficiency was defined based on serum and/or hair zinc respectively, after exclusion by inflammation. Anemia was defined as hemoglobin < 115 g/L.

Linear regression model of zinc status and hemoglobin concentrations, HAZ adjusted for age, gender, CRP and albumin are shown in Table 5. There was a positive relationship with between serum zinc and hair zinc (stand. Beta = 0.71, P < 0.001). No relationship was found between serum zinc, and Hemoglobin (stand. Beta = -0.03, P = 0.37) , HAZ (stand. Beta = -0.02, P = 0.66), respectively (Table 5).

Table 5 Linear regression model predicting serum zinc, hair zinc, HAZ and hemoglobin

Dependent variable	Adjusted R square	Independent variables	Standardized coefficients	sig.
Serum zinc	0.59	hair zinc	0.71	<0.001
		Hemoglobin	-0.03	0.37
		HAZ	0.02	0.66

Serum zinc was log transformed. The model was fitted by linear regression and adjusted for age, gender, CRP and albumin.

Discussion

In our study, we found that both stunting and zinc deficiency were not highly prevalent among primary school children in rural areas with low soil zinc in Jiangsu Province. Moreover, we found that boys were more vulnerable to zinc deficiency than girls.

Stunting has been suggested as a functional indicator of population zinc status[29]. The prevalence of stunting in the present study (4.7%) was lower than the cut-off of 20% that would indicate elevated zinc deficiency[14], although mild stunting was more prevalent. Older children were more likely to suffer from stunting. Prevalence of stunting in children of 0-5 years old has decreased from 31.9% in 1992 to 14.3% in 2000, mainly due to economic improvement since 1983 in China[30]. Shi *et al.*[31] reported the prevalence of stunting to be 2.9% in students aged 12-14 yrs in Jiangsu Province, which is in range with our data. Stunting in school children varies tremendously among different countries: 4.4% of stunting was shown among Turkish school children aged 7-10 years[32], whereas an average of 51% of stunting was reported among school children aged 6-17 years in five countries in rural Africa and Asia[33]. Stunting ranged from 2.9 to 40.2%, and mild stunting ranged from 31.4 to 75% among school children aged 8-11 years in South Africa[34]. Opposed to stunting, the prevalence of overweight and obesity among childhood in China has increased quickly from 1985 to 1992[35]. The prevalence of overweight and obesity in the present study were lower than those reported on the national level.

Up till now, many studies on zinc status and zinc supplementation have focused on preschool children or toddlers, whereas only a few have addressed school children. Thurlow *et al.*[24] reported a prevalence of low serum zinc concentration of 57% among school children aged 6-13 years in North-East Thailand. In Mexican school-age children below 12 years, 19-24% of zinc deficiency based on serum zinc was reported[36], whereas only 16% of zinc deficiency based on dietary zinc intake was found among children aged 5-14 years in New Zealand[37]. In China, Ma *et al.*[13] reported proportions of inadequate zinc intake to be 15.3% and 12.9% in age groups 4-6 years and 7-10 years, respectively, based on WHO recommendations. Wuehler *et al.*[38] estimated that 14.3% Chinese population were at risk of inadequate zinc intake by using national food balance data in food supplies. Until now there has been no estimation of zinc deficiency using a biomarker among school children in China. In our study we have used serum and/or hair zinc to estimate zinc deficiency. Our results show that a combination of serum and hair zinc would be in good agreement with the estimation from the dietary intake studies in China.

Although serum zinc has recently been suggested as a good biomarker for zinc deficiency, we found that zinc deficiency based on serum zinc was much lower, as compared to hair zinc Serum zinc level reflects short-term status, and hair zinc concentration reflects long-term zinc status. Serum zinc can be influenced by recent dietary intake, and has been shown repeatedly to be sensitive to changes in zinc intake in supplementation studies[9, 28]. However, zinc deficiency might take a long course to develop, and serum zinc may remain within the normal range in marginal zinc deficiency[39]. Another explanation for the low prevalence of zinc deficiency defined by serum zinc concentrations would be unexpected contamination, although we have taken all precautious measures following the current recommended procedures. In addition, cut-offs of serum zinc are estimated mostly from surveys in America, which might not apply in China. Defining zinc deficiency in China still remains an unresolved discussion.

The discrepancy of zinc deficiency between boys and girls was consistent with other reports[40]. Compared to girls, boys have a higher requirement for zinc to meet their higher growth rate, and a greater proportion of muscle per kilogram body weight. Muscle contains a higher content of zinc than fat[3].

Previously, anemia was suggested as an indicator of zinc deficiency because iron and zinc have a similar distribution in the food supply, similar food sources, and low bioavailability in cereals due to the presence of phytate. However, anemia is no longer recommended as an indicator of zinc deficiency due to inconsistencies, as recently advised by WHO/UNICEF/IAEA/IZiNCG[14]. In our study, anemia is highly prevalent among rural primary school children, and does not coincide with zinc deficiency based on serum zinc concentrations. This supports the current recommendation not to use anemia as an indicator of zinc deficiency.

There were some limitations in our study. Firstly, we only collected blood samples in one county, which might not be representative for zinc deficiency in the wider Province. Secondly, although we had a good sampling framework and sample size in Huai án County, the sample size for blood collections turned out slightly smaller planned for. We based the sample size on a prevalence of zinc deficiency of 19%, whereas in fact the prevalence was lower. In addition, although we have provided a fairly comprehensive assessment of risk of zinc deficiency in the selected population, we do not address the role of the diet in determining zinc status.

In conclusion, in our study, we did not find a high prevalence of zinc deficiency and stunting among primary school children in rural areas in Jiangsu Province despite the low soil zinc concentrations, which suggests that dietary pattern, interaction of micronutrients, or other factors may play more important roles than soil zinc concentrations only. Zinc deficiency was more prevalent in boys than girls. In addition, anemia is highly prevalent in the area, which should receive more attention.

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Chapter 4

Zinc bioavailability from intrinsically labeled biofortified rice compared with extrinsically labeled extruded fortified rice

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Abstract

Objective: To determine the bioavailability of zinc from intrinsically ^{70}Zn labeled biofortified rice, and to compare it with zinc bioavailability from both extrinsically ^{70}Zn labeled extruded fortified rice and normal rice.

Methods: Thirteen women were recruited for three test rounds with rice meals (biofortified rice, zinc extruded fortified rice and control rice) with a wash-out period of 4 weeks between meal administrations. Subjects were given each rice meal as breakfast in random order. ^{67}Zn was used as an intravenous tracer. Factional zinc absorption (FAZ) was assessed by measuring the isotope ratio in urine samples collected before and 3-day after meal administrations.

Results: FAZ from the biofortified rice was consistently higher than that from the control rice, and that from extruded fortified rice was consistently lower.

Conclusion: Due to adhesion of ^{67}Zn to the vials used for the infusions, we could not fully quantify FAZ from the three types of rice. However, we conclude that zinc from biofortified rice is well absorbed. The study should be repeated to quantify FAZ.

Introduction

More than 500 enzymes in the human body require zinc in adequate amounts in order to function properly[1]. Therefore, zinc deficiency is likely to have adverse effects on many body functions. The most apparent of those so far established are growth, immunity and cognition[2, 3]. In China, zinc deficiency of agricultural soils is prevalent, which results in low yields and low zinc content of crops[4]. Zinc deficiency is considered to be prevalent especially in rural areas of China and in segments of the population with low socio-economic status[5, 6].

Biofortification is an intervention strategy under development with the goal of increasing zinc content in the edible portion of staple food crops by agronomic or genetic means[7]. It offers a long-term, sustainable, food-based solution for alleviating micronutrient deficiencies[8]. Rice is a promising vehicle for biofortificaiton, since it is a leading staple food in China, as well in other South Asia countries, such as Philippines[9]. Another effective strategy is fortification with extrusion technology[10]. Using this technology, vitamin A and iron fortified rice has been reported[11, 12]. However, the efficacy of those strategies to improve nutrient status still needs to be proven. The bioavailability of zinc from cereals is usually inhibited by the presence of phytic acid[13]. The zinc content of Chinese brown rice (n=56) was recently reported to be 23 mg/kg (dry mass) and the phytic acid content to be 9.6 g/kg[14]. The molar ratio of phytic acid to zinc was reported to be 42.9 ± 7.5, whereas a molar ratio of 10 or higher is known to inhibit zinc absorption substantially[15].

Isotopic labeling of food has been used widely in many human studies for measurement of mineral absorption and evaluation of bioavailablility[16-20]. Extrinsic and intrinsic labeling are two distinct methods to label nutrients in foods[21]. For plant foods, the intrinsic labeling technique entails the incorporation of a labeled nutrient in the natural form of the food during growth[22]. The extrinsic labeling technique is based on the assumption that an labeled element added to the food right before consumption and the unlabeled element present in the food enter a common pool in the stomach and/or intestine, and has the same absorption pattern, which can be validated by comparison with an intrinsic labeling technique[23].

Zinc bioavailability from high phytate foods using extrinsic labeling has previously been measured for maize or maize-based diets[24-26], wheat[27] and mixed diets[28-32], but no study has been applied for biofortified rice. Therefore, in this study, our aim was to determine the bioavailability of zinc from biofortified rice using ^{70}Zn as an intrinsic label, and to compare it with the bioavailability of zinc from rice mixed with ^{70}Zn extruded fortified grains and with non-labeled rice to which ^{70}Zn was added immediately before consumption as an extrinsic tag.

Methods

Subjects

The sample size was calculated to detect a difference of 10% in the fractional absorption of zinc (FAZ) between biofortified and normal rice assuming a baseline FAZ of 0.15 and an SD of 0.08[24, 33], with 80% power and α=0.05[31]. At least 12 subjects would be required for this, and to allow for drop-out 15 healthy women aged 18 to 45 years were recruited in a village from Huai án County, Jiangsu Province. Before being accepted in the study, each woman completed a health questionnaire, and a 10-ml screening blood sample was taken to exclude those with biochemical and hematological indices outside the normal range with respect to the following parameters hepatitis B and C, HIV, blood glucose, cholesterol. Height, weight, and blood pressure were measured by trained health workers. Women suffering from gastro-intestinal diseases, or other acute and chronic diseases, such as diabetes, anemia, hepatitis, hypertension, cancer or cardiovascular diseases were excluded. Those smoking, pregnant or lactating, having a history of miscarriage or assisted conception, or when taking dietary supplements containing iron or zinc were also excluded. Informed consent was obtained after full explanation of the study protocol, and the involved risks and benefits.

A validated food frequency questionnaire (FFQ) over the previous year was used to collect data on habitual zinc intake. Portion size for each food was established by reference to food models. Intakes of foods were converted into g/week for data analysis [34]. The study protocol was approved by the Medical Ethical Committee of Huai án County, China.

Study design

The study was comprised of three test rounds with rice meals (biofortified rice, zinc extruded fortified rice and control rice) with a wash-out period of 4 weeks between meal administrations. Subjects were given each rice meal as breakfast in random order. After an overnight fast, women finished their rice meals under supervision of trained study staff in a local clinic. The test meal administration was followed by the intravenous (iv) administration of a second stable isotope (^{67}Zn). No food and drinks were allowed for 3 h after the administration of the iv dose.

Rice preparation

All rice for the bioavailability studies was produced in a greenhouse under controlled conditions at Wageningen University, the Netherlands, using Qinai-3-hun. This cultivar was selected on the basis of grain Zn loading ability under field conditions[35]. Experiments conducted with a series of cultivars had provided evidence that when grown on nutrient solution, rice grain Zn concentrations of around 50 mg kg^{-1} could be reached [36]. Further pilot studies were used to decide on timing of ^{70}Zn application to optimize its grain allocation versus allocation to vegetative tissues and thus avoid unnecessary costs (Stomph et al. Personnel communication). Rice with extruded fortified ^{70}Zn labeled rice grains as well as control rice was produced on nutrient solution in 165 L basins, by addition of a standard nutrient solution with 0.05 mg Zn L^{-1}. Rice for the biofortified ^{70}Zn enriched rice was produced by application of 3 mg Zn L^{-1} in the nutrient solution until panicle initiation, followed by 2 mg L^{-1} until harvest. The nutrient solution with normal Zn was replaced by a nutrient solution with ^{70}Zn from roughly one week before flowering onwards. All Zn was added to the nutrient solution as $ZnSO_4$. The purchased ^{70}ZnO (99.53% ^{70}Zn), was acidified with H_2SO_4 (0.01 M ZnO + 0.0195 M H_2SO_4) to form $^{70}ZnSO_4$. For each kg of biofortified ^{70}Zn enriched rice, 1 g of ^{70}Zn was required. Upon harvesting grains were hulled manually and subsequently polished using a Pearlest® laboratory scale grain polisher (purchased from Kett Electric Laboratory, Tokyo, Japan). Grains were polished for 60 seconds at 20 g at a time. In total, 3 kg of polished control rice and 1.5 kg of polished biofortified rice was produced. Extruded rice fortified with ^{70}Zn was produced at the Laboratory of Human Nutrition, ETH Zurich, Switzerland. For this, 1.5 kg of the control rice was milled into rice flour, and 300 g of this flour was mixed with natural $ZnSO_4$ and $^{70}ZnSO_4$ according to the isotopic levels and total Zn content reached in the biofortified rice.

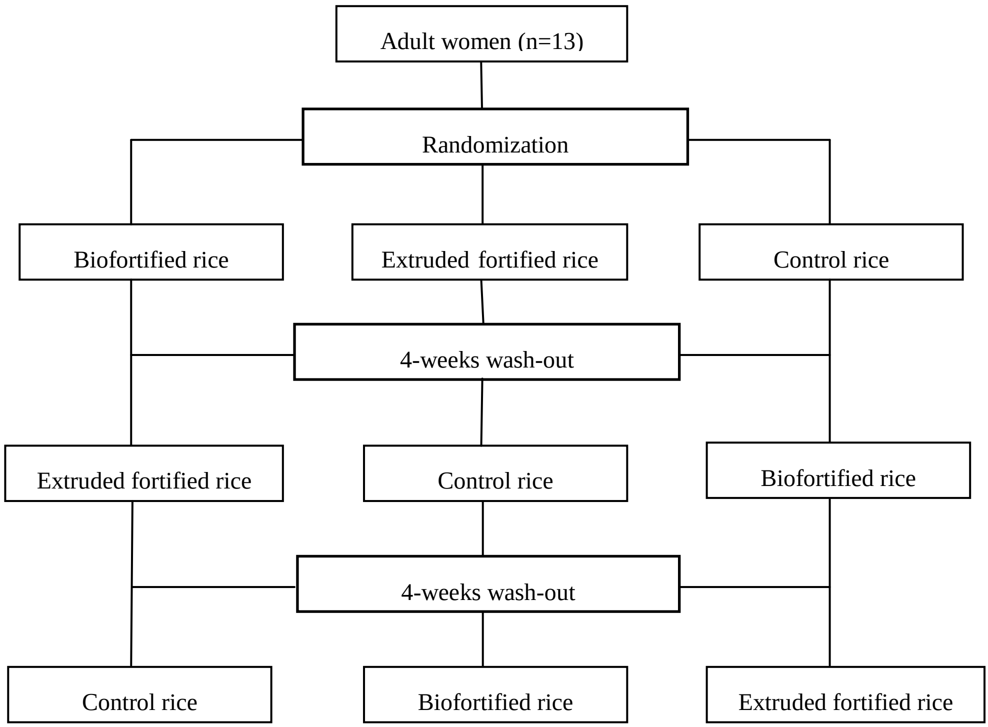

Figure 1. Flow diagram of the study design

Meal preparation

For each test meal, 50 g of rice and 300 g of pure water (Yibao) were weighed and put into an electric rice cooker, and the rice was cooked to porridge for exactly 2 hours. For extruded rice, 20 g of extruded and 30 g of control rice were mixed in order to match Zn content of the extruded rice meal with that of the biofortified rice meal. After cooking, the rice was transferred from the cooker into a container for test meal administration while rinsing the cooker three times with 50 mL of pure water to insure quantitative transfer. For the control rice, 50 mL of pure water was added to rinse after cooking. Then, 1mL of $^{70}ZnSO_4$ solution (1 mg/g zinc) and 100 mL of pure water were added and mixed into the meal just before administration. Rice and ^{70}Zn solutions were weighed using a 3-digit weighing scale (8953 Dietikon, Switzerland) that was calibrated daily. After consumption, the containers were washed with 120 mL pure water for at least twice time, and the pure water was drunk by the subjects.

Intravenous dose preparation and administration

Doses for intravenous administration were prepared from ^{67}ZnO in a sterile environment at the Cantonal Pharmacy of the University Hospital Zurich. Isotopically enriched zinc oxide was transformed to ZnCl with HCl, adjusted to pH 7 by adding $NaHCO_3$ and diluted by physiological saline. Individual doses of 9.5 g solution with a concentration of 22.2 ug Zn/ml were transferred to glass vials, septum sealed, labeled, sterilized and checked for sterility and pyrogens. Immediately after finishing the meal, the intravenous dose was administered over 5 min into the antecubital vein of the arm using a ‘butterfly’ infusion set. The butterfly tubing was flushed with 5 ml sterile saline solution to ensure that the entire tracer dose was infused. The exact amount of the tracer solution infused was determined by weighing the syringe before and after the infusion. The isotope infusions were done by a registered nurse. Some of the empty infusion capsules were sent back to ETH, Switzerland, for analysis of any remaining tracer and adjustment of the final FAZ.

Specimen collection

Overnight fasted urine samples were collected before meal administration. After each meal administration, urine samples were collected from 6 am to10 am during the next three days. The time of each collection was noted on the container and log sheets. A 5-ml blood sample was collected once-off into a Zn-free plastic tube before the first meal administration. Serum samples were separated from blood cells within 30 min. All of the specimens were stored at -20 ℃ until they were transported to the laboratory for measurement of serum zinc and C-reactive protein (CRP), and urinary zinc and isotope ratio analyses.

Laboratory analyses

The urine samples were dried into concentrated urine by a freeze dryer at –80 ℃ (Christ alphal-2, German) at the Jiangsu Provincial Center for Disease Control and Prevention in Nanjing, China, and transported to the Laboratory of Human Nutrition at the ETH, Zurich, Switzerland, for analysis. Serum and urine zinc concentrations were determined by ICP-AAS in Huai án Center. C-reactive protein (CRP) was measured by immunoturbidmetric method[37] in Jiangsu Provincial Hospital. Zinc and phytate contents of the biofortified and control rice were analyzed by the Laboratory of Human Nutrition, ETH Zurich, Switzerland. Furthermore, Zn isotope ratios (^{66}Zn:^{67}Zn and ^{66}Zn:^{70}Zn) in the urine samples were measured by inductively coupled plasma mass spectrometry (ICP-MS) on a Finnigan™Neptune (High Resolution, Multicollector Mass Spectrometer) in the Laboratory. The relative enrichment (amount of oral spike to amount of intravenous spike) was calculated from the measured ratios.

Statistical analysis

Data were presented as means ± SDs or percentage. Zinc deficiency was defined as serum zinc concentration below 74 µg/dL, and CRP ≥10 mg/L was defined as an indicator for inflammation[15, 38]. Insufficiency of zinc intake was determined as below 2/3 of the recommended nutrient intake (RNI) (11.5 mg/d) according to the Chinese Dietary Reference Intakes (DRIs)[39]. Phytate to zinc molar ratio of the two types of rice was calculated with the equation recommended by International Zinc Nutrition Consultative Group (IZiNCG) [15].

Fractional absorption of zinc (FAZ) was calculated according to the validated equation[32, 40-42].

$$FA = \text{enrichment (oral/iv)} \times \text{dose (iv/oral)}$$

$$= [(R^s-R^N)/R^N(\text{oral})]/[(R^s-R^N)/R^N(\text{iv})] \times \text{dose(iv)}/\text{dose(oral)}$$

Where R^s is the ratio of oral or iv isotopes to the reference isotopes in urine; R^N is the natural enrichment ratio of the oral or iv isotopes to the reference isotope; dose is the administered dose of the oral or iv isotopes.

Results

Thirteen women completed the whole trial. Two women dropped out because they were out of the village, or unwilling to continue participation. In table 1, the characteristics of the women in the study are described. The age of the subjects was all above 18 and below 45 years old. Serum zinc concentration of each subject was above the cut-off for zinc deficiency and CRP values below the cut-off for inflammation. Only one subject had low dietary zinc intake in reference to the Chinese DRIs.

Table 1 Characteristics of the study population

	Value
Age (yrs)	29.1 ±5.9
Height (cm)	156.2 ±5.5
Weight (kg)	55.1 ±5.1
BMI (kg/m^2)	22.6 ±1.5
C-reactive protein (mg/L)	1.6 ±1.8
Serum zinc concentration (μg/dL)	94.0 ±7.0
Dietary zinc intake (mg/d)	15.4 ±5.9
Insufficiency of zinc intake (%)	7.1

Insufficiency of zinc intake was determined as below 2/3 of the recommended nutrient intake (RNI) (11.5 mg/d) according to the Chinese DRIs [3].

Zinc and phytate content of each types of rice are presented in Table 2. The control rice had lower zinc and higher phytate content than the biofortified rice. The phytate to zinc molar ratio was 7.58 in the biofortified rice much lower than that in the control rice (23.10), and slightly lower than that in the extruded rice (8.72).

Table 2 Zinc, phytate in three types of rice

	Biofortified rice	Extruded fortified rice	Control rice
Zinc (mg/100g)	3.79	5.11	1.93
Phytate (g/100g)	0.29	0.45	0.45
Phytate/zinc molar ratio	7.58	8.72	23.10

The extruded rice is mixed with mixed with 20 g fortified rice and 30 g control rice. The concentration in the extruded rice is calculated by 9.89 ×40% + 1.93 ×60%. The phytate concentration in the extruded rice is the same with normal rice.

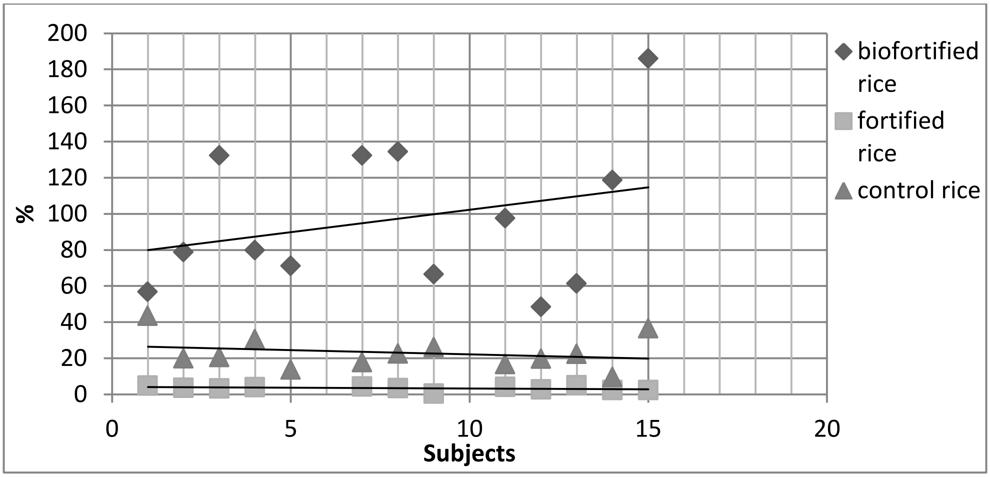

Figure 2 Fractional zinc absorption from three rice meals for each subject

Figure 2 and Table 3 show FAZ from the three types of rice meal in subjects. The mean FAZ from biofortified rice was consistently higher than that of control rice and extruded rice.

Table 3 Fractional absorption of zinc (FAZ) for three types of rice

	70Zn (%)	Zinc (mg/100g)	FAZ
Biofortified rice	16.02	3.79	0.97±0.41
Extruded rice	56.3	5.11	0.04±0.01
Control rice	49.01	4.93	0.23±0.09

Zinc in biofortified, extruded and control rice was 3.79, 9.89, and 1.93 mg/100g, respectively. Extruded rice meal was mixed with 20 g fortified rice and 30 g control rice. The zinc concentration in the extruded rice meal was calculated by 9.89 × 40% + 1.93 × 60%. The zinc concentration in the control rice meal was calculated by 1.93 + 3 (from 1ml of ^{70}Zn solution added to the meal).

Discussion

In the present study, the bioavailability of zinc was consistently highest from biofortified rice, intermediate from control rice, and lowest from extruded rice. However, due to technical constraints we could not reliably quantify differences in zinc absorption between the three types of rice.

An important limitation of the study is that it was difficult to determine the exact amount of ^{67}Zn stable isotope label in the infusion fluid for each subject. Although we used a 3-digit weighing scale to ensure the exact amount of ^{67}Zn solution infused to each subject to be measured, part of the ^{67}Zn stable isotope label remained in the infusion vial by adhesion to the vial wall, which inevitably influenced the results of the study. In some of the vials the remaining Zn content in the vial was measured and results have shown that this was pretty much the same in all the measured vials. However, it will result in a systematic error. Therefore, we just make assumption to estimate FAZ, but not quantify it. Despite this limitation which doesn't allow us to draw conclusions from this study, the results are consistent within each subject with respect to the FAZ for the three types of rice as shown in figure 2. We therefore think that zinc from biofortified rice has a higher bioavailability than zinc from the control rice which was extrinsically labeled or rice which was fortified and labeled during extrusion.

The efficiency of zinc absorption from the diet usually ranges from about 15-35% in adults, depending on the amount consumed and the presence of other dietary factors, such as phytate, that inhibit absorption[43]. Studies on zinc absorption from maize, wheat and mixed diets have been reported in adult women and men[24, 27, 29, 32], pregnant women[28], as well as in children[25, 26, 31], which are listed in table 4. The FAZ varied from 0.13 to 0.38. In our study, FAZ from the control rice was 23% and similar to the findings with a high-phytate maize-based diet[25], but lower than earlier found in toddlers and women consuming a representative Chinese diet[31, 32]. One study indicated higher zinc absorption (0.28-0.38) from low-phytate maize[24]. Vegetarian diets were found to contain lower amounts of absorbable Zn than meat-based diets[30]. Rosado et al[27] compared zinc absorption from biofortified wheat (extrinsically labeled) with that from control wheat with a typical zinc concentration, and found that the amount of absorbed zinc from biofortified wheat was higher, although FAZ was lower than that from control wheat.

Our study showed a much higher FAZ from biofortified rice, some of the values are more than 100%, which suggested a overestimation although we have a FAZ from control rice in a normal range. Moreover, we found that the phytate concentration in biofortified rice is lower than control rice. Whether biofortification increases zinc concentration simultaneously decreases phytate concentration in rice is still unclear. Another explanation for the possible high bioavailability would be that the labeled zinc may have been bounded to protein rather than to phytate. The metabolism of zinc in such rice may be different with the normal rice.

Unexpectedly, zinc from extruded fortified rice presented to be much less well absorbed than from the control rice, although the molar ratio of phytate/zinc is not largely different with the biofortified rice. A possible explanation for the lower FAZ is that zinc used to fortify the extruded rice was embedded in a very dense and strongly closed matrix and thus couldn't be easily released during digestion.

Table 4 Zinc absorption from different diets with extrinsic stable isotope labeling

Study	Subjects	Meal	FAZ
Hambidge, et al[24]	6 men	Low-phytate hybrid maize	0.28±0.04
		Nutridense low phytate maize	0.38±0.07
	4 women	Lpa-1 wild-type isohybirds	0.15±0.07
		ND wild-type idohybirds	0.13±0.05
Manary, et al[25]	10 children(43.6 ±7.7 mo)	High-phytate maize-based diet	0.24±0.04
Mazariegos, et al[26]	60 children	Low-phytate maize	0.32±0.07
	(8.9±1.3 y, 20 per group)	Wild-type maize	0.28±0.07
		Local maize	0.29±0.06
Rosado, et al[27]	26 adult women	95% extracted control wheat	0.20±0.05
	Randomization to 4 groups	95% extracted biofortified wheat	0.15±0.05
		80% extracted control wheat	0.38±0.14
		80% extracted biofortified wheat	0.31±0.07
Harvey, et al[28]	13 healthy pregnant women (18-40 y)	Meals with iron supplement (16wks of pregnant)	0.21±0.03
		Meals with iron supplement (24 wks of pregnant)	0.24±0.03
		Meals with iron supplement (34 wks of pregnant)	0.31±0.06
		Meals with placebo (16wks of pregnant)	0.22±0.07
		Meals with placebo (24 wks of pregnant)	0.24±0.03
		Meals with placebo (34 wks of pregnant)	0.31±0.05
Kim, et al[29]	7 healthy young women	High phytate diets	0.22±0.14
Sheng, et al[31]	43 toddlers (23.0±2.0 m)	Habitual diet at home	0.35±0.12
Yang, et al[32]	20 urban women of childbearing age (18-22 y)	Representative Chinese diet	0.36±0.13

In summary, it seems that zinc from biofortified rice is significantly better absorbed than zinc from control rice and from extruded fortified rice grains in adult women. However, the conclusion should be drawn carefully after the repeated measurement for FAZ of the three types of rice. Since rice is the main staple food in China, increasing the zinc content of rice by agricultural, agronomic or genetic means[8, 44-46], will potentially contribute to the prevention of zinc deficiency in high priority populations, such as infants, children and pregnant women.

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Chapter 5

Zinc biofortification of rice in China: a simulation of zinc intake with different dietary patterns

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Abstract

Objectives: To describe the effect of simulated biofortification of rice with zinc on dietary zinc intake and to compare the effect in different dietary patterns among adults in Jiangsu Province.

Methods: A cross-sectional survey with 2819 adults aged 20 years and older was undertaken in 2002. Zinc intake was assessed using a consecutive 3-day 24-h dietary recall method. Insufficient and excess zinc intake was determined according to the Chinese DRIs. Four distinct dietary patterns were identified namely “traditional”, “Macho”, “sweet tooth”, and “healthy”. Intake of zinc from biofortified rice was simulated at an intermediate zinc content (2.7 mg/ 100g) and a high zinc content (3.8 mg/ 100g) in rice.

Results: Average total zinc intake in the population was 12.0 ± 3.7 mg/d, and insufficient zinc intake was 15.4%. Simulated zinc intake from biofortified rice with intermediate and high zinc content decreased the prevalence of low zinc intake decreased to 6.5% and 4.4%, respectively. The effect was most pronounced in “traditional” pattern, with a 0.7% of insufficient zinc intake in the highest quartile of the pattern. An inverse trend in zinc intake was shown over “sweet tooth” pattern. Excess zinc intake was negligible for both simulated zinc levels.

Conclusion: Zinc biofortifed rice improves dietary zinc intake and lowers risk for insufficient zinc intake, especially for subjects with a more “traditional” food pattern among adults in Jiangsu Province. A “sweet tooth” food pattern may attenuate the positive effect.

Introduction

Zinc deficiency is prevalent and affects nearly two billion people in the developing world, where mainly cereals are consumed by the population[1]. Zinc deficiency results in retarded growth[2], higher morbidity and mortality from infectious diseases in children[3, 4], impaired pregnancy and infant outcome[5], and is also associated to several chronic diseases in adults, such as diabetes and malignancy[6]. Although there is still no accurate indicator for zinc deficiency, dietary zinc intake appears to adequately predict zinc status among adults[7].

In a previous study we have shown that daily dietary zinc intake in Jiangsu Province is low according to the Chinese Dietary Recommended Intakes, especially in children and adolescents[8]. Zinc content is highest in animal-source foods, relatively high in whole-grain cereals, and low in refined cereals and vegetables[9]. Rice is one of the most important staple foods and is an important source of minerals and trace elements in China. Cereal grains and vegetables together contribute 50-70% to dietary zinc intake in the Chinese population[10], which is higher than the proportion (27%) in western countries[11]. One of the strategies to improve dietary zinc intake is to increase zinc content in staple crops through genetic or agronomic strategies, or through selective breeding, which is called biofortification. These techniques have the potential to combat zinc deficiency, although there is still no direct evidence to demonstrate this[12].

Simulation models using dietary intake data in a representative target population is an useful tool to evaluate food-based interventions, such as biofortification or fortification[13]. Such models have been used in establishing safe and potentially efficacious fortification for some micronutrients, like folic acid, and calcium[13-16]. However, this way of analysis is still limited, and only a few of such studies have focused on zinc, one from Bangladesh[17] and one from Mexico[18].

In order to identify individuals at risk for nutrition related complaints, dietary patterns rather than the traditional single food item approach has been developed using principal factor analysis[19-23]. Such analyses are useful to evaluate associations between dietary patterns and risk of diseases. Such dietary patterns have been defined in relation to anemia and obesity for the population of Jiangsu Province, China, based on data from a sub-group of a national dietary intake survey[23, 24]. In the present study we aimed to describe the effect of simulated zinc intake from biofortified rice on dietary zinc intake and to compare the effect of biofortification between different dietary patterns among adults in the Province.

Materials and methods

Sample

The study was conducted in Jiangsu Province using a multistage cluster sampling method, as described before[8, 24], which was part of the 2002 National representative cross-sectional survey on nutrition and health. Six counties and two prefectures were included, which represented a geographically and economically diverse population for Jiangsu Province. From each of the six areas, three streets/towns were randomly selected. In each street/town, two villages/neighbourhoods were further randomly selected. In each village/neighbourhood, thirty households were randomly selected. All members in the households were invited to take part in the study and written consent was obtained from all the participants with a response rate of 90.8%. . Altogether, 2819 adults aged 20 years and older with complete data were included in our analysis. The study was approved by the Human Investigation Review Committee at the National Institute for Nutrition and Food Safety, Chinese Center for Disease Control and Prevention.

Dietary intake measurement

Trained interviewers from the local Center for Disease Control and Prevention visited subjects in their homes to collect the information on food intake using a 24-h dietary recall method on three consecutive days, including two weekdays and one weekend day. Energy and nutrient intake was calculated using the data of the dietary recall in conjunction with the China Food Composition Table which was updated in 2002[25].

Determination of dietary pattern

Dietary patterns were identified with data collected by a food frequency questionnaire (FFQ), using standard principal component analysis as described before for this population[24]. The FFQ was validated, and used to collect dietary information over the previous year[26]. The FFQ included a series of detailed questions regarding the usual frequency and quantity of intake of thirty-three foods and beverages. This was further merged into twenty-five food items in the analysis because of the low intake of some food items. Portion size for each food was established by reference to food models. Subjects were asked to recall the frequency of consumption of individual food items (number of times per day, per week, per month, per year) and the estimated portion size, using local weight units (1 liang = 50 g) or natural units (cups). Intakes of foods were converted into g/week for data analysis. Use of vitamin and mineral supplements was included in the questionnaire, but because these were very seldom used in the area, they were not included in this analysis.

For identification of the dietary patterns, factor loading for each food item was calculated, which is equivalent to a simple correlation between the food item and the factor (Table 1). Higher loadings (absolute value) indicate that the food shares more variance with that factor. The sign of the loading determines the direction of the relationship of each food to the factor. Food groups with absolute values of less than 0.20 are excluded from the table for simplicity. Only one food item (cheese) is missing owing to low factor loading. Wheat flour includes noodles and steamed dumplings, and beverages include soft drinks, coffee and tea.

Table 1. Factor loading for four food patterns among adults

Factor 1: "Traditional"		Factor 2:"Macho"		Factor 3:"Sweet tooth"		Factor 4:"Healthy"	
Food or food group	Factor loading	Food or food group	Factor loading	Food or food group	Factor loading	Food or food group	Factor loading
Rice	0.81	Poultry	0.56	Cake	0.60	Whole grains	0.54
Fresh vegetables	0.57	Beer	0.53	Juice	0.58	Fruits	0.49
Pork	0.37	Beef, lamb	0.46	Beverage	0.48	Pickled vegetables	0.46
Fish	0.21	Deep-fried products	0.45	Milk	0.48	Tofu	0.44
Root vegetable	-0.32	Pork	0.44	Yoghurt	0.44	Fresh vegetables	0.37
Wheat flour	-0.78	Liver	0.43	Beef, lamb	0.30	Root vegetable	0.34
		Alcohol	0.43	Nut	0.26	Milk	0.31
		Eggs	0.38	Poultry	0.25	Eggs	0.30
		Fish	0.26	Fruits	0.23	Fish	0.24
		Nuts	0.23	Pickled vegetables	-0.20	Wheat flour	0.23
		Fruits	0.22	Alcohol	-0.27	Milk powder	0.22
		Tofu	0.22			Beer	-0.21

Factor loadings are equivalent to a simple correlation between the food items and the factor. Higher loadings (absolute value) indicate that the food shares more variance with that factor. The sign of the loading determines the direction of the relationship of each food to the factor. Food groups with absolute values of less than 0.20 are excluded from the table for simplicity. Only one food item (cheese) is missing owing to low factor loading. Wheat flour includes noodles and steamed dumplings. Beverage includes soft drinks, coffee and tea.

Four different patterns were defined: 1) the "traditional" pattern, characterized by high intakes of rice and fresh vegetables and low intake of wheat flour; 2) the "macho" pattern, characterized by intake of animal foods and alcohol, i.e. foods commonly eaten by men; 3) the "sweet tooth" pattern, characterized by intake of cake, milk, yoghurt and beverages; and 4) the "healthy" pattern, characterized by intake of whole grain products, fruits, root vegetables, and fresh and pickled vegetables. The four patterns explained 30.5% of the variance in intake (10.6%, 8.6%, 5.9% and 5.4% for "traditional", "macho", "sweet tooth", and "healthy" patterns, respectively) (Table 1). Scores for each pattern were calculated as the sum of the products of the factor loading coefficient and the standardized weekly intake of each food associated with that pattern. Only foods with factor loadings of more than 0.20 or less than -0.20 were included in the calculation of pattern scores because these items represent the foods most strongly related to the identified factor.

Socio-economic status

Socio-economic status (SES) was assessed by the question “What was your family’s income per person in 2001?” Low SES was defined as an income of less than 1999 Yuan, “medium” as 2000–4999 Yuan and “high” as more than 5000 Yuan.

Simulated biofortification of rice

The mean zinc concentration in normal rice was 1.7 mg/100g (raw polished rice) based on the dietary China Food Composition Table (2002). Ma et al[27]. have reported that phytic acid concentration in four types of raw rice consumed in China ranged from 55-183 mg phytic acid/ 100g (average 115 mg/ 100 g), and the phytate/zinc molar ratio to range from 3.07 to 11.27 (average 8.4). A specific variety with a zinc concentration of 3.8 mg /100g and 290 mg/100g phytate (phytate/zinc molar ratio 7.6) in raw polished rice (Qinai-3-hun, a Chinese variety) has recently been produced in a greenhouse by breeding under controlled conditions at Wageningen University, the Netherlands (unpublished results). The limit of zinc in biofortified rice appears to be about 10 mg/100g, and zinc concentrations of 3.5 mg/100g have previously been reported in paddy-grown rice[28]. In our study, we simulated two levels of zinc in biofortified rice, namely a high level (3.8 mg/ 100g) and an intermediate level (2.7 mg/ 100g).

Statistical analysis

Variables are presented as mean ± standard deviations (SD) for numeric variables or as percentages for categorical variables. Insufficiency of zinc intake was determined as below 2/3 of the recommended nutrient intake (RNI) with age- and gender- specific values according to the Chinese DRIs, which is 15 mg/d for males (18-49 y), and 11.5 mg/d for males (> 49 y) and for females (>19 y)[29]. Furthermore, excessive zinc intake was defined as zinc intake higher than the cut-off of the tolerable upper intake level, which is 45 mg/d for males (20-50 y) and 37 mg/d for females (>20y) and for males (> 50 y)[29]. Absorbed zinc was estimated according to IZiNCG guidelines, with zinc absorption fractions of 26% in men and 34% in women consuming a mixed diet. Consequently, absorbed zinc inadequacy was defined as absorbed zinc levels lower than 2.69 mg/d for men and 1.86 mg/d for women[9]. Dietary pattern scores were categorized to quartiles from Q1 (the lowest) to Q4 (the highest).This implies that the highest quartile consists of subjects that best represented each of the dietary patterns. Paired t-test was applied to analyze differences in zinc intake from normal rice as compared to simulated zinc intake at the two levels biofortification. ANOVA and chi-squared test was used to determine group differences for continuous and qualitative variables, respectively. Linear regression was performed for zinc intake over the quartiles in dietary pattern scores, adjusted by household, age, gender, region and area of residence, SES, education and energy intake. All analyses were performed using SPSS 19.0 (SPSS Inc., Chicago, IL, USA). Statistical significance was set at $\alpha = 0.05$.

Results

The study included 2819 participants, with 1297 males and 1522 females. There were no differences in age, residence (urban/rural), region (north/south), socio-economic status and education level between genders. The average rice intake was 250.1 ± 145.8 g/d, with no difference between males and females. About 5% of the participants reported not to eat rice.

Table 2 Sample characteristics in the highest quartile of dietary patterns

		All (%)	"Traditional"	"Macho"	"Sweet tooth"	"Healthy"
Gender	Male	1297 (46.0)	46.9	44.5	45.9	48.5
	Female	1522 (54.0)	53.1	55.5	54.1	51.5
Age group	20-29	307 (10.9)	8.7	13.7	18.8	11.3
	30-39	589 (20.9)	16.4	25.2	22.0	21.1
	40-49	610(21.6)	23.1	25.5	19.3	23.3
	50-59	527(18.7)	26.3	16.8	16.6	17.0
	60+	786 (27.9)	25.4	18.7	23.3	27.3
Residence	Urban	703 (24.9)	18.3	39.8	49.6	38.2
	Rural	2116 (75.1)	81.7	60.2	50.4	61.8
Region	South	1486 (52.7)	82.3	54.8	68.0	41.9
	North	1333 (47.3)	17.7	45.2	32.0	58.1
SES	Low	911 (32.2)	10.9	23.2	14.4	39.8
	Medium	899 (31.9)	41.7	34.6	27.4	27.2
	High	984 (34.9)	47.4	42.2	58.1	33.0
Education	Primary	1343 (47.6)	45.9	29.2	28.3	41.4
	Junior school	1024(36.3)	36.4	47.4	39.1	35.8
	High school	451 (16.1)	17.7	23.4	32.6	22.8

SES, socio-economic status.

Data are presented as percentages of the total number of subjects in the fourth quartile of each of the dietary patterns.

Table 2 shows the characteristics in the highest quartile of each of the four dietary patterns. There were no gender differences in each of the dietary patterns. The 'traditional' pattern was associated with age, whereas the "macho" and "sweet tooth" patterns were inversely associated with age. Compared to rural residents, more urban residents scored high on the "macho", "sweet tooth" and "healthy" pattern. The "traditional", "macho" and "sweet tooth" patterns were more frequently represented in those with high SES. Compared to the South, subjects from the North reflected more the "healthy" pattern and less any of the other patterns. The "traditional" and "healthy" patterns were not related to education level, whereas the "macho" and "sweet tooth" patterns were more represented in the medium and high education categories.

Mean zinc intake in the population was 12.0 ± 3.7 mg/d and was not different for males and females (12.1 ± 3.7 mg/d vs. 12.0 ± 3.7 mg/d, respectively). The average zinc intake from normal rice was 4.1 mg/d, and represented 34.3% of total zinc intake. In 15.4%

of the population zinc intake was insufficient, i.e. below 2/3 of the recommended nutrient intake (RNI) with age- and gender- specific values according to the Chinese DRI. The estimated amount of absorbed zinc was 3.6 mg/d, with 16.2% of the population showing inadequate amounts of absorbed zinc. None of the subjects consumed zinc at higher intakes than the cut-off of the tolerable upper intake level (Table 3).

Table 3 Estimated zinc intake from a diet with normal rice and biofortified rice

	Normal rice	Biofortified rice	
		Intermediate level	High level
Zinc content in rice (mg/100g)	1.7	2.7	3.8
Zinc intake from rice (mg/d)	4.1 ±2.7	6.5 ±4.3†	9.2 ±6.1†
% of total zinc intake	34.3	45.4	53.9
% of insufficient zinc intake	15.4	6.5‡	4.4‡
Estimated absorbed zinc intake	3.6 ±1.2	4.4 ±1.4†	5.2 ±1.7†
% of absorbed zinc inadequacy	16.2	7.3‡	5.2‡

Insufficiency of zinc intake was determined as below 2/3 of the recommended nutrient intake (RNI) with age- and gender- specific values according to the Chinese DRIs. Absorbed zinc intake was estimated according to IZiNCG suggested, using zinc absorption of 26% for men and 34% for women with a mixed diet. Absorbed zinc inadequacy was defined as absorbed zinc intake lower than 2.69 mg/d for men and 1.86 mg/d for women.

†Paired t-test with normal rice, P<0.001.

‡ Chi-square test compared with normal rice, P<0.001.

Simulated zinc intake at the two levels of zinc from biofortified rice were significantly higher than that from normal rice, with 6.5 and 9.2 mg/d for the intermediate and high zinc level (P<0.001), respectively. The prevalence of insufficient zinc intake decreased to 6.5% and 4.4% for the intermediate and high level of biofortification (P<0.001), respectively. Estimated absorbed zinc intake improved accordingly, with 7.3% and 5.2% of subjects remaining with an inadequate intake of absorbable zinc, respectively. One out of 2819 (0.03%) participants had an excessive intake of zinc with a diet including the intermediate biofortification level, and four out of 2819 (0.1%) with the high biofortification level (Table 3).

Table 4 Dietary zinc intake and prevalence of insufficiency dietary zinc intake (%) with normal rice and biofortified rice in each of the dietary patterns

Dietary patterns		Energy (1000kcal/d)	Rice (g/d)	Normal rice (1.7 mg/ 100 g)		Biofortified rice (2.7 mg/100 g)		Biofortified rice (3.8 mg/ 100 g)	
				Zinc intake	%	Zinc intake	%	Zinc intake	%
"Traditional"	Q1	2.5 (0.7)	111.2 (97.9)	12.1 (4.0)	18.4	13.1 (4.2)	11.5	14.3 (4.7)	9.3
	Q2	2.2 (0.6)	215.1 (109.2)	11.0 (3.6)	23.3	13.2 (4.1)	10.9	15.5 (5.2)	7.1
	Q3	2.2 (0.6)	309.0 (104.0)	11.9 (3.3)	11.3	15.0 (3.5)	2.1	18.4 (4.1)	0.4
	Q4	2.5 (0.7)	367.0 (122.2)	13.1 (3.4)	8.4	16.6 (4.0)	1.6	20.4 (4.7)	0.7
P for trend		< 0.001	< 0.001	0.003	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
"Macho"	Q1	2.3 (0.7)	247.6 (12.4)	11.6 (3.7)	18.8	14.0 (4.1)	7.6	16.7 (5.1)	5.1
	Q2	2.3 (0.6)	152.9 (143.6)	11.7 (3.4)	14.4	14.2 (3.9)	6.7	16.9 (5.0)	4.0
	Q3	2.3 (0.7)	255.2 (138.0)	12.1 (3.8)	16.3	14.6 (4.3)	7.5	17.3 (5.4)	5.7
	Q4	2.5 (0.7)	244.6 (137.8)	12.7 (3.9)	12.0	15.1 (4.4)	4.3	17.7 (5.5)	2.9
P for trend		< 0.001	0.51	0.401	0.002	0.968	0.04	0.565	0.05
"Sweet tooth"	Q1	2.7 (0.7)	258.1 (166.8)	13.5 (4.0)	6.8	15.9 (4.4)	2.8	18.7 (5.4)	2.1
	Q2	2.4 (0.7)	261.8 (158.2)	12.0 (3.7)	15.5	14.5 (4.1)	6.4	17.3 (5.2)	4.3
	Q3	2.2 (0.6)	255.3 (138.4)	11.6 (3.3)	17.8	14.0 (3.9)	7.1	16.7 (5.0)	4.5
	Q4	2.1 (0.6)	225.0 (111.0)	11.0 (3.4)	21.4	13.3 (3.9)	9.8	15.9 (5.1)	6.7
P for trend		< 0.001	< 0.001	0.037	< 0.001	< 0.001	< 0.001	< 0.001	0.001

Table 4 Dietary zinc intake and prevalence of insufficiency dietary zinc intake (%) with normal rice and biofortified rice in each of the dietary patterns

Dietary patterns		Energy (1000kcal/d)	Rice (g/d)	Normal rice (1.7 mg/ 100 g)		Biofortified rice (2.7 mg/100 g)		Biofortified rice (3.8 mg/ 100 g)	
				Zinc intake	%	Zinc intake	%	Zinc intake	%
“Healthy”	Q1	2.3 (0.7)	290.2 (131.7)	11.9 (3.7)	16.9	14.5 (4.2)	6.6	17.4 (5.1)	4.9
	Q2	2.3 (0.6)	270.1 (151.5)	12.0 (3.5)	14.8	14.5 (4.0)	6.0	17.2 (5.1)	3.7
	Q3	2.4 (0.7)	226.0 (148.5)	11.8 (3.6)	16.2	14.1 (4.2)	8.3	16.7 (5.5)	5.5
	Q4	2.5 (0.7)	213.9 (137.6)	12.5 (4.0)	13.6	14.7 (4.4)	5.2	17.3 (5.3)	3.4
P for trend		< 0.001	< 0.001	0.821	0.65	0.021	0.13	0.001	0.16

Data are presented by mean (SD) or percentage. Q1 is the lowest and Q4 the highest quartiles of each dietary patterns. Insufficiency of zinc intake was determined as below 2/3 of the recommended nutrient intake (RNI) with age- and gender- specific values according to the Chinese DRIs and was analyzed by chi-square test over dietary patterns. Energy and rice intake was analyzed by ANOVA. Dietary zinc intake over dietary patterns was analyzed by linear regression, adjusted for household, age, gender, resident, region, SES, education and energy intake.

Energy intake decreased with the “sweet tooth” pattern, and increased with the other three patterns (Table 4). Daily rice intake was highest in the highest quartile of the “traditional” pattern, and decreased with the “sweet tooth” and “healthy” patterns. Average zinc intake increased with the “traditional” and decreased with the “sweet tooth” pattern. The prevalence of insufficient zinc intake was lower with the “traditional” and “macho” patterns, and higher with the “sweet tooth” pattern. No association was found between the “healthy” pattern and prevalence of zinc insufficiency (Table 4).

When replacing normal rice with biofortified rice, the largest increases in dietary zinc intake were seen in the highest quartile of the “traditional” pattern (+3.5 mg/d and +7.3 mg/d for biofortification at intermediate and high level, respectively). The prevalence of dietary zinc insufficiency after biofortification decreased for all of the dietary patterns. For the ‘traditional’ pattern, the prevalence of insufficient zinc intake decreased to only 0.7% in the highest quartile of the “traditional” pattern after simulating zinc intake with biofortified rice at the highest zinc level.

Discussion

In our study, simulated zinc intake from biofortified rice at two levels of zinc resulted in an increase in total zinc intake and a decrease in the prevalence of insufficient zinc intake in an adult population. Rice was the most important staple food in our study population, with 95% of subjects eating rice daily. Only 0.1% of the participants would have excessive zinc intake with rice biofortified at the highest level (3.8 mg/100g).

There are some limitations in the present study that should be mentioned. Firstly, day-to-day variation is the main random error of dietary recall, which we have minimized by using 3-day 24 hour dietary recalls, including two weekdays and one weekend day, in addition to a validated FFQ conducted by well-trained interviewers. Nevertheless, over- or underreporting may have occurred resulting in misclassification, thereby weakening the associations under study. Secondly, since data on dietary phytate was not available, we could not calculate the exact bioavailability of zinc for each individual. Instead, we have assumed that the mixed diets of people in Jiangsu Province are inhibitory for zinc absorption at the intermediate level based on the suggestions by IZiNCG[9]. When estimating intake of absorbable zinc, the adequacy of absorbed zinc intake was very similar to insufficiency of total zinc intake. Moreover, we used a cut-off of 2/3 Chinese RNI for assessment of insufficient zinc intake. This cut-off corresponds well with the IZiNCG EAR at the age of 18-49 years[9]. Thirdly, a common limitation for factor analysis is arbitrary decisions in determining the number of factors to retain and in labeling food patterns[20, 30]. Also, a causal relationship between dietary pattern and insufficient zinc intake cannot be derived from this cross-sectional study.

The use of simulated zinc intake in the design of programs for micronutrient fortification or biofortificaiton is limited. Consistent with our report, a Mexican study showed that simulated biofortification of maize and beans with additional amounts of zinc resulted in a significantly decreased prevalence of inadequate absorbed zinc intake[18]. Subjects in our study consumed 12 mg/d of total dietary zinc and 3.6 mg/d of estimated absorbed zinc, and the prevalence of insufficient zinc intake and absorbed zinc inadequacy was around 15-16%. Mexican women, however, only consumed 1.68 mg/d of absorbed zinc, and the prevalence of absorbed zinc inadequacy was between 40-50%[18]. Like our study, Arsenault et al.[17] also reported the effect of simulated increases in the zinc content of rice on improvements in total dietary zinc adequacy in rural Bangladeshi children and women In the women, mean intakes of total zinc and absorbed zinc after simulation were 5.5 and 1.3 mg/d, with a prevalence of absorbed zinc inadequacy of 76-99.8%, compared with 100% at baseline[17]. The discrepancies between studies show that the effect of biofortification depends on the magnitude of zinc deficiency in a specific population. Moreover, differences in the simulated amount of zinc from biofortification may also result in different effects. In the Bangladeshi population, an additional 0.8 mg of zinc/ 100 g of rice (raw weight, with with unknown baseline zinc concentration) was added[17], whereas we added 1.0 mg/100 g and 2.1 mg/100g in our simulations. The modest increment of zinc in rice may have contributed to the remaining high prevalence of absorbed zinc inadequacy in the Bangladeshi study.

Biofortification of rice provides a potential approach to improve zinc intake in populations with rice as a staple food, because it does not require a change in the choice of staple foods. Since the phytate/zinc molar ratio is lower than 15 in rice in China[27], it can be expected that zinc from rice is well absorbed. For this study, we have used two levels of biofortification: 2.7 mg/100g and 3.8 mg/100 g. The latter level was derived from biofortified rice that has been grown at Wageningen University on Zn-fortified solution culture medium in a greenhouse (unpublished results). It still needs to be shown whether this high level of biofortification can also be reached under field conditions, although there is some evidence[28]. Since a biofortified staple food is more difficult to adjust to the specific needs of populations than commercial fortified foods[12], it is very important to set efficacious target breeding levels. According to our simulations, the largest reduction in low insufficient zinc intake prevalence was achieved with the intermediate level of zinc. Therefore, a level of 2.7 mg/100g may be set as preliminary goal for the first phase of development of biofortified rice, which is very close to the target zinc content in rice (2.8 mg100g) that has recently been recommended by HarvestPlus[28].

With respect to dietary patterns, the positive effect of biofortification on dietary zinc was much more significant in the highest quartile of the ‘traditional’ pattern, which can be attributed to the higher intake of rice in this population segment. In contrast, there was an inverse trend between zinc intake and the ‘sweet tooth’ pattern. High intake of beverages, milk and cake may contribute to poor zinc intake. Zinc concentrations have been reported to be low in distilled drinks and milk[9, 31]. Moreover, calcium is abundant in milk, which may have an inhibitory effect on zinc absorption[32].

In conclusion, we found that zinc biofortifed rice improves dietary zinc intake and lowers the risk for insufficient zinc intake, especially for subjects with a “traditional” dietary pattern among adults in Jiangsu Province, where rice is the main staple food. The positive effect is attenuated with a “sweet tooth” dietary pattern due to the lower intake of rice. Biofortification of rice offers a promising opportunity to reduce zinc deficiency in China, in addition to other diet-based strategies such as dietary diversification and health education. Further studies should focus on the breeding level of Zn that can actually be achieved in rice when grown under various field conditions in China, as well as its cost-effectiveness. Furthermore, the absorption of zinc from zinc biofortified rice should be assessed.

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Chapter 6

Association of dietary pattern and body mass index with blood pressure in Jiangsu province, China

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Abstract

Objectives: To identify risk factors, associations between dietary patterns, body mass index (BMI), and hypertension in a Chinese population were investigated.

Methods: Habitual dietary intake was assessed in 2518 adults by a 3-day 24h recall. Salt and oil intakes were measured by weighing records. Four dietary patterns were identified using principal component analysis. Obesity was determined according to the Chinese BMI categories. High blood pressure was defined as systolic blood pressure ≥ 140 mmHg and/or diastolic blood pressure ≥ 90 mmHg. Prevalence ratios (PR) were calculated using Poisson regression.

Results: Of the subjects, 26.7% had high blood pressure. Subjects with overweight and obesity were more likely to have high blood pressure than those with normal weight (PR, 95% CI: 1.60, 1.40-1.87; 2.45, 2.11-2.85, respectively). About 25% of subjects consumed salt below 6 g/d, and blood pressure increased with salt intake ($P_{\text{for trend}} < 0.01$). A “traditional” dietary pattern was positively associated with high blood pressure ($P_{\text{for trend}} = 0.001$). Subjects consuming “Macho” and “sweet tooth” dietary patterns were less likely to be high blood pressure ($P_{\text{for trend}} = 0.004$ and $P_{\text{for trend}} < 0.001$, respectively).

Conclusion: A traditional dietary pattern is associated with to high blood pressure among the population of Jiangsu Province, which may be mainly due to high salt intake. Moreover, high BMI is an important determinant of high blood pressure. Both issues need to be addressed by lifestyle interventions.

Introduction

Hypertension has been identified to be the first leading risk factor of mortality and the third leading risk factor of the total burden of disease globally[1]. It was estimated that a quarter of the world's adults had hypertension in 2000, and that the proportion will increase to 29% by 2025[2]. Hypertension contributes to premature death and disability from cardiovascular diseases and stroke; peripheral vascular disease; and kidney failure[3]. In China, the prevalence of hypertension in the adult population has quadrupled from 5% in 1959 to nearly 19% in 2002[4]. In addition, awareness of hypertension is poor. According to data from the China National Nutrition and Health Survey of 2002, less than one quarter of the hypertensive population are aware of having hypertension, and only one quarter is adequately treated and controlled [5].

Many studies have indicated that body mass index is generally positively associated with blood pressure[6]. Overweight and obesity is an emerging epidemic in China, with almost 30% of the adult population being classified as either overweight or obese[7]. The high prevalence of hypertension as well as of overweight and obesity in China can be attributed to the recent economic development and urbanization accompanied by unfavorable changes in diet and lifestyle.

Multiple dietary factors affect blood pressure. A large-scale intervention study on Dietary Approaches to Stop Hypertension (DASH) revealed that a dietary pattern rich in fruits and vegetables, rich in low-fat dairy products, and reduced in saturated fat and cholesterol reduces the risk of hypertension[8]. Furthermore, dietary sodium reduction, regular aerobic physical activity, and moderation of alcohol consumption do help to maintain normal blood pressure[9]. Hypertension has been reported in relation to some western and South-East Asian dietary patterns[10-12]. A cohort study from Shanghai showed that high consumption of fruit and milk was inversely associated with blood pressure among middle-aged and elderly men[13]. Wang et al. indicated that a typical traditional Southern dietary pattern, characterized by high intakes of fruit, pork, poultry, rice, vegetables, aquatic products and nuts, was inversely related with hypertension independent from body mass index[14].

Four distinct dietary patterns have previously been identified for the population of Jiangsu Province, China[15, 16]. In this study, we aimed to investigate the associations between the dietary patterns, body mass index, and hypertension in the Chinese population.

Materials and methods

Sample

The study was conducted in Jiangsu Province using a multistage cluster sampling method, as described before[16, 17], which was part of the 2002 National representative cross-sectional survey in nutrition and health. Six counties and two prefectures represented a geographically and economically diverse population for Jiangsu Province. From each of the six areas, three streets/towns were randomly selected. In each street/town, two villages/neighbourhoods were further randomly selected. In each village/neighbourhood, thirty households were randomly selected. All members in the households were invited to take part in the study. Written consent was obtained from all the participants, and adults aged 20 years and above in our study. Those already diagnosed with hypertension, dyslipidemia, stroke and cardiovascular diseases were excluded from the study, because they may have changed their dietary habits. Among 2832 subjects, 311 had already been diagnosed with hypertension (11.0%; males: 11.7%, females: 10.4%). In total, 2518 subjects with 1146 males and 1372 females were included in the data analysis.

Dietary intake measurement

Trained interviewers from the local Center for Disease Control and Prevention visited subjects in their homes to collect the information on food intake using a 24-h dietary recall method on three consecutive days, including two weekdays and one weekend day. Energy and nutrient intake was calculated using the data of the dietary recall in conjunction with the China Food Composition Table published in 2002[18].

Salt and other condiments which contributed to salt intake, such as soy sauce, as well as cooking oil were weighed at the beginning and end of a 3-day household visit. The household salt and oil consumption was calculated as the difference between the two weights. Individual salt and oil intake was estimated based on the proportion of each household member's food consumption, and categorized as quartiles.

Dietary pattern

A validated food frequency questionnaire (FFQ) was used to collect dietary information over the previous year[19]. The FFQ included a series of detailed questions regarding the usual frequency and quantity of intake of thirty-three foods and beverages. This was further merged into twenty-five food items in the analysis because of the low intake of some food items. Portion size for each food was established by reference to food models. Subjects were asked to recall the frequency of consumption of individual food items (number of times per day, per week, per month, per year) and the estimated portion size, using local weight units (1 liang =50 g) or natural units (cups). Intakes of foods were converted into g/week for data analysis. Use of vitamin and mineral supplements was included in the questionnaire, but because these were very seldom used in the area, they were not included in this analysis.

Dietary patterns were identified by factor analysis, using standard principal component analysis as described before for this population[16]. Four different patterns were defined: 1) the "traditional" pattern (characterized primarily by consumption of rice and fresh vegetables; secondary of pork and fish; and lastly of root vegetable and wheat flour); 2) the

"Macho" pattern (characterized primarily by consumption of animal foods and alcohol; and secondary of eggs, fish, nuts, and fruits); 3) the "sweet tooth" pattern (characterized primarily by consumption of cake, milk, yoghurt and drinks; secondary of animal foods, nuts and fruits; and lastly of pickled vegetables and alcohol); and finally 4) the "healthy" pattern (characterized primarily by consumption of whole grains, fruits, pickled vegetables, and secondary by fresh vegetables, milk, eggs and fish). The four factors explained 30.5% of the total variance in intake (10.6 %, 8.6%, 5.9% and 5.4% for "traditional", "Macho", "sweet tooth", and "healthy" patterns, respectively). Scores for each pattern were calculated as the sum of the products of the factor loading coefficient and the standardized weekly intake of each food associated with that pattern. Only foods with factor loadings of more than 0.20 or less than -0.20 were included in the calculation of pattern scores because these items represent the foods most strongly related to the identified factor. Factor scores were divided into quartiles. The scores (intakes) increased from quartile 1 (Q1) to quartile 4 (Q4).

Anthropometric measurement

Weight was measured in light indoor clothing without shoes to the nearest 10th of a kilogram. Height was measured without shoes to the nearest 10th of a centimeter with a stadiometer. Waist circumference was measured midway between the inferior margin of the last rib and the crest of the ilium, in the mid-axillary line in a horizontal plane. All measurements were measured twice during the visit by trained observers using a standard protocol and techniques[20]. Body mass index (BMI) was calculated as weight in kilograms divided by height in squared meters. Subjects were classified by BMI categories as underweight (BMI < 18.5), normal weight (BMI $> 18.5 < 24$), overweight (BMI $\geq 24 < 28$) and obese (BMI ≥ 28) according to the Chinese standards[21].

Blood pressure measurement

Blood pressure was measured twice on the right arm by trained investigators with the participants in a seated position after 5 minutes of rest, using a standard mercury sphygmomanometer and appropriate-sized cuff according to a standard protocol[22]. The mean of those 2 measurements was used for analyses, with the coefficient of variation of 1.28% and 1.78% for systolic and diastolic blood pressure, respectively. High blood pressure was defined as systolic blood pressure $\geq$ 140 mmHg and/or diastolic blood pressure $\geq$ 90 mmHg.

Physical activity

Information on physical activity was collected using a validated physical activity questionnaire covering a time period of 1 year[23]. Questions on daily commuting to and from work were categorized into three categories: (1) using motorized transportation (0 min of walking or cycling); (2) walking or cycling 1-29 min; (3) walking or cycling for >30 min. Daily leisure-time physical activity was classified into 0; 1-29; $\geq$ 30 min.

Alcohol use and socio-economic status

Alcohol use was assessed by asking the participants about the frequency and amount of alcohol/beer intake, which was categorized into three (categories <0, 0-9, and ≥ 10 g/d). Low socio-economic status (SES) was defined as an income of less than 1999 Yuan, "medium" as 2000–4999 Yuan and "high" as more than 5000 Yuan.

Statistical analysis

Variables were presented as percentage or mean ± standard deviations (SD). T-test, ANOVA and chi-square test was used to determine subgroup differences for continuous and qualitative variables, respectively. Poisson analysis was conducted to analyze association between BMI, salt intake, dietary pattern and high blood pressure, and was performed in SAS 9.2. All other analyses were performed using SPSS 13.0 (SPSS Inc., Chicago, IL, USA). Statistical significance was set at $\alpha = 0.05$.

Results

The mean age of the subjects was 47.0 ± 14.5 years old, and 26.7% had high blood pressure. Older subjects had a higher prevalence of high blood pressure than younger subjects ($P_{for\ trend}$ <0.001). The prevalence was lower in subjects reporting more active commuting activities ($P_{for\ trend}$ <0.001), and increased with duration of leisure time activities ($P_{for\ trend}$ <0.001). Compared with never drinkers, alcohol drinkers had a higher prevalence of high blood pressure ($P_{for\ trend}$ <0.001). The prevalence increased with salt intake ($P_{for\ trend}$ <0.001) and BMI categories ($P_{for\ trend}$ <0.001). No difference was found in gender, SES and potassium intake (Table 1).

Table 1 Subject characteristics by blood pressure status

		Blood pressure		P
		Normal	High	
N		1845	673	
gender	Male	831 (72.5)	315 (27.5)	0.43
	Female	1014 (73.9)	358 (26.1)	
Age	20-29	270 (96.4)	10 (3.6)	< 0.001
	30-39	459 (90.0)	51 (10.0)	
	40-49	423 (77.3)	124 (22.7)	
	50-59	292 (20.5)	191 (39.5)	
	60+	401 (57.4)	297(42.6)	
SES	Low	570 (72.5)	216 (27.5)	0.31
	Middle	583 (72.2)	225 (27.8)	
	High	672 (74.7)	228(25.3)	
Active commuting	None	628 (65.9)	325 (34.1)	< 0.001
	1-30 min/d	966 (78.3)	(21.7)	
	>30min /d	251 (75.8)	80 (24.2)	
Leisure time activity	None	1704 (74.7)	577 (25.3)	< 0.001
	1-30 min/d	70 (61.9)	43 (38.1)	
	>30min /d	71 (57.3)	53 (42.7)	
Alcohol drinking	Never	1590 (74.2)	552 (25.8)	0.005
	Low	142 (70.0)	61 (30.0)	
	High	113 (65.3)	60 (34.7)	
Salt intake	< 6	474 (75.5)	154 (24.5)	< 0.003
	6-9	428 (75.4)	140 (24.6)	
	9-14	523 (74.6)	178 (25.4)	
	≥14	420 (67.6)	201 (32.4)	
Potassium intake	< 1.28	478 (74.7)	162 (25.3)	0.57
	1.28-1.56	463 (74.0)	163 (26.0)	
	1.56-1.94	437 (69.8)	189 (30.2)	
	≥1.94	467 (74.6)	159 (25.4)	
BMI	<18.5	110 (85.9)	18 (14.1)	<0.001
	18.5-24	1134 (81.0)	266 (19.0)	
	24-28	485 (67.1)	238 (32.9)	
	≥28	116 (43.4)	151 (56.6)	

Data are presented as N (%). Differences between groups were analyzed by chi-square test.
High blood pressure is defined as SBP≥140 mmHg and /or DBP≤90 mmHg.

The prevalence of overweight and obesity was 28.8% and 10.6% among subjects, respectively. Subjects with overweight and obesity were more likely to have high blood pressure than those with normal weight (PR: 1.60, 95% CI: 1.40-1.87; PR: 2.45, 95% CI: 2.11-2.85, respectively), after adjustment for household, age, gender, SES, salt and potassium intake, physical activity and alcohol use (Table 2).

Table 2 Prevalence ratios (95% CI) of high blood pressure among BMI categories

BMI	Model 1	Model 2	Model 3
Underweight	0.74 (0.48-1.15)	0.68 (0.44-1.03)	0.71 (0.46-1.08)
Normal	1	1	1
Overweight	1.73 (1.49-2.01)	1.60 (1.38-1.85)	1.60 (1.40-1.87)
Obesity	2.98 (2.56-3.46)	2.46 (2.12-2.85)	2.45(2.11-2.85)
P for trend	<0.001	<0.001	<0.001

Model 1 crude model

Model 2 adjusted by household, age and gender.

Model 3 additionally adjusted by SES, salt intake, potassium intake, alcohol use and physical activity.

The average salt intake was 11.4 ± 9.6g/d, and there was a positive association between salt intake and high blood pressure ($P_{for\ trend} < 0.01$), independent of household, age, gender, SES, potassium intake, physical activity, BMI and alcohol use (Table 3).

Table 3 Prevalence ratios (95% CI) of high blood pressure among salt intake categories

Salt intake （g/d）	Model 1	Model 2	Model 3
< 6	1	1	1
6-9	1.00 (0.82-1.23)	1.01 (0.84-1.22)	0.98 (0.81-1.17)
9-14	1.03 (0.86-1.25)	1.06 (0.89-1.27)	1.00 (0.84-1.18)
≥14	1.32 (1.10-1.58)	1.32 (1.10-1.56)	1.21 (1.02-1.43)
P for trend	0.003	0.003	0.01

Model 1 crude model

Model 2 adjusted by household, age and gender.

Model 3additional adjusted by SES, BMI, potassium intake, alcohol use and physical activity.

Salt intake increased over quartiles of the “traditional” pattern, and decreased over quartiles of the “sweet tooth” pattern. Fresh vegetable intake was highest in the highest quartile of the “traditional” pattern. Potassium and energy intake increased over quartiles of the “traditional”, “Macho” and “healthy” patterns, and decreased over the “sweet tooth” pattern (Table 4).

Table 5 shows that the prevalence of high blood pressure increased over quartiles of the “traditional” dietary pattern ($P_{for\ linear\ trend}=0.006$), and decreased over quartiles of the “Macho” dietary pattern ($P_{for\ linear\ trend}=0.02$) and the “sweet tooth” dietary pattern ($P_{for\ linear\ trend} <0.001$). There was no trend in the prevalence over quartiles of the “healthy” dietary pattern. The trends remained similar after adjustment for household, age, gender, SES, physical activity, BMI and energy intake.

Table 4 Food and nutrient intakes related to high blood pressure in the lowest and highest quartiles of dietary patterns

	"Traditional"		"Macho"		"Sweet tooth"		"Healthy"	
	Q1	Q4	Q1	Q4	Q1	Q4	Q1	Q4
Salt (g/d)	11.4±8.9	12.3±11.9*	11.2±9.2	11.3±9.1	13.7±11.8	9.4±6.6*	10.9±9.1	11.5±9.5
Meat (g/d)	42.9±57.9	113.7±89.2*	54.0±62.8	119.9±93.0*	74.9±86.5	110.3±81.9*	103.1±79.4	85.1±87.2*
Fresh vegetable (g/d)	256.5±173.6	326.7±144.5*	288.4±177.2	282.8±158.7	302.9±167.3	251.4±127.6*	285.1±142.5	285.9±169.6
Oil (g/d)	44.7±29.1	43.7±28.5*	40.7±28.7	42.5±27.5	48.2±30.4	38.2±23.2*	37.6±27.1	44.8±28.7*
K (g/d)	1.8±0.6	1.8±0.7*	1.6±0.6	1.7±0.6*	1.8±0.6	1.6±0.5*	1.6±0.6	1.8±0.7*
Energy (KJ/d)	2510.2±734.9	2505.7±669.6*	2306.0±675.0	2476.8±718.1*	2677.3±688.5	2111.7±599.1*	2273.5±654.5	2465.8±731.5*

*P<0.05 over quartiles analyzed by ANOVA.

Table 5 Prevalence ratios (PRs) of high blood pressure among dietary patterns

Dietary pattern	%	Model 1	Model 2	Model 3
"Traditional"				
Q1	21.1	1	1	1
Q2	28.2	1.34 (1.09-1.65)	1.37(1.10-1.71)	1.30 (1.05-1.61)
Q3	28.0	1.33 (1.09-1.62)	1.37(1.10-1.71)	1.51(1.21-1.88)
Q4	28.7	1.36 (1.11-1.66)	1.39(1.12-1.71)	1.47(1.18-1.82)
P for trend		0.006	0.007	0.001
"Macho"				
Q1	29.9	1	1	1
Q2	27.0	0.90 (0.76-1.08)	0.90 (0.76-1.07)	0.92(0.77-1.09)
Q3	25.8	0.86 (0.72-1.03)	0.85 (0.71-1.02)	0.82(0.69-0.98)
Q4	24.1	0.81 (0.67-0.97)	0.80(0.66-0.96)	0.78(0.65-0.94)
P for trend		0.02	0.02	0.004
"Sweet tooth"				
Q1	31.8	1		
Q2	27.5	0.86 (0.73-1.02)	0.84 (0.71-1.00)	0.86 (0.73-1.02)
Q3	24.0	0.76 (0.63-0.91)	0.72 (0.60-0.86)	0.75 (0.62-0.90)
Q4	23.4	0.74 (0.61-0.89)	0.67(0.55-0.82)	0.71 (0.58-0.86)
P for trend		0.0004	< 0.0001	0.0001
"Healthy"				
Q1	26.5	1	1	1
Q2	26.1	0.98(0.82-1.18)	0.99(0.82-1.19)	0.92(0.77-1.10)
Q3	25.7	0.97(0.80-1.17)	0.97(0.81-1.17)	0.88(0.74-1.06)
Q4	28.6	1.08(0.90-1.29)	1.09(0.91-1.30)	0.89(0.74-1.07)
P for trend		0.45	0.19	0.19

Q1 is the lowest quartile, Q4 is the highest quartile.

Model 1 crude model

Model 2 adjusted by household, age and gender.

Model 3 additionally adjusted by SES, BMI, physical activity and energy intake.

Discussion

Our study shows that overweight and obese subjects are more likely to have high blood pressure, also after adjustment for possible confounders. Moreover, salt intake is a determinant of blood pressure. The prevalence of high blood pressure was positively associated with the "traditional" pattern, and inversely with the "Macho" and "sweet tooth" patterns.

A main strength of the study is that we used dietary weighing in combination with a 3-day 24-h recall in the study which provided relatively accurate estimation of salt intake. As a cross-sectional study, the main limitation is that we have not established a causal relationship between dietary patterns and high blood pressure. However, we excluded subjects with diagnosed hypertension and related diseases to avoid possible dietary change following clinician's suggestions and thereby maintaining a natural association between dietary intake and blood pressure in the study population. Misclassification may have occurred, although we used a validated food frequency questionnaire in the survey.

In our study, BMI was directly associated with blood pressure. Maintaining a normal body weight (BMI 18.5-24.9 kg/m^2) is recommended for prevention and management of hypertension[9]. Two meta-analyses of randomized controlled trials showed that weight loss contributed to a reduction in both systolic and diastolic blood pressure[6, 24]. It has been estimated that weight loss of 10 kg can reduce 5-20 mmHg of systolic blood pressure[9]. Obesity is associated with hyperleptinemia by secreting several immunomodulators and bioactive molecules from adipose tissue[25, 26]. Leptin, which helps to maintain blood volume and pressure homeostasis in normal conditions, increases blood pressure through activation of the sympathetic nervous system during chronic hyperleptinemia[26, 27].

Consistent with a Korean study[12], we found a positive association between the "traditional" pattern and high blood pressure, despite a high vegetable intake. As a model of DASH, the Mediterranean Diet is effective in prevention and treatment of hypertension in which abundant amounts of vegetables are usually consumed in the form of salads without cooking[10]. However, eating raw vegetables is not common in many other parts of the world. For example, in Korea, subjects with a traditional dietary pattern had higher blood pressure, which is explained by subjects mostly consuming salted vegetables resulting in high sodium intake[12]. In China, vegetables are usually cooked and then stir-fried with a large amount of oil and salt, which may contribute to the high energy and sodium intake in subjects with a "traditional" dietary pattern in our study. However, the "traditional" pattern in our study in Easter China is not similar to the traditional South-Chinese pattern which was shown to be inversely related to hypertension[14]. The discrepancy may be due to different cooking methods between populations.

Salt intake has been acknowledged as a direct risk factor for hypertension[28]. Salt (sodium chloride) is distributed predominantly to the extracellular space. The rise in extracellular volume by excess amounts of salt intake results in increased cardiac output and rising blood pressure[29]. A meta-analysis of 17 randomized trials showed that modest and long-term reduction of salt intake lowers blood pressure in both hypertensive and normotensive individuals[30]. In addition, dietary sodium reduction is related to decreased blood pressure[31, 32] and reduces the risk of cardiovascular outcomes by 25-30%[33]. WHO recommends a salt intake of no more than 5 g per day[34], however, the majority of our study population consumed salt at amounts higher than the recommendation. The inverse association between "sweet tooth" pattern and hypertension that we found may be due to the relatively low intake of salt in combination with low vegetable and oil intake.

Our study showed that consuming the "Macho" pattern with a high intake of meat can reduce blood pressure, which is inconsistent with other reports[35-37]. A cross-sectional study in Europe showed that meat eaters had a higher prevalence of hypertension than non-meat eaters, especially vegans[35]. An international collaborative cross-sectional study found that a high intake of red meat (103 g/d) results in both higher systolic and diastolic blood pressure[36]. A 10-year follow-up study indicated that red meat intake, but not poultry, was positively associated with the risk of hypertension, compared to those who consumed no red meat[37]. Meat products, particularly red meat, are a major source of saturated fat, animal protein, cholesterol, which may contribute to the development of hypertension[38]. However, other studies reported that a higher intake of meat lowers blood pressure[39, 40]. The effect of animal meat therefore remains uncertain[41].

Whereas physical activity for commuting was inversely related to blood pressure, leisure time activity showed a positive association with blood pressure. This may be explained by age as a major confounder. Older people tended to have more activities in leisure time than younger people (27.7% aged 60 and above, vs 11.1% aged 30 and below).

In conclusion, we found that a “traditional” dietary pattern is associated with high blood pressure, which may mainly be due to a higher intake of salt. The “Macho” and “sweet food” dietary patterns were inversely associated with high blood pressure. Overweight and obesity were also directly associated with blood pressure. Our findings may be generalized to other parts of China and other Asian countries with comparable cooking methods. Public health measures including mass education campaigns with dietary recommendations should be conducted to promote healthy lifestyles including reduction of salt and improved cooking practices in China.

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Chapter 7

Iron status in relation to body mass index and waist circumference among Chinese women

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Abstract

Objectives: To investigate the relationship between iron status and body mass index and waist circumference among women in Jiangsu Province, China.

Methods: Data collected in a cross-sectional survey with 1,537 women aged 20 years and above were included in the analyses. Subjects were classified by body mass index categories as underweight, normal weight, overweight and obese according to the Chinese standard. Central obesity was defined as a waist circumference ≥ 80 cm. Anemia was stratified to iron-deficiency anemia (IDA), the presence of both anemia and iron deficiency, and non-iron deficiency anemia (non-IDA). Prevalence ratios (PRs) of iron deficiency or anemia were calculated using Poisson regression.

Results: The prevalence of iron deficiency and anemia was 15.2% and 31.1%, with 6.3% of IDA and 24.8% of non-IDA. Overweight and obese subjects had a lower chance of having anemia (PR: 0.72, 95% CI: 0.62-0.89; PR: 0.59, 95% CI: 0.43-0.79), and non-IDA (PR: 0.67, 95%CI: 0.54-0.84; PR: 0.60, 95%CI: 0.43-0.83). Subjects with underweight were more likely to suffer from IDA than those with normal weight. Central obesity was inversely associated with anemia and non-IDA.

Conclusion: Overweight/obesity and central obesity was inversely associated with anemia and non-IDA. As it seems, no measures are required currently to target iron status specifically in overweight and obese people in China.

Introduction

Iron deficiency remains one of the most prevalent nutritional disorders in the world, and 50% of the cases of anemia are due to iron deficiency[1]. Globally, anemia affects 1.62 billion people, and most of them are preschool-age children and non-pregnant women[2]. Anemia can increase risk of maternal and child mortality, and iron deficiency anemia can impair cognitive and physical development in children, and endanger physical performance, particularly work productivity, in adults[1, 3]. A National survey in 2002 showed that 20% of preschool children and of non-pregnant women of reproductive age in China were affected by anemia, which can be classified as a moderate public health problem according to WHO[3].

The global obesity epidemic is estimated to affect between 1 and 2 billion people, among them one in five is Chinese[4, 5]. As an economic transition country, China is also undergoing nutrition and lifestyle transition. Increased fat intake, decreased intensity of physical activity, and different modes of transport has contributed to the increase of overweight and obesity in adults, adolescents and children in China[6-9]. Obesity has been recognized as a leading risk factor for chronic diseases which lead to 66% of deaths worldwide[10]. Obesity-related chronic diseases include hypertension, hyperlipidemia, type 2 diabetes, cardiovascular disease, COPD, chronic kidney disease and some cancers[11-14]. China has experienced an alarming increase in these obesity-related chronic diseases over the past decade[15]. It is expected by 2020 that China will have passed the US in the number of obese subjects[6].

Iron deficiency has been reported in some countries to be associated with obesity in children, adolescents[16-20] and adults[21-27]. However, the association between adiposity and iron status is not equivocal for all iron status indicators. Since obesity could add to the burden of iron deficiency in China, we investigated the relationship between obesity with iron deficiency and/or anemia among a female Chinese population in Jiangsu, which was part of the China National Nutrition and Health Survey in 2002.

Method and materials

Study population

The study was conducted in Jiangsu Province using a multistage cluster sampling method, as described before[28, 29], which was part of the National survey on nutrition and health conducted in 2002. Six counties and two prefectures represented a geographically and economically diverse population for Jiangsu Province. From each of the areas, three streets/towns were randomly selected. In each street/town, two villages/neighborhoods were further randomly selected. In each village/neighborhood, thirty households were randomly selected. All household members were invited to take part in the study. Written consent was obtained from all the participants. For the current study, only women aged 20 years and above were included in the data analyses. Altogether, 1,537 out of 1,652 women with complete data were included in the survey. The response rate of the study was 93.0%. The study was approved by the Human Investigation Review Committee at the National Institute for Nutrition and Food Safety, Chinese Center for Disease Control and Prevention.

Data collection

Trained interviewers from the local Centers for Disease Control and Prevention (CDC) visited subjects in their homes to collect information on food intake using a 24-h dietary recall method on three consecutive days, including two weekdays and one weekend day. Energy and nutrient intake was calculated using the data of the dietary recall in conjunction with the China Food Composition Table published in 2002[30].

Socio-economic status (SES) was assessed by the question "What was your family's income per person in 2001?" Low SES was defined as an income of less than 1,999 Yuan, "medium" as 2,000–4,999 Yuan and "high" as more than 5,000 Yuan.

Weight was measured in light indoor clothing without shoes to the nearest 10^{th} of a kilogram. Height was measured without shoes to the nearest 10^{th} of a centimeter with a stadiometer. Waist circumference was measured at 1 cm above the navel at minimal respiration. All measurements were measured twice during the visit by trained observers using a standard protocol and techniques[31]. Body mass index (BMI) was calculated as weight in kilograms divided by height in squared meters. Women were classified by BMI categories as underweight (BMI < 18.5), normal weight (BMI $> 18.5 < 24$), overweight (BMI $\geq 24 < 28$) and obese (BMI ≥ 28) according to the Chinese standard[32]. Central obesity was defined according to guidelines of the International Diabetes Federation for Chinese populations as a waist circumference ≥ 80 cm for women[33].

Laboratory measurements

All participants were requested to provide a fasting venous blood sample, which was preserved and analyzed in the Jiangsu Provincial CDC in Nanjing. Blood sample collection, transportation, storage and testing in standardized laboratories in each province were performed according to a standardized protocol developed by the China CDC. All reagents and blood-sampling tools were provided by the China CDC, as well as the supervision of quality of examination. Hemoglobin (Hb) level was measured by the cyanmethemoglobin method (Dallman, 1984). Serum ferritin was analyzed by the China CDC in Beijing using a commercially available radioimmunoassay kit (Beijing North Institute of Biological Technology). Iron deficiency was defined as a serum ferritin level < 15 g/L. Anemia was defined as a Hb level of below 12 g/dl for this population, including iron-deficiency anemia (IDA), the presence of both anemia and iron deficiency, and non-iron deficiency anemia (non-IDA)[1, 34].

Statistical analysis

Those with incomplete data on anthropometric and blood indices were excluded from this analysis. Variables were presented as percentage or means ± standard deviations (SD) by BMI categories, with chi-square test for categorical variables and ANOVA for continuous variables. Poisson regression was used to estimate the association as prevalence ratios (PRs) between BMI categories and iron deficiency and anemia, respectively, with normal weight as reference and controlling for confounders, including age, residence, socio-economic status, educational level, and daily energy and iron intake. Poisson regression was performed by using SAS (SAS Institute Inc., Cary, NC, USA), all others by SPSS 13.0 (SPSS Inc., Chicago, IL, USA).

Table 1 General information for Chinese women by body mass index categories

	Underweight	Normal weight	Overweight	Obese	P value
n (%)	89 (5.8)	834 (54.3)	424 (27.6)	190(12.4)	
Age (years) (%)					
< 35	38.2	29.5	16.5	9.5	< 0.001
35 to 44	13.5	25.4	21.9	21.6	
45 to 54	13.5	19.9	33.7	26.3	
≥ 55	34.8	25.2	27.8	42.6	
Residence					
Urban city	23.6	22.1	27.4	31.1	0.03
Rural city	76.4	77.9	72.6	68.9	
SES					
Low	26.1	32.4	33.9	34.0	0.39
Medium	38.6	31.0	34.8	33.0	
High	35.2	36.6	31.3	33.0	
Education					
Low	50.6	54.9	59.4	65.3	0.002
Medium	27.0	32.3	30.9	21.1	
High	22.5	12.8	9.7	13.7	
Height (cm)	155.8 (6.8)	155.4 (5.9)	154.9 (5.6)	154.6 (5.67)	0.160
Weight (kg)	42.3 (4.5)	52.3 (5.2)	61.5 (5.1)	72.9 (7.0)	< 0.001
BMI (kg/m^2)	17.3(1.3)	22.0 (1.7)	27.0 (1.4)	32.2 (1.8)	< 0.001
Waist circumference (cm)	65.0 (5.3)	72.4 (6.1)	82.5 (6.4)	93.5 (8.4)	< 0.001
Hemoglobin (g/L)	125.0 (14.6)	124.9 (15.4)	128.4 (15.0)	131.9 (1 5.1)	< 0.001
Serum ferritin	74.3 (77.1)	64.8 (67.1)	72.3 (69.7)	94.0 (86.1)	< 0.001
Energy (kcal/d)	1916.5(562.7)	2124.4(581.7)	2179.31(638.8)	2198.5(637.2)	0.001
Iron (mg/d)	20.0(7.4)	23.4(9.6)	24.2(9.8)	23.8(9.5)	0.003
Zinc (mg/d)	10.0(3.1)	10.9(3.1)	11.2(3.4)	11.1(3.3)	0.016
Vitamin C (mg/d)	60.5(33.4)	61.1(37.9)	59.5(39.1)	59.6(36.3)	0.886
Calcium (mg/d)	359.7(169.5)	385.25(194.5)	393.21(190.6)	407.2(187.6)	0.228

BMI, body mass index; SES, socio-economic status. Chi-square test for categorical variables and ANOVA for continuous variables.

Results

The mean age of the subjects was 46.4 ± 14.7 yrs. The average BMI was 23.6 ± 3.7, with 5.8% being underweight, 27.6% overweight, and 12.4% obese (Table 1). Being overweight or obese increased with age. Overweight and obese women were more present in urban residences (30.5% and 15.5%), and subjects with lower education. No difference in BMI was found between socio-economic status classifications. The average height was 155.8 ± 22.3 cm in women, with no differences among BMI groups. Waist circumference increased over BMI categories. The obese group had the highest concentrations of Hb and serum ferritin, compared with other BMI groups. Overweight and obese groups ingested more energy and iron per day than other BMI groups. Zinc intake was highest in the overweight group, whereas, vitamin C and calcium intake showed no differences among groups.

Table 2 Prevalence of iron deficiency and anemia among Chinese women

		Iron deficiency	Anemia	IDA	Non-IDA
Altogether		15.2	31.1	6.3	24.8
Age (yrs)	<35	26.1	31.8	10.9	20.9
	35-45	21.8	27.7	8.4	19.3
	45-55	12.1	32.1	6.2	25.9
	>=55	3.2	32.5	0.9	31.6
χ^2 test		< 0.001	0.452	< 0.001	< 0.001
Linear trend test		< 0.001	0.517	< 0.001	< 0.001
Residence	Urban	12.6	30.0	7.6	22.4
	Rural	16.0	31.5	5.9	25.6
P value		0.11	0.59	0.22	0.21
Socio-economic status	Low	21.5	21.5	5.4	16.1
	Medium	14.6	35.9	7.4	28.5
	High	9.8	35.4	6.1	29.4
χ^2 test		< 0.001	< 0.001	0.416	< 0.001
Linear trend test		< 0.001	< 0.001	0.689	< 0.001
Education	Illiterate	13.7	31.1	4.8	26.3
	Low	19.0	32.1	8.4	23.7
	More	12.9	28.9	8.2	20.6
χ^2 test		0.023	0.714	0.017	0.207
Linear trend test		0.374	0.757	0.011	0.077
BMI	Underweight	19.1	39.3	12.4	27.0
	Normal	16.2	35.3	6.4	28.9
	Overweight	15.6	25.7	6.6	19.1
	Obese	7.9	21.1	2.6	18.4
χ^2 test		0.022	< 0.001	0.019	<0.001
Linear trend test		0.008	< 0.001	0.015	<0.001
Waist circumference	Normal	16.9	33.9	7.3	26.5
	Central obesity	12.1	26.1	4.3	21.8
χ^2 test		0.011	< 0.001	0.015	0.039

BMI, body mass index; IDA, iron deficiency anemia. Analyzed by chi-square test.

The prevalence of iron deficiency and anemia was 15.2% and 31.1%, respectively. Only 6.3% of the sample had IDA. In contrast, the prevalence of non-IDA was 24.8%. Overall, there was no significant association between age and anemia. However, age was inversely associated with iron deficiency and IDA but positively with non-IDA. There was no difference in the prevalence of iron deficiency, anemia and IDA between urban and rural residence. SES was inversely associated with iron deficiency and, positively associated with anemia and non-IDA, but no association with IDA. Education level was positive with IDA. The prevalences of iron deficiency, anemia, IDA and non-IDA showed a significant decreasing trend with increasing BMI. Central obesity was associated with significantly lower prevalences of iron deficiency, anemia, IDA and non-IDA than normal waist circumference (Table 2).

Table 3 Prevalence ratio (95% CI) from Poisson regression of iron deficiency and anemia as a function of BMI categories

	Adjusted PR	95%CI*
iron deficiency		
Underweight	1.37	0.85-2.19
Normal weight	1.00	
Overweight	1.10	0.85-1.43
Obesity	0.63	0.38-1.07
Anemia		
Underweight	1.05	0.80-1.39
Normal weight	1.00	
Overweight	0.74	0.62-0.89
Obesity	0.59	0.43-0.79
IDA		
Underweight	1.92	1.03-3.58
Normal weight	1.00	
Overweight	1.10	0.71-1.71
Obesity	0.41	0.15-1.14
Non-IDA		
Underweight	0.90	0.63-1.28
Normal weight	1	
Overweight	0.67	0.54-0.84
Obesity	0.60	0.43-0.83

BMI, body mass index; IDA, iron deficiency anemia.

Prevalence ratios (PR) are significant when 95% CIs do not include 1.00.

*adjusted for age, residence, socio-economic status, educational level, daily energy and iron intake.

Compared to normal weight women, overweight and obese women had lower PRs for anemia (PR: 0.72, 95%CI: 0.62-0.89; PR: 0.59, 95%CI: 0.43-0.79) and non-IDA (PR: 0.67, 95%CI: 0.54-0.84; PR: 0.60, 95%CI: 0.43-0.83) (Table 3). Furthermore, obesity showed an inverse association with iron deficiency (PR: 0.63, 95%CI: 0.38-1.07) and IDA (PR: 0.41, 95%CI: 0.15-1.14) but this association did not remain statistically significant after adjustment for confounders. Subjects with underweight were more likely to suffer from IDA (PR: 1.92, 95%CI: 1.03-3.58) than those with normal weight.

Central obesity was inversely associated with anemia (PR: 0.75, 95% CI: 0.63-0.89) and non-IDA (PR: 0.75, 95% CI: 0.62-0.90) in multivariate analysis. However, no association between central obesity and ID or IDA was found (Table 4).

Table 4 Prevalence ratio (95% CI) from Poisson regression of iron deficiency and anemia as a function of waist circumference categories

	Adjusted PR	95%CI*
iron deficiency		
Normal waist circumference	1.00	
Central obesity	0.96	0.74-1.26
Anemia		
Normal waist circumference	1.00	
Central obesity	0.75	0.63-0.89
IDA		
Normal waist circumference	1.00	
Central obesity	0.74	0.46-1.17
Non-IDA		
Normal waist circumference	1.00	
Central obesity	0.75	0.62-0.90

IDA, iron deficiency anemia.

Prevalence ratios (PR) are significant when 95% CIs do not include 1.00.

*adjusted for age, residence, socio-economic status, educational level, daily energy and iron intake.

Discussion

In this cross-sectional study, we found that both overweight/obesity and central obesity were inversely associated with anemia and non-IDA, but not with iron deficiency or IDA. Underweight was positively associated with non-IDA.

Results are inconsistent from different countries on overweight/obesity and iron deficiency or anemia. Eckhardt et al[23] collected data from Mexico, Peru and Egypt, where the prevalence of anemia was between 23-31% among women. Overweight women were at lower risk for anemia than non-overweight women in Egypt and Peru, but not in Mexico. Ausk et al[21] found overweight and obese subjects were less likely to be anemic and had higher serum ferritin levels compared with normal-weight US adults. Also in a US study, high body fat concentration was associated with a reduced prevalence of iron deficiency defined by serum ferritin and transferrin saturation in pre-menopausal women[27]. In addition, serum ferritin concentrations were higher in overweight and obese as compared to normal weight in urban Malian women[24]. However, higher rates of iron deficiency were found in obese Mexican women by using indicators of serum iron and total-iron-binding capacity[22]. A study with a large sample of US adults reported an inverse relationship between serum iron levels and individual BMI[26].

The discrepancies in the iron-obesity findings would be partly due to different indicators for measurement of iron deficiency, different statistical methods and level of dietary iron intake. In our study, iron intake ranged from 20 to 24 mg/d, almost similar to the study in Mali (18 mg/d)[24]. However, in the Mexican population iron intake was reported to be in in the range of 8-9 mg/d[22] and the prevalence of iron deficiency was much higher (50-60%) than in our study (15%). Intake of vitamin C, the most potent enhancer of non-heme iron absorption, was present in sufficient amounts in the diets of Chinese women (60 mg/d), whereas vitamin C intake in Mexican women was low (30 mg/d). It may be that the Chinese diet conveys enough absorbable iron to lower the risk of anemia in contrast to the Mexican diet [23].

Moreover, all of those studies did not take non-IDA anemia into account. Our study showed a significantly higher proportion of non-IDA than IDA[35]. Non-IDA may result in other nutrient deficiencies, such as vitamin A, vitamin B, and chronic inflammatory conditions such as chronic infections, cancer or autoimmune diseases[36, 37], which could partly explain high prevalence of anemia in our population, whereas dietary iron intake was sufficient[28]. For this study, we have used serum ferritin as an indicator of iron status. Ferritin is responsible for the storage of iron, and is responsive to inflammation. Usually for subjects with anemia of chronic disease, serum ferritin levels are normal or increased, due to immune activation, and reflecting increase storage and retention of iron within the reticuloendothelial system[36]. On the other hand, obesity, as a chronic inflammatory condition and a leading risk factor of chronic diseases, would up-regulate the hepcidin expression[38]. Hepcidin can inhibit intestinal absorption of iron, and diminish the amount of bioavailable body iron[39, 40]. Consistent with other studies, we found higher serum ferritin levels in overweight and obese subjects. However, it may be that this is due to inflammation by either chronic or acute diseases, rather than iron status as well as markers of inflammation, would be required to unravel this. We did not find a positive association between body weight and non-IDA, which indicates that overweight or obesity in the population does not decrease red-cell survival or impair erythropoiesis[21].

Our study showed that women who were underweight were approximately twice as likely to have IDA as compared to women of normal weight. This may be due to lower intakes of energy and iron from the diet. Moreover, vitamin B12 or vitamin K intake, which is important in erythropoiesis, might be relatively low in persons with low BMI[21]. Another explanation could be that those with underweight had an underlying disease causing the IDA.

Waist circumference reflects intra-abdominal fat mass, and is related to cardiovascular diseases in adults[41]. In our study, the prevalence of central obesity was 36.2% (not shown in the result part), and women with central obesity were less likely to have anemia and non-IDA, consistent with the result in men reported by Gillum et al[25].

In our study, the inverse association between overweight/obesity and non-IDA was most pronounced in the middle and high SES group (not shown in the result part). Since overweight and obesity was not different among SES groups, other mechanisms, including dietary iron intakes, other nutrient deficiencies or inflammation caused by diseases should be further investigated.

There are some potential limitations to our study. First, we used anemia and serum ferritin as indicators of iron status, which only represents a part of the complex assessment of true iron status. Therefore, we cannot truly distinguish anemia of chronic disease and anemia caused by iron deficiency. Second, serum ferritin, as an acute-phase reactant, may be confounded by the adipose-related inflammation or infectious diseases, chronic diseases. Hence, we may have underestimated iron deficiency anemia due to inflammation in our population. However, a study in the province including 1230 subjects showed that just 2.9% were suffering from inflammation defined by C-reactive protein higher than 10 mg/L (Zuo, H. Personal communication). Inflammation in the population may not disturb our results. Finally, because of its cross-sectional nature, our study cannot determine a causal relationship between body mass index and iron status.

In conclusion, in this study we found an inverse association between overweight/obesity, central obesity and non-iron deficiency anemia, defined as normal serum ferritin and low hemoglobin, in Chinese women from Jiangsu Province. Underweight women were at higher risk for iron deficiency anemia. Our study contributes to the existing knowledge base on the complex association between adiposity and iron status. Inclusion of multiple iron and inflammation markers in future studies could possibly unravel the true meaning of our findings. As it seems, no measures are required currently to target iron status specifically of overweight and obese people in China. Since causes of anemia are multifactorial and related to socio-economic status, additional strategies to iron supplementation should be considered in Chinese population.

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Chapter 8

General discussion

As a consequence of the nutrition transition, non-communicable diseases (NCDs) increasingly coexist with persisting malnutrition in China. In this thesis, dietary zinc intake and zinc status have been assessed in Jiangsu Province, a Chinese Province where zinc deficiency is expected to be present because of its low soil zinc concentration. Biofortified and fortified rice with zinc, as potential strategies to combat zinc deficiency in rice consuming countries, were evaluated for their fractional zinc absorption (FAZ) using intrinsic and extrinsic labeling. Further, the potential effect of zinc biofortified rice on dietary zinc intake was simulated for four distinct dietary patterns that were identified in Jiangsu Province. In addition, these dietary patterns were examined in relation to blood pressure, thereby also taking the presence of obesity into account. Both of these conditions nowadays add importantly to the disease burden in China. Finally, we examined the presence of anemia among school children in Jiangsu Province. Since several studies have previously shown that obesity is positively associated with anemia, we have also looked into that issue.

Main findings

The overall prevalence of insufficient intake of zinc was 22.9%, with a higher prevalence in children (64.6%) and adolescents (64.9%), and in those with low SES (27.3%) (Chapter 2). The prevalence of zinc deficiency measured by serum and hair zinc was 0.7%, and 15.2% in primary school children, respectively, and 32.3% of them had anemia (Chapter 3). In Chapter 4, biofortified ^{70}Zn enriched rice with an intrinsic label was found to have higher FAZ than extrinsically labeled fortified extruded rice. However, the results were not accurate because we could not assess the exact amount of ^{67}Zn isotope infused to subjects because of ^{67}Zn remaining on the wall of the vials. When replacing normal rice with zinc biofortified rice with 2.7 and 3.8 mg/100g of zinc in a simulation analysis, the prevalence of insufficiency of zinc intake decreased from 15.4% to 6.5% and 4.4%, respectively (chapter 5). In chapter 6, we found to our surprise that a “traditional” dietary pattern in Jiangsu Province was most strongly associated with high blood pressure ($P_{for\ trend} = 0.005$). Such pattern is characterized primarily by consumption of rice and fresh vegetables, secondary of pork and fish, and lastly of root vegetables and wheat flour. The association is probably due to high salt intake. Obesity was also positively and independently related to high blood pressure. Chapter 7 showed an inverse association between overweight / obesity and anemia.

Internal validity

Errors associated with dietary assessment

Measurement errors, including systematic and random errors, may occur across all stages of a study. Such errors refer to the sampling of subjects, the methods and time frame of a study, the respondents’ ability to report data, and the investigators’ skills in collecting, coding, computing and interpreting data[1].

The data for the Jiangsu population were derived from a sub-national representative survey in 2002, based on four-stage stratified random sampling considering geographic characteristics and economic development. The sample was considered as representative for the Province by comparison with demographic indexes with the 2000 China National Population Census. The sample for primary school children was selected using cluster sampling from three rural areas with low soil zinc content, but was not checked for representativeness for local school-age children in these areas.

Day-to-day variation is the main random error of dietary recall， and can be minimized by increasing the number of observations. In our study, we took this into account by using 3-day 24-hour dietary recalls including two weekdays and one weekend day. It is important that weekend dietary intake data are included to ensure a better representation of dietary intake[2]. Direct weighing, in combination with 24-h dietary recall, can more accurately estimate salt and oil consumption at the household level. We had trained interviewers to help respondents to remember in detail the food and drink and portion size they consumed previously. We have coded and computed the data based on the China Food Composition Table 2002[3].

The validated FFQ was used to assess usual food consumption patterns over a relatively long time period, and four dietary patterns have been determined, which could explain 30.5% of the total variance in dietary intake. However, over- or under- reporting may have resulted in misclassification, thereby weakening the associations under study.

In conclusion, in view of the limitations of available procedures and reference instruments for adjustment of measurement errors[4], we minimized the errors by using standardized measurement techniques, training personnel, and quality control procedures.

Assessment of zinc deficiency

So far, there is lack of simple, specific and accurate indicators to assess zinc deficiency. We used dietary zinc intake, serum zinc concentration, and stunting prevalence for assessment of zinc status, which have been jointly recommended[5]. In addition, we included hair zinc and anemia as indicators for zinc deficiency. All of these various indicators were used and helped us to assess comprehensively zinc deficiency in the population.

Since phytate content in only some cereal foods has been measured in China[6], and so far no reference method is available to analyze phytate content in foods, we cannot calculate phytate/zinc molar ratio from the common diet consumed by the population. Therefore, insufficiency of zinc intake may have been misclassified by only using total zinc intake, dependent of bioavailability from diets. Another possible limitation is that zinc content of foods in Jiangsu Province with low soil zinc concentration may be lower than the national data presented in the China Food Composition Table 2002[3], so that we may have overestimated zinc intake especially in the rural population because they rely more on local foods.

Serum zinc can be affected by recent meals, inflammation, and stress[7], and may remain within the normal range in the situation of marginal zinc deficiency[8]. In our study, we have included c-reactive protein as an indicator of inflammation, which showed a low prevalence of inflammation (2%). It is known that zinc measurement can be influenced by contamination with zinc from many sources, such as ordinary lab equipment. We have taken precautions to avoid this during sample collection, transportation and analysis according to the international Zinc Nutrition Consultative Group (IZiNCG) guideline[8], and had used certified control sera for quality control. Such measures helped us to reduce bias during laboratory measurement.

Measurement of fractional zinc absorption

We have used the double isotopic tracer ratio (DITR) technique to measure fractional absorption of zinc (FAZ) with relatively simple procedures, for which only a spot urine sample is required at an appropriate time after tracer administration in order to define the response of the orally and intravenously administered tracers[9, 10]. This method has successfully been applied by others, but for us this was the first time for application. We could not foresee that part of stable isotope label remained in the infusion vial by adhesion to the vial wall, due to the neutral pH used for the intravenous (iv) doses. In the meantime, it has been sorted out that by decreasing the pH in the iv doses from 7 to 6 prevents this problem. We were able to measure the amount of zinc remaining in some of the iv vials after they were used in our study, and found that this amount of zinc was rather stable with a coefficient of variation of CV 4.6%. We could correct for this systematic error in our calculations, but not all error could be taken away. Therefore, we could not quantify the FAZ from the three types of rice in the study. In some subjects, FAZ from biofortified rice was higher than 100%, which is biologically impossible. However, the FAZ from biofortified rice was consistently higher than from the other types of rice in all of the 13 subjects. We can conclude that zinc from biofortified rice is well absorbed.

Assessment of iron status

Serum ferritin was measured as an indicator of iron status in our study. However, we did not assess inflammation status for subjects. Serum ferritin may be confounded by obesity-related inflammation, or inflammation due to infection and other diseases. Therefore, we cannot distinguish anemia caused by iron deficiency or by other factors such as infection or inflammation, which would be important to unravel the reasons for high prevalence of anemia in China. Unknown factors such as vitamin B deficiency may also exist in the relationship between obesity and anemia in our study.

External validity

Prevalence of zinc deficiency

We estimated inadequate zinc intake by using 2/3 of the Recommended Nutrition Intakes (RNIs) in a sub-National representative sample. This kind of cut-off has been used before in other National Surveys, such as in South Africa[11], the UK[12, 13], and the US[14, 15]. Nowadays, Estimated Average Requirements (EARs) are suggested for evaluation of insufficient intakes, and for zinc, IZiNCG has developed EARs by life stage and diet type for international use and comparison[16]. The EAR is the median intake in a population indicating that 50% of the subjects meet the RNI[17]. The cut-offs we used (2/3 Chinese RNI) are almost similar to the IZiNCG EARs for subjects aged 18-49 years, somewhat higher for children and adolescents, and lower for males aged 50 and above (Table 1). Despite the discrepancies, we prefer to use the 2/3 of the Chinese RNI as a reference, because it is based on the Chinese population.

Table 8 Comparison of the IZiNCG EARs with a mixed or refined plant-based diet and 2/3 of the Chinese RNI for zinc by age group

EARs			2/3RNIs		
Age group (yrs)	Sex	Female	Age group(yrs)	sex	Female
4-8	M+F	3	4-6	M+F	8
9-13	M+F	5	7-10	M+F	9
			11-13	M	12
				F	10
14-18	M	8	14-18	M	12.7
	F	7		F	10.3
>19	M	10	18-49	M	10
	F	6		F	7.7
			50+	M+F	7.7

We collected non-fasting serum samples from children in the morning, which may be influenced by recent dietary zinc intake and used the recommended cut-off for non-fasting blood samples of 65 μg/dL[18]. However, this cut-off point of zinc deficiency is based on the data of US adults[19], which may not well be applied for Chinese children. In addition, higher serum zinc concentrations were reported for morning blood samples as compared to afternoon samples[8]. Moreover, the prevalence of low hair zinc (15.2%) was higher than that of low serum zinc, and anemia was prevalent (32.2%) in this sample. So, we may have underestimated the prevalence of low serum zinc in the sample. The prevalence of low serum zinc in primary school children in the present study was much lower than sometimes found in other parts of the world, such as North-East Thailand (57%)[20], Mexico (19-24%)[21], and New Zealand (15-21%)[22]. The prevalence was also lower than others have reported in China, such as 34% in children aged 1-6 years[23] and 19% in rural school children aged 7-14 years in 2002[24] in Beijing. A recent study conducted in a rural area in the Southern part of Jiangsu Province showed 38.2% of low serum zinc in children aged 3-5 years[25].

Stunting is not specific for zinc deficiency alone, and can also be attributed to maternal short stature, frequent infections, and other nutritional deficiencies[26]. However, it has still been considered as the best functional indicator for zinc deficiency[27]. We found a prevalence of around 4% of stunting in rural primary school children with stunting measurements conducted according to the WHO Growth Standards published in 2006[28]. This is much lower than we expected beforehand, because we selected our study population from rural areas with low soil zinc content hypothesizing that stunting would be prevalent due to zinc deficiency. The prevalence of stunting in children below 5 years has sharply decreased from 34% in 1992 to 14% in 2000 in China due to economic development and nutrition transition[25]. However, it varies largely with different age groups and areas. In the National Nutrition and Health Survey in 2002, the prevalence of stunting among urban children <5 years was 4.9%, which was lower than among rural children (14.3%)[29]. The prevalence was 16.5% in children aged 5-12 years[30]. A study from 50 counties of 13 mid-western provinces showed 30.2% of stunting in children aged <5 years[31]. The prevalence of stunting presented 6.4% in school children with a mean age of 14 years from three towns in a rural area of Guangdong Province, Southern part of China[32], and 2.9% in children aged 12-14 years from two cities of Jiangsu Province, Eastern part of China[33].

From these assessments, the low prevalence of low serum zinc and stunting indicates that zinc deficiency may not be a large problem of public health significance in the areas where we conducted the research on stunting and serum zinc. However, the situation may be different in other parts of Jiangsu Province.

Zinc absorption

Because the internal validity of the bioavailability study was threatened, we cannot make valid comparisons with other published data. However, FAZ from the control rice (23%) in the study was in the range of 13-38% reported in other studies[34-41]. All of these studies have used an extrinsic labeling isotope, which is different with the intrinsic labeling that we used for the biofortified rice in our study. Compared with the extrinsic technique, an intrinsic isotope is more likely to be incorporated into the plant and to become a natural part of the plant food[42]. As extrinsic labels are frequently added just before the administration of meals, and may not equilibrate well with the native minerals, they are considered to be inferior to predict absolute values for absorption in comparison with intrinsic labels[43, 44]. This may partly explain the consistent discrepancy in FAZ that we found between the biofortified rice and the other two types of rice. Moreover, the phytate/zinc molar ratio in the biofortified rice was lower (7.6) than that in the control rice (23.1), which would predict a higher bioavailability of zinc in the biofortified rice. However, FAZ from the extruded fortified rice was much lower than that from the control rice, although the phytate/zinc molar ratio (8.7 vs 7.6) was not different from the biofortified rice. The lower FAZ may have occurred because fortified zinc was embedded in a very dense and strongly closed matrix with the extruded rice and could not be easily released during digestion. Another explanation for the high FAZ from biofortified rice is that the labeled zinc may have been bound to proteins rather than to phytate, and may be absorbed differently from zinc salts. In conclusion, our study suggests a higher bioavailability of intrinsically labeled zinc from biofortified rice which needs to be further investigated.

It has been reported that simulated increases in the zinc content of rice improves total dietary zinc adequacy in rural Bangladeshi children and women[45]. Our simulation analysis with biofortified rice also shows a significantly increased zinc intake, especially in subjects with the “traditional” pattern. The positive effect of biofortified rice would probably be larger in children and adolescents, provided that they consume enough rice. Because dietary patterns are difficult to assess for this age group, our study has therefore focused on adults.

Dietary pattern and health outcomes

Table 2 Dietary patterns and health outcomes in Jiangsu Population

	Zinc insufficient intake	Anemia	Obesity	High blood pressure
“Traditional”	-	+	N	+
“Macho”	-	N	N	-
“Sweet tooth”	+	+	N	-
“Healthy”	N	-	+	N

+, positive relationship; -, negative relationship, N, no relationship.

Table 2 shows all published[46, 47] and unpublished (this thesis) findings on dietary patterns in the Jiangsu population with related health outcomes. These results are different from what we expected, and seem to be in contrast with our knowledge on nutrition transition concomitant with diseases transition. The positive relationships between the traditional dietary pattern and hypertension, and between the “healthy” dietary pattern and obesity suggests that traditional cooking habits, such as using high amounts of salt and oil, play a role despite the otherwise healthy components of these patterns. Consistent with our study, a study from Korea also showed a positive association between a “traditional” dietary pattern and high blood pressure[48]. The fact that both the “Macho” and the “sweet tooth” dietary patterns did not show an association with Non-Communicable Diseases (NCD) may be explained by the short duration that these patterns have been common practice in China. Nowadays, more and more children and youngsters change their pattern to a more western pattern with more meat and sugar[49]. Therefore, the effect of dietary transition on NCDs in this population may only become emergent in the next decades. In addition, the “sweet tooth” dietary pattern included more sweets and sugar with poor essential elements is associated with poor zinc intake and anemia, and will likely contribute to a double burden of malnutrition in this population. On the other hand, the positive association between the “traditional” pattern and zinc intake suggests that a dietary pattern with cereals, medium meat intake and healthier food preparation methods will be helpful in the prevention of both undernutrition and overnutrition.

Obesity and anemia

In the study, both overweight/obesity and central obesity were inversely associated with anemia. These results are consistent with studies from Peru, Egypt, and the US, but not with a Mexican study[50, 51]. Also in a US study, high body fat concentration was associated with a reduced prevalence of iron deficiency defined by serum ferritin and transferrin saturation in pre-menopausal women[52]. However, higher rates of iron deficiency were found in obese Mexican women by using indicators of serum iron and total-iron-binding capacity[51]. A study with a large sample of US adults reported an inverse relationship between serum iron levels and individual BMI[53]. The current hypothesis is that obesity, as a chronic inflammatory condition and a leading risk factor of chronic diseases, would up-regulate the hepcidin expression[54]. Hepcidin can inhibit intestinal absorption of iron, and diminish the amount of bioavailable body iron[55, 56]. The inverse results in our study may be explained by a relatively high intake of iron from the diet, as well as high vitamin C intake which enhances iron absorption. In the Mexican study, iron and vitamin C intake were 8-9 and 30 mg/d, whereas this was 20-30 and 60 mg/d in our study. Moreover, vitamin B12 and vitamin K intake were also high, which are important vitamins for erythropoiesis[50].

In conclusion, we found that especially children and adolescents had low dietary zinc intake, in Jiangsu Province, where the soil is deficient in zinc[57]. However, these findings did not match with stunting and serum zinc in primary school children from three rural areas of the Province, where soil zinc is relatively lower. Zinc is better absorbed from biofortified rice than from control rice or extruded fortified rice. Simulated biofortified rice with zinc at a level of 2.7 mg/kg has the potential to significantly improve zinc intake, especially in the “traditional” dietary pattern with high rice and vegetable intake and medium meat intake. However, this pattern contributes to a high blood pressure, which may be due to high salt intake during the cooking process. Obesity is positively and independently related to high blood pressure, and inversely related to anemia.

Implications for public health

The low prevalence of low serum zinc and stunting in our study indicates that zinc deficiency is not a large problem in primary school children in the three counties. Despite this, half of the children and adolescents from Jiangsu Province as a whole had insufficient zinc intake. At the same time, we saw in our dietary pattern analysis that each pattern may be related with under-nutrition or over-nutrition except for the “Macho” pattern. From a scientific perspective, the “traditional” and “healthy” patterns have healthier food components than the other two patterns. Healthier cooking methods, such as reducing salt and oil during the cooking process, would bring these patterns in better line with the dietary guidelines. In this way, over-nutrition can be prevented and adequate nutrient intake can be maintained. To achieve this, food preparation methods need to be changed. This change can be initiated through effective communication to bridge the gap between technical experts, policy-makers and the general public. Sustainable effective communication should be done to improve knowledge and awareness of healthy diets on an individual and society level.

Individual change also requires changes in society to give better information and offer healthier choices. Good policy needs to be formulated and implemented to positively influence the public. Nutrient-dense, rather than energy-dense foods should be promoted and supported. Food labeling should not only show the essential nutrients but also information on fat quality, salt and sugar content. Food products with an added value should be widely available tailored to specific population groups, such as young children and elderly. Mass media should air the messages on health promotion to the public at large.

The National Nutrition and Health Surveys should be used continuously to track the trends in food consumption, lifestyle and related health outcomes in the public, as also done in this thesis. Such data can help to evaluate current policy, help policy-makers to develop new strategies to change people’s behavior into healthy diets and lifestyles. Since women generally make decisions about household nutrition, especially in rural areas, such strategies should be gender sensitive.

Suggestions for future research

Assessment of zinc deficiency

There is a clear need for a specific, simple indicator for assessment of zinc status as has also been recognized by IZiNCG. As long as no good biochemical indicators are available, phytate content in local foods needs to be measured and incorporated into food composition databases to better estimate zinc absorption, not just total zinc intake. Combination with dietary intake measurement, absorbed zinc intake can then be estimated with phytate/zinc molar ratios. Appropriate reference values of serum zinc for the Asian population, especially for children needs to be further investigated.

Biofortification of rice with zinc

Biofortification of rice with an adequate zinc content as a suitable and sustainable vehicle can help to combat zinc deficiency also in other rice consuming and zinc deficient areas. The breeding level of zinc that can actually be achieved in rice or other staple foods grown under various field conditions needs to be determined. Cost-effectiveness and acceptability also requires further study. The efficacy of zinc biofortified rice to improve zinc status and related health outcomes needs to be evaluated.

Causes of anemia

Although dietary iron intake is sufficient, anemia is still highly prevalent in China. Causes of iron deficiency and anemia are usually multifactorial and include nutritional deficiencies, inflammation, and NCDs. Comprehensive indicators, including soluble transferrin receptor, are needed to investigate the causes of iron deficiency and anemia. It is clear, however, that obesity is not among the causes for anemia.

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Summary

Summary

China is undergoing a rapid nutrition transition from diets high in carbohydrate and fibre to diets high in meat and fat. Such a transition is characterized by persisting micronutrients deficiencies, and increasing non-communicable diseases (NCDs).

Jiangsu Province is one of the most prosperous provinces in China, but soil zinc in the province is lower than the national level. One of the aims of this thesis was to assess dietary zinc intake and zinc status using different indicators in a representative population and a sample of primary school children, respectively. Zinc absorption from biofrtified rice was analyzed, and the effect of biofortified rice on dietary zinc intake was simulated for four distinct dietary patterns in Jiangsu Province. The other aim was to investigate the association of these dietary patterns with blood pressure, as well as of obesity with anemia.

In chapter 2, as part of 2002 National Nutrition and Health Survey, 3,867 subjects aged 4-89 years were representatively sampled in two urban and six rural areas of Jiangsu Province. Zinc intake was calculated using data from 3-day 24-hour dietary recall in conjunction with the Chinese Food Composition Table 2002. Insufficient zinc intake in specific population groups were determined as below 2/3 of the recommended nutrition intakes (RNI). The prevalence of insufficient intake of zinc was 22.9%, with a higher prevalence in children (64.6%) and adolescents (64.9%), and in those with low socio-economic status (27.3%).

Two data collection rounds were conducted in chapter 3. In the first data collection round, 2,268 primary school children aged 6-9 years were included by cluster sampling from three counties with low soil zinc concentrations in the Province. The prevalence of stunting was defined as a z-score of height-for-age below -2 according to the WHO new Growth Standards in 2006. For the second data collection round, the county with the highest prevalence of stunting was selected. From this county, 297 children aged 6-9 years were recruited by cluster sampling. Anthropometric measures, serum and hair zinc, and hemoglobin were measured at this stage. Around 4% of the primary school children were stunted. The prevalence of zinc deficiency measured by serum and hair zinc was 0.7%, and 15.2% respectively, and 32.3% of them had anemia.

In Chapter 4, thirteen women were recruited for three test rounds with rice meals in a stable isotope study. Intrinsically ^{70}Zn labeled biofortified rice, and extrinsically ^{70}Zn labeled extruded fortified or control rice were served to subjects in random order as a breakfast meal. ^{67}Zn was used as an intravenous tracer. Urine samples were collected before and 3-day after the administrations. Fractional zinc absorption (FAZ) was measured with the use of the double isotope tracer ratio method. The results were not accurate because we could not estimate the exact amount of isotope infused to subjects due to ^{67}Zn remaining on walls of the vials. Nevertheless, the biofortified ^{70}Zn enriched rice with an intrinsic label was found to have higher FAZ than extrinsically labeled fortified extruded rice.

Chapter 5 presents the effect of replacing normal rice with simulation biofortified rice with zinc at an intermediate level (2.7 mg/ 100g) and a high level (3.8 mg/ 100g) on dietary zinc intake. Zinc intake was assessed from 2819 adults aged 20 years and older, a subsample of 2002 Nutrition and Health Survey in Jiangsu Province, using a consecutive 3-day 24-h dietary recall method. Insufficient zinc intake was determined by the cut-off of 2/3 Chinese RNI. Four distinct dietary patterns were identified namely “traditional”, “Macho”, “sweet tooth”, and “healthy”. When replacing normal rice with zinc-biofortified rice with 2.7 and 3.8 mg/100g of zinc in a simulation, the prevalence of insufficiency of zinc intake decreased from 15.4% to 6.5% and 4.4% at the intermediate and high level, respectively. We found that the “traditional” pattern was associated with higher zinc intake.

Association between the four dietary patterns and blood pressure is shown in chapter 6. A total of 2518 adults without diagnosed hypertension and other related diseases were included in the study. Salt and oil consumption was calculated by dietary weighing in combination with 24h recall. Subjects were classified by body mass index categories as underweight, normal weight, overweight and obese according to Chinese standard. High blood pressure was defined as systolic blood pressure ≥ 140 mmHg and/or diastolic blood pressure ≥ 90 mmHg. The “traditional” dietary pattern in Jiangsu Province was most strongly associated with high blood pressure ($P_{for\ trend} = 0.005$). Such pattern is characterized primarily by consumption of rice and fresh vegetable, secondary of pork and fish, and lastly of root vegetable and wheat flour. The association is probably due to the high salt intake. Obesity was also positively and independently related to high blood pressure.

In chapter 7, we investigated the association between iron status and obesity among 1537 women. Anemia was stratified to iron-deficiency anemia (IDA), which is the presence of both anemia and iron deficiency, and non-iron deficiency anemia (non-IDA). Central obesity was defined as a waist circumference ≥ 80 cm. The prevalence of iron deficiency and anemia was 15.2% and 31.1%, with 6.3% of IDA and 24.8% of non-IDA. Overweight and obesity was inversely associated with anemia and non-IDA. Subjects with underweight were more likely to suffer from IDA than those with normal weight. Central obesity was inversely associated with anemia and non-IDA.

The main findings of our study are summarized in chapter 8. Internal and external validity are discussed. Implications for public health and suggestions for future research are proposed. In conclusion, zinc intakes are lower than the Chinese Dietary Intakes in Jiangsu Province, where the soil is deficient in zinc. Zinc deficiency in high-risk populations segments needs to be further investigated. Biofortified rice has the potential to improve zinc intake and status, especially in the “traditional” pattern with high intake of rice and vegetable, and medium intake of meat. However, this pattern needs to be modified with healthier cooking methods, such as reducing salt and oil in the cooking process, to prevent hypertension.

Samenvatting

Het Chinese voedingspatroon zit in een overgangsfase van een dieet rijk aan koolhydraten en vezels naar een dieet met veel vlees en vet. Een dergelijke overgang wordt in de regel gekenmerkt door een aanhoudend tekort aan micronutriënten zoals zink en ijzer, met tegelijkertijd een snelle toename in welvaartsziekten.

Jiangsu is één van China's meest welvarende provincies, maar het zink gehalte in de bodem is onder het nationale gemiddelde. Eén van de doelen in dit proefschrift was daarom het vaststellen van de zink inname via de voeding en het bepalen van zink status in Jiangsu, daarbij gebruikmakend van verschillende indicatoren in zowel een representatieve steekproef van de totale bevolking als in een geselecteerde groep basisschool kinderen. In een experimentele studie-opzet werd de absorptie van zink uit biologisch verrijkte rijst bepaald en werd vervolgens het effect hiervan op zink inname via de voeding gesimuleerd voor vier verschillende voedingspatronen in de provincie Jiangsu. Een ander doel was te onderzoeken of er een verband bestond tussen deze voedingspatronen en bloeddruk, alsook een verband tussen overgewicht en bloedarmoede.

In hoofdstuk 2 werden, als deel van de '2002 National Nutrition and Health Survey', 3.867deelnemers in de leeftijd 4-89 jaar gerekruteerd in respectievelijk twee stedelijke en zes rurale gebieden van de provincie Jiangsu. Zinkinname werd berekend door de data van een 3-daagse voedingsnavraag methode te combineren met de Chinese Voedingsmiddelentabel uit 2002. Onvoldoende zink inname in specifieke populatie groepen werd gedefinieerd als minder dan 2/3 van de Chinese aanbevolen hoeveelheid. De prevalentie van onvoldoende zink inname was 22,9%, met een hoge prevalentie bij kinderen (64,6%), adolescenten (64,9%) en personen met een lage sociaaleconomische status (27,3%).

In hoofdstuk 3 worden twee rondes van datacollectie beschreven. Bij de eerste ronde werden 2.268 basisschoolkinderen van 6-9 jaar geselecteerd door middel van cluster sampling uit drie provinciedistricten. De prevalentie van stunting werd gedefinieerd als een z-waarde in lengte-voor-leeftijd lager dan -2 in overeenstemming met de groei standaarden van de WHO. Voor de tweede datacollectie ronde werd het district met de hoogste prevalentie van stunting geselecteerd. Vervolgens werden 297 kinderen in de leeftijd van 6-9 jaar geselecteerd, en antropometrische waarden, en gehaltes aan serum zink, haar zink en hemoglobine bepaald. Slechts 4% van de basisschoolkinderen bleek stunted te zijn. De prevalentie van zinktekort berekend aan de hand van serum en haar zink was respectievelijk 0.7% en 15,2%, en verder had 32,3% anemie.

In hoofdstuk 4 werden dertien vrouwen gerekruteerd om deel te nemen aan een studie naar de biobeschikbaarheid van zink uit verrijkte rijst met behulp van een dubbel gemerkt zink methode. Hierbij werden met een interval van vier weken drie verschillende rijstmaaltijden verstrekt, elk gemerkt met een stabiel gelabeld zink isotoop. De maaltijden bestonden uit intrinsiek ^{70}Zn gelabeld biologisch verrijkte rijst, extrinsiek ^{70}Zn gelabeld verrijkte rijst middels een extrusie proces, en controle rijst. De maaltijden werden in willekeurige volgorde geserveerd aan de participanten als een ontbijt. ^{67}Zn werd gebruikt als een intraveneuze indicator. Urine monsters werden vooraf aan het toedienen van de maaltijd tot drie dagen erna verzameld. Fractionele zinkabsorptie (FAZ) werd berekend, maar de resultaten waren niet accuraat omdat een groot deel van het intraveneus toegediende isotoop achterbleef in het toedieningsvaatje. Desondanks kunnen we concluderen dat intrinsiek ^{70}Zn gelabelde biologisch verrijkte rijst systematisch een hogere FAZ had dan extrinsiek ^{70}Zn gelabeld verrijkte rijst.

Hoofdstuk 5 beschrijft het effect op zinkinname met de voeding wanneer consumptie van gewone rijst met 1.7 mg zink per 100 g werd vervangen door rijst met een gesimuleerd intermediair zink gehalte (2,7 mg / 100g) of een hoog zink gehalte (3,8 mg / 100g). Zinkinname werd gesimuleerd voor 2819 volwassenen ouder dan 20 jaar, als een subpopulatie van de ‘2002 National Nutrition and Health Survey’ de provincie Jiangsu, wederom gebruikmakend van de 24-uurs voedingsnavraag methode tijdens drie opeenvolgende dagen. Onvoldoende zink inname werd gedefinieerd als inname minder dan 2/3 van de Chinese aanbevolen hoeveelheid. De voedingspatronen werden onderverdeeld in vier categorieën namelijk ‘traditioneel’, ‘macho’, ‘zoet’ en ‘gezond’. Door het vervangen van normale rijst door rijst met een intermediair of hoog zink gehalte daalde de prevalentie van onvoldoende zink inname van 15,4% tot respectievelijk 6,5% of 4,4%, waarbij de grootste daling optrad in de groep gekenmerkt door een ‘traditioneel’ voedingspatroon.

Hoofstuk 6 beschrijft de relatie tussen de vier verschillende voedingspatronen en bloeddruk. In totaal werden 2518 volwassenen zonder diagnose van hypertensie of andere gerelateerde ziekten in de studie opgenomen. De zout en olie consumptie werd berekend middels het wegen van deze voedingsmiddelen in combinatie met de 24-uur voedingsnavraag methode gedurende drie dagen. Participanten werden ingedeeld naar body mass index klasse volgens de Chinese standaarden. Hoge bloeddruk werd gedefinieerd als een systolische blood druk ≥ 140 mmHg en/of een diastolysche druk ≥ 90 mmHg. In de provincie Jiangsu was het ‘traditionele’ voedingspatroon het sterkst geassocieerd met hoge bloeddruk (P voor trend = 0.005). Dit voedingspatroon wordt gekenmerkt door de consumptie van rijst en verse groenten, met daarnaast varkensvlees en vis en als laatste knollen en tarwemeel. Het verband met hoge bloeddruk wordt wellicht veroorzaakt door de hoge zoutinname. Overgewicht was ook positief en onafhankelijk gerelateerd met hoge bloeddruk.

In hoofdstuk 7 werd het verband tussen ijzerstatus en overgewicht bij 1537 vrouwen onderzocht. Bloedarmoede, of anemie, werd onderverdeeld in anemie die wel of niet veroorzaakt wordt door ijzertekort. Overgewicht werd zowel gedefinieerd op basis van body mass index als op basis van middelomtrek (> 80 cm). De prevalentie van ijzertekort en anemie was respectievelijk 15,2% en 31,1%, waarvan 6,3% veroorzaakt door ijzertekort en 24,8% door andere factoren. Er bestond een omgekeerd evenredige relatie tussen overgewicht en anemie. Participanten met ondergewicht leden vaker aan anemie door ijzertekort dan participanten met een normaal gewicht.

De belangrijkste bevindingen uit dit proefschrift worden weergegeven in hoofdstuk 8. Verder worden hier de interne en externe validiteit uitgebreid besproken en worden implicaties gegeven voor de volksgezondheid, alsmede suggesties voor verder onderzoek genoemd. Geconcludeerd kan worden dat de zinkinname in de provincie Jiangsu lager is dan de gemiddelde inname van zink in China. Tekort aan zink in hoog risico groepen moet verder onderzocht worden. Biologisch verrijkte rijst heeft de potentie om de zink- inname en status te verbeteren. Dit is vooral zo bij het traditionele voedingspatroon welke rijk is aan rijst en groenten. Dit voedingspatroon moet echter worden aangepast aan gezondere bereidingsmethoden, zoals het reduceren van de hoeveelheid zout en olie, om hypertensie tegen te gaan.

总　　结

中国正处于快速营养转型期，体现在饮食结构由高碳水化合物高纤维转变为高动物肉类和高脂肪，这种转型导致了持续性的微营养素缺乏和不断增加的慢性非传染性疾病（以下简称“慢性病”）。

江苏省是中国经济发达的省份之一，然而，其土壤中锌的含量低于国家水平。本论文的目的之一旨在用不同的指标评估代表性样本人群饮食锌摄入量和部分地区小学生锌营养状态。同时，分析食用生物强化大米后锌的吸收情况，并结合该省现有的四种膳食模式评价模拟生物强化大米对饮食锌摄入的影响。另一个目的旨在探讨现有的四种膳食模式与血压、肥胖与贫血之间的关系。

在第二章里，从江苏省 2 个城区和 6 个农村地区抽取有代表性的样本 3867 人，通过三天 24 小时饮食回顾法和食物称量法，并结合 2002 年中国食物成分表计算出锌摄入量。锌摄入量低于我国年龄别推荐营养摄入量的 2/3 即判断为锌摄入不足。抽样人群中锌摄入不足的比例为 22.9%，在儿童和青少年中最高，分别为 64.6%和 64.9%；低收入人群中锌摄入量不足的比例也较高，为 27.3%。

第三章包含了两次抽样过程。第一次抽样，选择三个土壤锌含量相对较低的三个县，运用整群抽样的方法在 6-9 岁小学生中抽取 2268 人，根据世界卫生组织 2006 年生长标准，年龄别升高 z 评分小于-2 即定义为生长迟缓。第二次抽样，在生长迟缓比例最高的县，通过整群抽样抽取 297 名 6-9 岁小学生，测量身高、体重、血清和发锌、血红蛋白。结果表明，约 4%的小学生生长迟缓，由血清和发锌低于参考值的比例为别为 0.7%和 15.2%，32.3%的小学生患有贫血。

第四章，我们征集了 13 名妇女参加稳定性核素标记试验估计不同大米锌的吸收率。参加对象按照各自的随机顺序，分别食入三种大米煮成的粥，这三种大米分别为内源性 ^{70}Zn 标记的生物强化大米、外源性 ^{70}Zn 标记的压榨强化大米和普通对照大米。通过静脉注射 ^{67}Zn，在每次试验的前一天和后三天收集尿样，锌吸收率用双核素比的方法进行估算。由于在静脉注射同位素时，部分 ^{67}Zn 残留在注射瓶的瓶壁上，本次研究未能准确估算锌吸收情况。尽管如此，从计算结果来看，内源性标记的生物强化大米锌吸收率高于其他两种外源性标记大米。

第五章分析了两种水平模拟锌生物强化大米代替普通大米后对锌摄入的影响，强化大米锌含量分别为 2.7 mg/ 100g 和 3.8 mg/ 100g。研究对象为 2002 年营养调

查人群中 2819 名 20 岁及以上的成人，通过三天 24 小时膳食回顾调查方法计算锌摄入量，如锌摄入小于我国年龄别推荐营养摄入量的 2/3 即判断为锌摄入不足。确定了该省人群四种类型膳食模式，包括“传统型”、“肉食型”、“甜食型”和“健康型”。当以模拟生物锌强化大米取代普通大米时，锌摄入不足的比例从 15.4%下降到 6.5%（锌含量为 2.7 mg/ 100g ）and 4.4%（锌含量为 3.8 mg/ 100g ）。我们同时还发现“传统型”膳食模式与锌摄入量呈正相关。

第六章分析了上述四种膳食模式与血压之间的关系，研究对象为 2002 年营养调查人群中 2518 名未诊断高血压及其它相关疾病的成人。膳食盐和食用油的摄入量通过称重法和 24 小时膳食回顾法进行推算，根据体质指数将研究对象分为体重低下、体重正常、超重和肥胖。收缩压≥ 140 mmHg 和/或舒张压≥ 90 mmHg 者判断为血压过高。“传统型”膳食模式与血压过高呈正相关，这种类型膳食模式主要包括较多的大米和蔬菜、中等量的猪肉和鱼类、以及少量的根茎类蔬菜和小麦粉，与血压过高有关可能与盐摄入较多有关。另外，肥胖是血压过高的独立危险因素。

在第七章中，我们在 1537 名女性中分析了铁营养状况与肥胖的关系。将贫血分为缺铁性贫血（贫血和铁缺乏同时存在）和非缺铁性贫血，腰围≥ 80 cm 者为中心性肥胖。铁缺乏和贫血的流行率分别为 15.2% 和 31.1%， 其中缺铁性贫血流行率为 6.3%，非缺铁性贫血流行率为 24.8%。超重和肥胖与贫血、非缺铁性贫血呈显著负相关，体重低下者更容易罹患缺铁性贫血。中心性肥胖也与贫血、非缺铁性贫血呈显著负相关。

第八章概括了本研究的主要结果，讨论了内部和外部有效性，提出了公共卫生意义和未来研究的思路。总之，在土壤锌缺乏的背景下，江苏人群膳食锌摄入量低于中国膳食摄入推荐量，高危人群锌缺乏状况尚待进一步探讨。生物强化大米有望改善膳食锌摄入和锌营养状况，尤其是以“传统型”膳食模式（多大米和蔬菜及中等量肉类）为主的人群。然而，该膳食模式需要调整烹饪方法，例如在烹饪过程中应减少盐和食用油的使用量，以预防高血压的发生。

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Curriculum Vitae

QIN Yu was born in Jiangdu County, Jiangsu Province, P. R. China on January 1st 1973. In 1990, she was enrolled in Jiangxi Medical College, where she graduated in 1995 with a bachelor of Preventive Medicine. She enrolled in a master program in 1997 at Nanjing Medical University and did a master in cancer epidemiology. Her master thesis was entitled "case-control study on risk factors of stomach cancer". In 2000, she got her master degree in Epidemiology.

From August 2000, she has been working at the Department of Non-communicable Chronic Disease Control at Jiangsu Provincial Center for Disease Control and Prevention. During this period, she was involved in several research projects. In November 2002, she joined Wageningen University as a sandwich PhD student at the Division of Human Nutrition, financed by the Interdisciplinary Research and Education Fund (INREF).

She will defend her thesis on October 1st.

Overview of completed training activities

Discipline specific activities

Assessment of Iron Status, Wageningen University, 2003

Cancer Project training, Nanjing, P. R. China, 2007

Cancer Prevention Conference, Shanghai, P. R. China, 2007

Workshops and meetings

Nutrigenomics Symposium, Wageningen University, 2003

Nutrition and Lifestyle Epidemiology, Beijing, 2004

Dietary Influences on Blood Pressure, Wageningen University, 2006

Farewell Symposium Martijn Katan: Nutrition's Top Three, Wageningen University, 2006

Zinc Crops 2007 (Poster), Istanbul, Turkish, 2007

Mass Media Planning Workshop, Beijing, P. R. China, 2009

Global Tobacco Control Leadership Program, Baltimore, America, 2010

Fatty Acids Ventricular Arrhythmias and Sudden Death, Wageningen University, 2011

General courses

English course, Wageningen University, 2002-2003

Working with EndNote, Wageningen University, 2006

Advanced EndNote, Wageningen University, 2006

Project Management, Beijing, P. R. China, 2007

Project Evaluation, Dalian, P. R. China, 2008

Optionals

Preparation of Research Protocol

Nutritional Epidemiology, Wageningen University, 2003

Analytic Epidemiology, Wageningen University, 2003

Literature Study Program, 2002-2003

Journal Club, 2006

Oldsmobiles, 2006

INREF Program meeting, 2006

List of Publications

Qin Y, Melse-Boonstra A, Pan XQ, Zhao Jk, Yuan BJ, Dai Y, Zhou MH, Geleijnse JM, Kok FJ, Shi ZM. Association of dietary pattern and body weight with blood pressure in Jiangsu Province, China. Submitted for publication.

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