

Nutrient intake by kindergarten children in Beijing and Xian, China in 2004-2005, with special reference to sources of high sodium intake

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Abstract

Background and objectives: China has been experiencing dramatic social changes in recent years. From nutritional view, nutrient intakes of children should be clarified as baseline data at the early stage of the change. Food intake records were available to this study group for this purpose.

Methods: In practice, 120 children (60 boys and 60 girls) were studied, who were at the ages of 5 to 6 years attending kindergartens in Beijing (in 2004 and 2005) and Xian (in 2005). Guardians provided informed consents on behalf of children and submitted 24-hour duplicates of foods and drinks at the amounts served to children.

Results: Children took 1373 kcal energy, 59.6 g protein, 39.8 g lipid and 204.2 g carbohydrate per day. Cereals accounted for 39.1% of energy and 23.3% of protein. When compared with nationally set AI (adequate intake) or RNI (recommended nutrient intake), energy and macro-nutrient intakes were barely sufficient. Vitamin A and vitamin C intakes were quite sufficient, whereas thiamin intake was only barely so. Intake of Na was as high as more than twice AI. The Na intake was confirmed also by instrumental analysis. A close association of high Na intake with wheat-based foods was detected. Consumption of fast foods was quite limited.

Conclusions: Intakes were adequate for energy and macro-nutrients already early in this century, but improvements were necessary for several micro-nutrients. Na intake was especially quite in excess; an association with wheat-based foods was detected. Food contamination with Cd or Pb was not detectable.

Introduction

It is well known that the societies in China have been dramatically changing in recent years; the changes involve economy of every family and may affect the nutrition and health of people, especially of children [1-4]. Improved affordability for better foods may contribute favorably in general. Nevertheless, it was also pointed out that economic development may induce nutritionally unfavorable side effects [1]. For example, the trend of obesity among school children and adolescents coincides in time with economic development [3]. Increased intake of processed food due to income growth may cause overweight and even obesity in young people [2,4].

Thus, it is quite conceivable that the records on the nutrient intakes at the beginning of the steady socio-economic development early this century are important as the baseline knowledge to understand the changes in nutrition. Nevertheless, literatures in English on nutrient intakes of children are still scarce to clarify the situation in e.g. early 2000 to 2010.

Data were available to this research group on food intake of 5-6 years-old children in kindergartens in the capital city of Beijing and

one provincial capital of Xian for estimation of nutrient intake in 2004-5. The results will be presented in this article to fill the information gap even in part. Nutrient intakes of adult women in late 1990s have been published previously based on the surveys conducted in Shandong [5], Shaanxi [6], and Jilin provinces [7], China.

Materials and methods

Survey sites, survey subjects and ethical considerations

Surveys were conducted in 2004-5 in kindergartens (KGs in short) in the national capital of Beijing and one provincial capital of Xian (Shaanxi province) (for locations, Figure 1).

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Figure 1. Map of survey site locations in central China

In Beijing, surveys were conducted once in 2004 (to be abbreviated as Beijing 04) and then in 2005 (Beijing 05) at different KGs. The survey in Xian was in 2005. In total, 120 children at the age of 5–6 years [40 children (20 boys and 20 girls) each for Beijing 04, Beijing 05 and Xian] participated in the survey (Table 1). The children were apparently healthy and came to KGs regularly. Guardians (mothers in most cases) provided informed consents (on behalf of the children), offered food duplicate samples (for details, see below), and allowed to measure body sizes of the children.

This survey was conducted in accordance with Declaration of Helsinki, 1947. The study protocol was approved by the Institutional Review Board of Peking University, Beijing, China on 29th March 2004 (the approval number; IRB 00001052-0215).

Collection of food duplicate samples and estimation of nutrient intake

Each guardian prepared a 24-hour food duplicate sample by collecting foods (including water and drinks) at the exact amounts the child was served on the day [8] following the method previously detailed [6,9–11]. Food items in each duplicate sample were separated manually, and weights were recorded. Then, the food item was coded after China Food Composition Tables (the second edition) [12] by veteran nutritionists who knew local foods well. The nutrient intake was estimated from the code and weight of each food item followed by summation for intake per day [9–13]. The nutritionists estimated the amounts of unmeasurable food items (such as cooking and table salt) to supplement the food intake records [5–7].

Cadmium (Cd) and lead (Pb) in the duplicate samples were measured by inductively coupled plasma-mass spectrometry (ICP-MS) after homogenization and wet-ashing of each sample [11]. Sodium (Na) was measured by flame atomic absorption spectrometry [13] to validate the estimation. Successful quality assurance for the analytical

methods employed was also previously described for Cd and Pb [11,13].

Statistical analyses

Because the number of cases studied were limited (see above for the numbers), data for three groups (i.e., Beijing 04, Beijing 05 and Xian) were combined for nutritional evaluation (n=120). Normal distributions were assumed for nutrient intakes so that arithmetic means (AMs) and arithmetic standard deviations (ASDs) were taken as parameters to represent the distribution. As Cd and Pb values are known to distribute log-normally (rather than normally) e.g. [9,14,15], geometric means (GMs) and geometric standard deviations (GSDs) were calculated for these two pollutant elements. For statistical evaluation, $p < 0.05$ was taken as the point of significance.

Results

Demography of children

Demographic characteristics of the children are summarized in Table 1 by gender of children.

Calculation with data for Beijing and Xian in combination showed that boys were significantly ($p < 0.05$) taller and heavier than girls (Table 1). The difference was by 2.2 cm in height and by 1.2 kg in weight on an average

Daily dietary intake of energy and nutrients

On an average, children took 1373 kcal energy, 59.6 g protein, 39.8 g lipid, and 204.2 g carbohydrate per day. Further perusal of the data showed that Beijing children took more energy from rice-based foods (293 ± 101 kcal/day as $AM \pm ASD$) than from wheat-based foods (170 ± 75 kcal/day) ($p < 0.01$), whereas the reverse was the case for Xian children (i.e., 191 ± 83 kcal/day from rice and 375 ± 219 kcal/day from wheat) ($p < 0.01$ for both rice and wheat intake). One child in Xian took no rice and 10 others took < 170 kcal rice /day. These findings suggest that they depended primarily on non-rice cereals (e.g., wheat) as staple foods of the day.

With regard to micro-nutrient intakes (Table 2), of worthy to note was very high Na intake, i.e., 2250 mg/day. The intake was higher for children in Xian (i.e., 2828 ± 588 mg/day) than for children in Beijing (i.e., 1961 ± 472 mg/day) ($p < 0.01$ for the difference).

Dietary intake of Cd and Pb

In Table 3, dietary intake of Cd and Pb are presented in terms of GM and GSD. As for locality in heavy metal intake was concerned, Beijing

Table 1. Demography and food intake

	Height (cm)	BW (kg)	BMI (kg/m ²)	Total food weight (g/day)
Boys				
No. of cases	60	60	60	60
AM	116.5	21.7	15.9	1703
ASD	5.3	3.7	1.8	251
Girls				
No. of cases	60	60	60	60
AM	114.3	20.0	15.3	1704
ASD	4.2	3.3	1.8	257
Boys+Girls				
No. of cases	120	120	120	120
AM	115.4	20.8	15.6	1703
ASD	4.9	3.6	1.8	253

Table 2. Dietary intake of energy, macro-nutrients, and selected vitamins and minerals

[A] Energy and macro-nutrients							
Parameter	Energy from			Macro-nutrients			
	Total food	Wheat	Rice	Protein	Lipid	Carbo-hydrate	
(Unit/day)	(kcal)	(kcal)	(kcal)	(g)	(g)	(g)	
AM	1373	239	259	59.6	39.8	204.2	
ASD	283	170	107	13.2	14.4	43.9	
CV (%)	20.6	71.3	41.2	22.2	36.2	21.5	
[B] Vitamins							
Parameter	Vitamin A	Thiamin	Ribo-flavin	Niacin	Vitamin C	Vitamin E (Total)	
(Unit/day)	RE (µg)	(mg)	(mg)	(mg)	(mg)	(mg)	
AM	779	0.7	1.1	13.5	104.9	14.7	
ASD	385	0.3	0.4	3.1	44.8	5.7	
CV (%)	49.4	38.4	34.9	22.8	42.8	38.5	
[C] Minerals							
Parameter	Ca	Fe	K	Mg	Na	P	Zn
(Unit/day)	(µg)	(mg)	(mg)	(mg)	(mg)	(mg)	(mg)
AM	717	19.4	1973	297	2250	974	8.5
ASD	286	5.6	551	73	656	240	2.0
CV (%)	39.9	28.7	27.9	24.6	29.1	24.6	23.0

Table 3. Daily intake of Cd and Pb; comparison by survey site

Survey cite	No. of cases	Parameter	Cd-D (µg/day)	Pb-D (µg/day)
Beijing	80	GM	10.0	11.9
		GSD	1.7	1.6
Xian	40	GM	7.1	12.2
		GSD	1.3	1.7
Significance in difference ^a			p<0.01	ns
Total	120	GM	8.9	12.0
		GSD	1.6	1.6

Statistical tests were conducted after logarithmic conversion; ns stands for $p \geq 0.05$.

and Xian data were presented separately as well as in combination. Comparison between Beijing and Xian showed that children in Xian had less Cd intake than those in Beijing ($p < 0.01$) (7.1 µg/day for Xian children vs. 10.0 µg/day for Beijing children both as GM). Cd intake was 8.9 µg/day when all cases were combined. In case of Pb, the intake for all children in combination was 12.0 µg/day with no significant difference ($p > 0.10$) between Beijing (11.9 µg/day) and Xian (12.2 µg/day).

Validation of dietary Na intake

Accurate assessment of daily Na intake was considered important because health impact of high Na intake should be notable as to be discussed later. Thus, Na intake was evaluated as Food Composition Table [12]-based estimates as supplemented by nutritionists (E in short) and also as instrumentally measured values (M). The AM±ASD values were 2250±656 mg/day for E and 2117±516 mg/day for M. The E/M ratio was 1.06. The p value was between 0.05 and 0.10 by unpaired t-test and 0.042 by paired t-test. The M and E values correlated significantly with each other (Pearson's correlation coefficient $r = 0.286$, $p < 0.01$, $n = 120$). Thus, the estimated values were considered to be valid.

Food groups as leading sources for energy, major nutrients, Ca and Fe

Food items were classified by food groups. Then, accounts (in percentages) for food groups in contributing energy and macro-nutrients were calculated (Table 4). Attention was extended to two important micro-nutrient elements of Ca and Fe.

As it was quite conceivable, cereals were the major source for energy (39.1%) and carbohydrate (56.9%). In addition, cereals were the largest source for protein (23.3%). In contrast, none of animal-

based foods of meat (14.9%), poultries (2.3%), milks (13.1%) and eggs (10.5%) contributed more than 15% of total protein when evaluated individually, although they accounted for nearly 41% when combined. Consumption of fish (and shellfish) was very limited (1.8 % on an energy basis) possibly because both Beijing and Xian are inland cities. Rather unexpectedly, pulses such as soy beans contributed less than 10% each of protein (8.1%) and lipid (6.1%). Lipid came from animal-based food items (54.2%) together with fat and oil used as cooking material (14.9%). Ca intake was basically from milk (36.8%) and vegetables (20.9%), whereas cereals and vegetables were the leading sources for Fe (23.8 and 18.6%, respectively).

It should be worthy to note that the consumption of fast foods (2.3% on an energy basis) were quite limited both in Beijing (1.7%) and Xian (3.5%). Confectioneries were also consumed only a little (2.6% on an energy basis).

Analyses for cereal types and accounts for cereals as sources of nutrient minerals

Four types of cereals were identified in food records, i.e., wheat, rice, maize and millet. Consumption of barley was never detected. With regard to food efficiency, the calculation for the ratio of energy over weight (kcal/g) gave 1.52 for wheat, 1.60 for rice, 3.10 for maize and 0.46 for millet. The ratio was highest for maize, which was rather contrary to expectation.

The consumption of wheat and rice were common in three survey areas of Beijing 04, Beijing 05 and Xian. As discussed above, Beijing children depended more on rice (58.2% of total cereals on the energy basis) as compared with 34.1% on wheat. In contrast, Xian children depended 35.1% on rice and 60.3% on wheat. Distribution of maize or millet consumption was limited and quite biased.

The observation that Xian children consumed more wheat-based foods (see above) and ingested more Na (Table 3) appeared to suggest that the intake of wheat-based foods might be associated with Na intake (see below for further discussion). Cereals were the major sources for Fe intake but poor sources for Ca (Table 4). In addition, cereals are known to be the major or substantial sources of dietary Cd and Pb intake, as to be discussed below. Thus, possible correlation of major cereals (i.e., wheat and rice) with Na, Ca, Fe, Cd and Pb intakes were examined.

Table 4. Contribution of food groups as sources of energy, major nutrients, calcium and iron

No.	Food group	Contribution (%)					
		Energy	Protein	Lipid	Carbo- hydrate	Ca	Fe
1	Cereals	39.1	23.3	5.6	56.9	7.8	23.8
2	Potatoes and starches	2.7	1.1	0.2	4.3	1.0	2.7
3	Pulses	4.6	8.1	6.1	3.3	7.7	8.3
4	Vegetables	4.3	7.1	1.6	6.0	20.9	18.6
5	Mushrooms and algae	0.5	1.4	0.1	0.9	1.1	4.9
6	Fruits	6.3	2.2	1.0	10.4	3.0	3.7
7	Nuts and seeds	0.8	0.6	1.7	0.4	0.5	0.4
8	Animal meats	7.6	14.9	17.6	0.5	0.6	6.6
9	Poultry	1.3	2.3	3.1	0.2	0.2	0.7
19	Milks	11.0	13.1	19.0	6.2	36.8	4.4
11	Eggs	5.9	10.5	14.5	0.3	4.1	6.3
12	Fishes and shellfishes	1.8	9.2	0.6	0.1	5.0	1.7
13	Baby foods	0.5	0.6	0.6	0.4	0.3	0.3
14	Confectionaries	2.6	1.0	3.4	2.7	0.5	1.4
15	Fast foods	2.3	1.2	2.4	2.6	0.8	0.9
16	Beverages	0.8	0.1	0.1	1.2	0.7	0.1
17	Alcoholic beverages	0.0	0.0	0.0	0.0	0.0	0.0
18	Sugars and sweeteners	1.4	0.1	1.3	1.9	0.5	0.4
19	Fats and oils	3.7	0.0	14.9	0.0	0.1	0.6
20	Seasonings	2.9	3.4	6.1	1.6	8.3	14.1
21	Chinese medicine foods	0.0	0.0	0.0	0.0	0.0	0.0
22	Others	0.0	0.0	0.0	0.0	0.0	0.0
	Total	100	100	100	100	100	100

The analyses (Table 5) made it clear that Na intake was closely ($p < 0.01$) associated with intake of wheat-based foods (Figure 2a) in contrast to poor association with rice-based foods ($p > 0.10$; Figure 2b). Fe correlated with cereals intake significantly ($p < 0.01$) whereas Ca did not show significant correlation ($p > 0.10$) in agreement with the observation in Table 4 as a poor source for this element.

Cd intake correlated significantly with intakes of total foods, cereals, and rice ($p < 0.01$ for each of the three cases) although not with wheat intake. Pb intake however did not show significant correlation ($p > 0.10$) with cereals, rice or wheat intake, although a weak correlation was observed with total food intake (Figure 3).

Discussion

The present survey conducted in 2004-5 disclosed that 120 children at the ages of 5-6 years took 1373 kcal energy and 59.6 g protein per day on an average (i.e., as AM). The values were lower and higher, respectively, than the values [1679 kcal (=7053.1 J) and 51.6 g] reported by Cui and Dibley [16] for 7-10 years-old children for the year 2004. The National Survey [17] reporting current situation states that cereals account for 53.1% on the energy intake basis and the counterpart value from the present survey was 39.1% (Table 4). The account for cereals as a protein source is 47.3% in the National Survey [17] and 23.3% in the present survey (Table 4). Thus, the values are not unanimous among the reports. The differences might be attributable to the fact that the National Survey [17] covers people of all ages in both urban and rural areas, whereas the present survey was on kindergarten children in the most urbanized areas of Beijing and Xian. In addition, the survey time was different, i.e., the National Survey [17] was in 2015 and the present survey was in 2004-5 with a gap in time of about 10 years. Nevertheless, it appears likely that the nutritional dependency (both for energy and for protein) on cereals was no higher in the past than that at the present time.

Comparisons with AI (adequate intake) and RNI (recommended nutrient intake) values [12] (Table 6) suggest that the protein intake

was sufficient but energy intake was barely so (Table 2). Fe intake (19.4 mg/day on average) was also sufficient. Nevertheless, thiamin (0.7 mg/day) and Ca intakes (717 μ g/day) were either barely sufficient (thiamin) or in short (Ca) (Table 2). Na intake (2250 mg or about 5.6 g/day as sodium chloride; Table 2) was quite in excess, more than two times recommended AI (900 to 1000 mg) for 4 or 7 years-old children. The Na intake was even higher for Xian children (2828 mg Na or 7.2 g sodium chloride). It should be noted that high salt intake will be an independent factor of obesity [18] in addition to the well-known adverse effect of induction of hypertension later in life [19]. Attention to high salt intake as an etiology of hypertension among aged people has been growing in recent years in China [20-24].

The present survey disclosed that dependency on wheat-based foods as staples was associated with increased Na intake (Table 5, Figure 2a). To the knowledge of the authors, this observation is the first in reporting the association between wheat-based foods and salt intake in English. No significant effect was observed with intake of rice-based foods. It should be added that this observation is on line with the description in China Food Composition that wheat-based typical Chinese bread (Code Nos. 01-1-404 to 406) contains about 165 mg Na/100 g [12]. In contrast, Na contents in cooked rice (Code No. 01-2-402), rice grain (Code Nos. 01-2-101 to 104) and wheat flour (Code Nos. 01-1-201 to 203) are all as low as < 4 mg/100 g [12].

In previous surveys in 1996-1999 on adult women in three urban and rural pairs in Jilin, Shandong, and Shaanxi provinces in China [5-7], energy and Fe intakes were sufficient in common but clear urban-rural difference was detected in intakes of protein and lipid, as well as Na. Ge [25] also reported better nutritional conditions for urban residents than for villagers in the past. Wang et al. [26], however, observed that urban-rural difference in dietary habits had been gradually disappearing in their 25-year follow-up in Tianjin and its suburbs.

Possibly since 1991, China has been nation-wide conducting Nutrition and Health Surveys once every 2-3 years involving 15 to 20 thousand people at the ages of 7 years and above per occasion [27]. In the survey, data on younger children at pre-school ages were not presented separately, unfortunately. Based on the 1992 data, Ge and Chang [27] concluded that the intakes of vitamin A, thiamin, riboflavin and Ca did not meet with RNI, and that the problem was most serious in insufficient intake of Ca. Through following-up the data up to 2004, Zhai et al. [28] observed that the problems of low intakes of vitamin A and Ca persisted. The same shortages in Ca intake, although not in vitamin A, was detected also in the present analysis (Table 2). With regard to Ca, insufficient dietary Ca intake may induce unbalance in Ca metabolism in bones, especially in absence of proper physical exercise [29]. It is well-known that Ca loss is a risk factor for osteoporosis among post-menopausal women [29], although such may not be an issue for children.

Cereals are known to be major dietary sources for two environmental pollutant elements of Cd and Pb [14,15,30-32]. The present analysis proved that Cd intake was associated with intakes of cereals, rice in particular, despite the fact that Cd intake in the present survey was as low as 8.9 μ g/day (Table 3), which is only 1.5% of the levels reported for adult residents in Cd-polluted areas (e.g., 541 or 684 μ g/day [33]), because foods are exclusive sources of Cd exposure among general populations [14]. The lack of association of Pb exposure levels with any type of cereals is conceivable, although the level, 12.0 μ g/day (Table 3) was about 19% of the levels reported for polluted areas, e.g., 63 μ g/day [31]. Foods are not always major sources of exposure to Pb, and air-borne Pb in polluted atmosphere could be a substantial source [15].

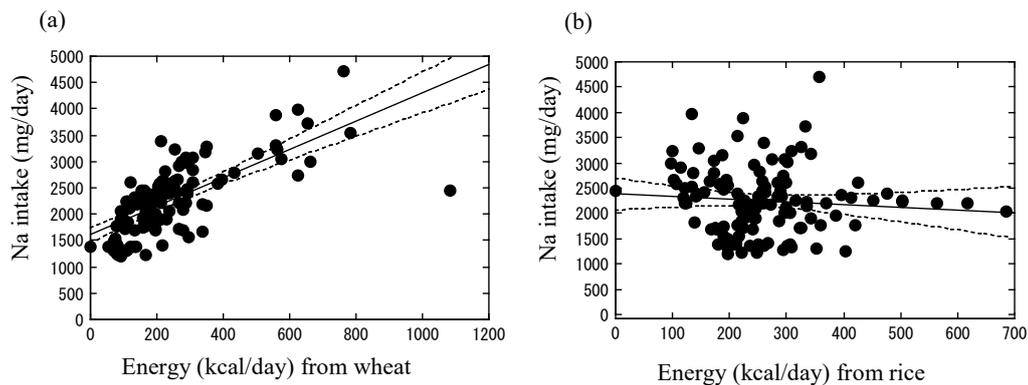


Figure 2. Relation of Na intake with intakes of energy from (a) wheat and (b) rice [The line in the middle is a calculated regression line. Two dotted curves on both sides show 95% confidence ranges. The equation for the line is; (a) $Y = -172 + 0.182 X$ ($r=0.703$, $p<0.01$, $n=120$), where X is energy (kcal/day) from wheat, and Y is Na intake (mg/day). (b) $Y = 290 - 0.014 X$ ($r=-0.085$, $p>0.10$, $n=120$), where X is energy (kcal/day) from rice, and Y is Na intake (mg/day)].

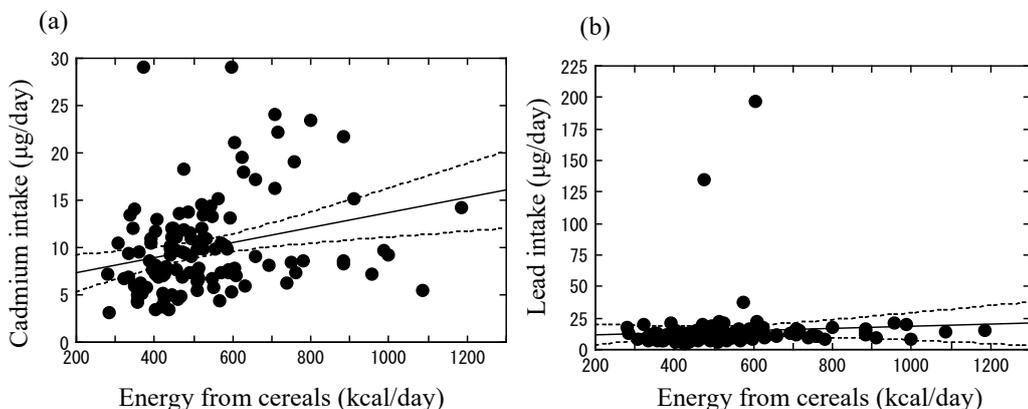


Figure 3. Relation of (a) Cd and (b) Pb intakes with cereals intakes [The line in the middle is a calculated regression line. Two dotted curves on both sides show 95% confidence ranges. The equation for the line is; (a) $Y = 440 + 9.09 X$ ($r=0.270$, $p<0.01$, $n=120$), where X is energy (kcal/day) from cereals, and Y is Cd intake ($\mu\text{g/day}$). (b) $Y = 523 + 0.521 X$ ($r=0.063$, $p>0.10$, $n=120$), where X is energy (kcal/day) from cereals, and Y is Pb intake ($\mu\text{g/day}$)].

Table 5. Correlation of nutrient and pollutant elements with energy sources

Energy intake (kcal/day) from	Intake of nutrient elements (mg/day)						Intake of pollutant elements ($\mu\text{g/day}$)			
	Na		Ca		Fe		Cd		Pb	
	r	p	r	p	r	p	r	p	r	p
Total foods	0.303	<0.01	0.707	<0.01	0.837	<0.01	0.430	<0.01	0.202	<0.05
Cereals	0.563	<0.01	0.163	>0.10	0.448	<0.01	0.269	<0.01	0.063	>0.10
Rice	-0.085	>0.10	0.381	<0.01	0.409	<0.01	0.530	<0.01	0.023	>0.10
Wheat	0.703	<0.01	-0.055	>0.10	0.155	>0.10	-0.065	>0.10	0.049	>0.10

There are several limitations in the present survey. First of all, the number of cases and sites surveyed was apparently too small. In this sense, the present results should be taken as a case report. Nevertheless, the present survey succeeded to show barely sufficient intake of energy and excess intake of Na, which should be taken as baseline findings. In addition, limited intake of fast foods was also of interest, because the small intake may suggest that food market revolution [4] had not prevailed yet in 2004-5 even in urban areas such as Beijing and Xian. Second point may be the quality of food duplicate samples. The answer to the question if the samples collected really represented daily food consumption depended largely on the honesty of each guardian who prepared the duplicate sample. Sample donors were suggested to avoid over- or under-preparations, but possibilities of such bias could not be excluded. It was not possible to collect food duplicate samples repeatedly for several days. The practice might be too bothering and nerve-taxing for sample donors. Such difficulties should be taken as a problem common to food duplicate-based surveys. In relation to

etiology of metabolic syndrome, Xu et al. [34] pointed out gender difference in food habits such that men were prone to take animal-based and fried foods whereas foods for women were energy-rich and more salted. However, such gender-related difference in food habits were not considered in the present analysis because the population surveyed was young children at 5-6 years of age.

Conclusion

The present analyses on nutrient intakes for children early in this century made it clear that intakes of macro-nutrients such as protein (although cereals-based, primarily) were sufficient even before the social changes in economy took place. With regard to micro-nutrient intakes, problems yet to be solved included insufficient intake of Ca. Very excess intake of Na may be a long-standing yet most urgent problem to be managed for the future health of children. A significant correlation of Na intake with wheat-based food consumption was detected.

Table 6. RNI and AI values for nutrients for 5–6 years-old children

[A] Energy and macronutrients (Unit)							
Item Unit	Energy (kcal)		Protein (g)		Fat (g)		
Classification	RNI		RNI				
	Boys	Girls	Boys	Girls			
5 years ^a	1600	1500	55	55	NV		
6 years ^a	1700	1600	55	55	NV		
[B] Vitamins							
Item	Vit. A	Thiamin	Riboflavin	Niacin	Vit. C	Vit. E (total)	
Unit	RE µg	mg	mg	mg	mg	mg	
Classification	RNI	RNI	RNI	RNI	RNI	AI	
4 years ^a	600	0.7	0.7	7	70	5	
7 years ^a	700	0.9	1.0	9	80	7	
[C] Minerals							
Item	Ca	P	K	Na	Mg	Fe	Zn
Unit	µg	mg	mg	mg	mg	mg	mg
Classification	AI	AI	AI	AI	AI	AI	RNI
4 years ^a	800	500	1500	900	150	12	12.0
7 years ^a	800	700	1500	1000	250	12	13.5

Values are cited from National Institute of Nutrition and Food Safety, China [12].

AI, adequate intake; NV, no value; RNI, recommended nutrient intake.

^aAges of children.

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Conflict of interests

The authors declare that no conflict of interests exists.

Ethical standards

This survey was conducted in accordance with Declaration of Helsinki, 1947. The study protocol was approved on 29 March 2004 by the Institutional Review Board of Peking University, Beijing, China (the approval number; IRB 00001052–0215).

Informed consent

All guardians provided informed consents on behalf of the children.

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