used to measure intra and inter-assay coefficients of variation in laboratory analyses for serum retinol and found to be 6.5% and 3.3%, respectively. VAD was defined as serum retinol level below <0.7 μmol/l or 20 mg/dl [1].

Serum albumin

Serum albumin was determined by end point colorimetric assay in which at pH value of 4.2 albumin display a sufficiently cationic character to be able to bind with bromcresol green (BCG), an anionic dyestuff, to form a blue green complex. The intensity of the blue green colour is directly proportional to the concentration of albumin and was determined photometrically (AUTOLAB PM 4000/3, Analyser Medical System, Italy). The analytical sensitivity (lower detection limit) of the assay which represents the lowest measurable albumin concentration that can be distinguished from zero is 0.2 g/dl or 2 gm/l. Hypoalbuminemia was defined as serum albumin level below 3.5 gm/dl or 35 gm/l [31].

Total Protein

Total serum protein (TSP) was determined by enzymatic reaction sequence in which protein in serum forms a blue coloured complex when reacted with cupric ions in an alkaline solution (Teco Diagnostics, USA). The intensity of the violet colour was proportional to the amount of protein present when compared to a solution with known protein concentration and was determined photometrically (AUTOLAB PM 4000/3, Analyser Medical System, Italy). Hypoproteinemia was defined as serum protein level below 6.2 gm/dl or 62 gm/l [31].

Total Cholesterol

Total cholesterol was determined by enzymatic colorimetric test using cholesterol esterase and cholesterol oxidase. Briefly, cholesterol was converted to cholest-4-en 3-one and hydrogen peroxide by oxygen with the aid of cholesterol esterase. The produced hydrogen peroxide under the catalytic action of peroxidise formed a red dyestuff when reacted with 4- aminoantipyrine and phenol (Biocon Diagnostic, Germany). The colour intensity was directly proportional to the concentration of cholesterol and was determined photometrically (AUTOLAB PM 4000/3, Analyser Medical System, Italy). The analytical sensitivity (lower detection limit) of the assay which represents the lowest measurable cholesterol concentration that can be distinguished from zero was mg/dl (0.08 mmol/Lt). Hypocholesterolemia was defined as serum level below 154 mg/dl [31]. Cholesterol level of below 200 mg/dl was considered as indicator for the absence of lipid metabolic disturbance and cholesterol level of 200-300 mg/dl with HDL cholesterol below 35 mg/dl and above 300 mg/dl is considered as indicator for the presence of lipid metabolic disturbance.

Quality control

In all cases commercially available control materials with established values were used for quality control purpose and no failure was observed to obtain the assigned values. In addition, pooled sera were used to measure intra and inter-assay coefficients of variation in laboratory analyses for serum retinol and found to be less than 5%. Sufficient sera for the determination of albumin, protein and cholesterol were only found for the 107 pregnant women. And also it was not possible to find extra sera in the control group.

Data analysis

Data were analyzed using SPSS version 17 statistical package (SPSS, Inc., Chicago, IL, USA). The significance of differences in the serum vitamin A, total protein, albumin and total cholesterol levels between pregnant women with or without HIV infection and healthy controls was evaluated using a one way analysis of variance with post hoc Tukey test to determine pairs of means which differ significantly. In all cases a P value of < 0.05 was considered statistically significant.

Results

A total of 423 pregnant women were included in this study. Among the 423 pregnant women, 44 (10.4%) were infected with HIV. From the total of 55 non pregnant voluntaries blood donors, who were living in the same geographic locale with the pregnant women, 30 (54.5%) were found to be HIV positive. The mean age of pregnant and the non pregnant women was 25.38 ± 5.68 (range 16-45 years) and 28.07 ± 7.2 (range 18-41 years), respectively. The mean age of pregnant women with and without HIV infection was 25.50 ± 4.90 (range 18-42 years) and 25.37 ± 5.76 (range 16-45 years), respectively. Whereas mean age of asymptomatic HIV positive healthy non pregnant women and healthy HIV negative controls was 29.57 ± 6.77 (range 18-38 years) and 26.28 ± 5.9 (range 18-41 years), respectively, with no significant difference in age distributions (*P value* >0.05).

Table 1 shows the concentrations of serum retinol in pregnant women and non pregnant controls. After controlling for total serum protein, albumin and demographic variables, the mean ± SD serum vitamin A in HIV seropositive pregnant women (0.96 ± 0.42 μmol/L) was significantly lower than that in pregnant women without HIV infection (1.10 ± 0.45 μmol/L, $P < 0.05$). A significant interaction between HIV positive group and level of TSP was observed. To further explore this interaction, we compared the mean retinol concentrations of those with a high TSP and those with hypoproteinemia by HIV status. The mean retinol concentration was significantly lower in the HIV positive pregnant women

Table 1 Socio-demographic characteristics and serum parameters of pregnant and non pregnant women

Characteristics	Pregnant (n = 423)		Non pregnant (n = 55)	
	With HIV (44)	Without HIV (379)	With HIV (30)	Without HIV (25)
Age (years)	25.50 ± 4.90	25.37 ± 5.76	29.57 ± 6.7	26.28 ± 7.4
BMI (kg/m^2)	23.56 ± 3.04[¶]	23.45 ± 3.05[¶]	18.67 ± 4.31	20.55 ± 3.1
Serum retinol (μmol/L)	0.96 ± 0.42*	1.10 ± 0.45[$]	0.74 ± 0.39[$]	1.18 ± 0.59
£Albumin gm/dl	3.65 ± 0.68*	3.99 ± 85	ND	ND
£Total protein (gm/dl)	6.12 ± 0.94	5.99 ± 1.22	ND	ND
£Cholesterol (mg/dl)	144.5 ± 54.20*	168 ± 51.52	ND	ND

Keys: ¶P value <0.001 versus HIV positive non pregnant women and HIV negative non pregnant women; *P value = 0.04 versus HIV negative pregnant women, [$]P value = 0.02 versus HIV positive non pregnant women, [$] P value = 0.000 verse HIV negative non pregnant women, ND: Not done, £ N = 107

with high TSP (P < 0.05) but was not significantly different from that of HIV negative group.

The mean concentration of serum vitamin A was not significantly different between HIV positive pregnant women and HIV positive non pregnant women. It was also not significantly different between HIV negative pregnant women and HIV negative apparently health non pregnant controls. However, mean concentration of serum vitamin A level was significantly higher in HIV positive pregnant women (0.96+/-0.42) than that in HIV positive non pregnant women (0.74+/-0.39) (*P = 0.02*). Irrespective of pregnancy, HIV positive women had significantly lower serum concentration of vitamin A compared with HIV negative women (P < 0.05). Non pregnant women without HIV infection had significantly higher serum vitamin A concentration compared to non pregnant controls with HIV infection, P < 0.001. The mean serum vitamin A level of HIV positive pregnant women was 0.96 ± 0.42 where as the mean serum vitamin A level of HIV negative pregnant mothers was 1.1 ± 0.45. This mean difference in serum vitamin A level between the groups was statistically significant (*P = 0.01*).

Of the total 478 pregnant and non pregnant women (including controls), VAD was observed in 99 (20.7%) with a serum retinol level of less than 0.7 μmol/L (Table 2). The proportion of HIV positive pregnant women with serum retinol levels consistent with severe (0.00-0.34 μmol/L) and moderate (0.35-0.69 μmol/L) VAD was significantly higher than that in healthy controls (without HIV infection) and asymptomatic HIV infected controls (*p*< 0.05). From the total 74 HIV positive women, 33.8% (25/74) had a retinol level of less than 0.7 μmol/L and were vitamin A deficient.

Of 423 pregnant women, VAD was observed in 78 which accounted for 18.4% prevalence rate of VAD among pregnant women. Out of the 44 pregnant women with HIV infection, 11 (25%) were deficient for vitamin A with serum retinol level of below 0.7 μmol/L of which, 3 (6.8%) and 8 (18.2) had moderate (0.35-0.69 μmol/L) and severe (0.00-0.34 μmol/L) vitamin A deficiency, respectively. Similarly, among 30 asymptomatic HIV infected non pregnant voluntaries, 46.7% (14/30) were vitamin A deficient with serum retinol level of below 0.7 μmol/L, of which, 4 (13.3%) and 10 (33.3%) had moderate (0.35-0.69 μmol/L) and severe (0.00-0.34 μmol/L) vitamin A deficiency, respectively.

As indicated in Table 1 and 3, serum albumin and cholesterol concentrations were significantly lower in the HIV positive pregnant women compared with HIV negative pregnant women (P < 0.001). There was no significant difference in TSP value within the two groups (P = 0.06). Although the mean serum levels of vitamin A decreases with age, the difference between was not statistical significance (P = 0.07) (Table 4). Mean serum vitamin A level was significantly lower among women with BMI < 18.5 kg/m^2 than among those with BMI of 18.5 kg/m^2 or above, P value = 0.024 (Table 4). Although, BMI is not a surrogate for nutritional assessment during pregnancy, undernourished (BMI < 18.5 kg/m^2) was observed in 4% of the pregnant women. Body weight and BMI were significantly lower in HIV infected pregnant woman compared to HIV non infected pregnant woman (P < 0.05). Undernourished (BMI < 18.5 kg/m^2) was observed in 50% of asymptomatic HIV positive non pregnant woman (data not shown).

Table 2 Vitamin A deficiency in pregnant and non pregnant women by HIV serostatus

Vitamin A Status	Pregnant women				Non pregnant women			
	HIV +ve	HIV -ve	OR (95%CI)	*P value*	HIV +ve	HIV -ve	OR (95%CI)	*P value*
VAD	11 (25)	67(17.7)	2.50(1.38-4.60)	0.002	14(46.7)	7(28)		
Non VAD	33 (75)	312(82.3)	1.30(1.14-1.50)	0.003	16(53.3)	18(72)		

Keys: +ve: Positive; -ve: Negative

Table 3 Prevalence of micronutrient deficiencies and distribution of pregnant women and controls by HIV infection and serum status of micronutrients

Parameters	Cutoff value	HIV positive pregnant women (44)		HIV negative pregnant women (379)		HIV positive non pregnant women (30)		HIV negative non pregnant controls (25)	
		Deficient	Non deficient	Deficient	Non deficient	Deficient	Non deficient	Deficient	Non deficient
Vitamin A	0.7 μmol/L	33 (75)* 0.79 ± 0.32**	11(25) 1.45 ± 0.25	234 (61.7) 0.82 ± (0.26)$	145 (31.8) 1.54 ± (0.29)	26(86.7) 0.63 ± 0.28)	4(13.3) 1.45 ± (0.16)	12(48) 0.68 ± (0.27)	13(52) 1.6 ± (0.39)
TSP (107)	6.2 gm/dl	8/13(61) 5.6 ± (0.68)	5/13(39) 7.1 ± (0.35)	56/94(59) 5.2 ± (0.55)	38/94(41) 7.2 ± (0.85)	ND	ND	ND	ND
Albumin (107)	3.5 gm/dl	6/33 (18) 3.06 ± (0.39)	27/33(82) 3.04 ± (0.51)	7/74(9) 4.15 ± (0.39)	67/72(91) 4.3 ± (0.64)	ND	.ND	ND	ND
Cholesterol (107)	154 mg/dl	9/13(69 119.8 ± (28)	4/13(31) 200.5 ± (59)	38/94(40) 119.5 ± (20.8)	56/94(60) 200.8 ± (38)	ND	ND	ND	ND

Keys: *Number (%), ** Mean ± SD, $P value< 0.05 versus HIV negative non pregnant controls, ND: Not done

Discussion

This cross-sectional study is the first of its kind in Ethiopian pregnant mothers with and without HIV infection in which serum levels of vitamin A has been measured. In this study, we found biochemical evidence that VAD was common in pregnant women, regardless of HIV status in Northwest Ethiopia. VAD has been defined as a public health problem when the prevalence of VAD, judged by serum retinol less than 0.7 μmol/l, among pregnant women is 15% [32]. In the present study, the overall prevalence of VAD among pregnant and non pregnant women was 18.4%, thus indicating a marginally major public health problem but neglected among these pregnant women in tropical settings of Northwest Ethiopia.

Due to multiple guidelines and lack of precise recommendation, nutritional surveys have used diverse reference values as a cut off indicating deficient vitamin A status. This in turn has jeopardized comparability of results of population-based prevalence studies. Although international consensus has yet to be establishes, serum retinol concentration below a cutoff of 1.05 μmol/l has been proposed to reflect low vitamin A status among pregnant and lactating women [21]. While the distribution of serum retinol concentrations below appropriate cut-offs are considered to reflect inadequate states of vitamin A nutriture, a low biochemical concentration of retinol in circulation is not considered a VAD. Thus, it is difficult to compare rates of VAD among previous studies. Even with this limitation, the 18.4% prevalence of VAD in this study population of pregnant women is a lower rate compared with previous report in a settings where about 40% rate of VAD has been reported [33] and previous reports from Bangladesh where VAD as high as 69% had been reported [34,35]. However, it is in agreement with a South African study which showed 16% VAD among mothers during the first 6 months of delivery [36]. The high rate of VAD in our study group could be due to dietary factors including inadequate vitamin A intake in Ethiopian population [24] and the relatively poor socio-economic and nutritional status of Ethiopian mothers compared with mothers in some other parts of developing nations.

Table 4 Relationship between serum vitamin A level with age and body mass index (BMI)

Variables	N	Serum retinol (μmol/L) Mean ± SD	*P value*
Age (Years)			
< 20	20	1.09+0.44	0.070
21-30	274	1.06+0.45	
31-40	73	1.04+0.48	
41+	4	0.88+0.57	
BMI (kg/m^2)			
<18.5	34	0.89 ± 0.43	0.024
>18.5	444	1.08 ± 0.45	

This study showed that, HIV positive pregnant women had significantly lower mean serum retinol concentration than did HIV negative pregnant women ($P<0.05$). This finding was consistent with a study from Malawi [37], Rwanda [38,39] and Zimbabwe [40]. The low levels of serum vitamin A might be due to a low release of vitamin A from the liver during HIV infection, which would lead to low levels of vitamin A in the plasma despite the body having enough vitamin A liver stores [16]. The mean serum vitamin A level of HIV seropositive pregnant women was 0.96 ± 0.42 where as the level in HIV negative pregnant mother was 1.1 ± 0.45 (P value <0.001). This significant difference in serum vitamin A between HIV positive and negative pregnant women remained after measures of total serum protein, albumin and demographic variables (age, weight, BMI) were controlled for, which was also in agreement with a Zimbabwean study [40]. Low serum vitamin A level among HIV infected pregnant women was reported to be associated with a higher risk of vertical transmission

of HIV [37-39], increased risk of preterm delivery and maternal anaemia [41].

HIV positive women had significantly lower serum concentration of vitamin A compared with HIV negative women ($P < 0.01$), irrespective of pregnancy. Serum retinol may transiently be low due to reduced production of retinol binding protein, which is often observed as part of the acute-phase response to infection [42]. Furthermore, it is also important to note that sub-clinical infection or inflammation has been found to be associated with the reduction of serum retinol level due to reduced production of retinol binding protein [42]. Although, we do not have any indication on the presence of sub-clinical infection on our study group and we did not measure acute inflammatory responses in the present study. It should be noted that, times of blood collections for all pregnant women was their routine antenatal follow up and all participants were healthy. Other possible confounding factors such as intestinal helminths [42,43] may influence serum retinol concentrations as high prevalence of helminthic infection was reported in the study area [26,27,43]. Low intake of green vegetables, fruit and dairy products could also be contributing factors for the low serum vitamin A level, as the staple food in Northwest Ethiopian is mainly a pancake named *enjera* made from a cereal called Tef (*Eragrostis teff*). Usually the *enjera* is eaten with stew made from legumes (mainly pea and beans).

In this study, overall prevalence of hypoalbuminaemia in pregnant women was relatively low (12%). This finding is similar to that of an earlier study carried out among Ethiopian pregnant mothers in the same locality [25]. No significant interaction between HIV sero-positive status during pregnancy and level of TSP was observed among vitamin A deficient women suggesting the lack of contribution of general protein-energy undernourished to VAD. In the present study, undernourished (BMI <18.5 kg/m^2) women were found to have statistically significantly lower serum retinol level than well-nourished (BMI > 18.5 kg/m^2) women. This is consistent with other studies from Bangladesh and Nepal [9,35].

Conclusions

The present study shows that VAD among pregnant women in tropical settings of northwest Ethiopia is a major public health problem. Irrespective of pregnancy, HIV positive women had significantly lower serum concentration of vitamin A levels compared with HIV negative women. Considering the possible implications of VAD during pregnancy, we recommend multivitamin (which has a lower level of vitamin A) supplementation in the care and management of pregnant women with or without HIV infection.

Limitations

This study has the following basic limitations. First, due to the presence of multiple guidelines and lack of precise recommendation, nutritional surveys have used diverse reference values as a cut off between normal and deficient vitamin A status. As a result, VAD in this study was defined based on WHO recommendation which are wildly accepted and used [1], as serum retinol level below 0.7 μmol/L. Second, micronutrient status is difficult to assess in the presence of infection, because biochemical indicators of several micronutrients are affected by the acute phase response. For instance, albumin, and retinol are "negative" responders, which increase during an acute phase response markers [36]. Thus, since in the present study, acute phase response (for example, C-reactive protein (CRP) and alpha 1- acid glycoprotein (AGP)) were not measured and controlled. Such markers were not measured in these subjects, due to the lack of laboratory facility. Hence, the results may be confounded by increased rates of acute phase response measures in the HIV positive women and may lead to an underestimation of vitamin A and albumin status. Third, body mass index (BMI) was used to determine the nutritional status of the study subjects though it is not a surrogate for nutritional assessment during pregnancy. Lastly, the low number of sample size among the control group resulted inflated HIV prevalence and hence might influence the results.

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Authors' contributions

AM: conception of the research idea, study design, data collection and analysis and interpret the data and the draft of the manuscript; AK: conception of the research idea, study design, data collection and analysis, interpret the data and reviewed the manuscript; KH, BT, GY: data collection, part of laboratory work, data analysis and reviewed the manuscript; SY, MN, NN, FO: data analysis and interpretation and reviewed the manuscript; AB, YW: study design, interpret the data and reviewed the manuscript. All authors have read and approved the final version of the manuscript.

Competing interests
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ORIGINAL

Dietary zinc intake and its effects on zinc nutrition in healthy Japanese living in the central area of Japan

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Abstract : In the present study, we first examined the dietary zinc intake from food groups in 109 healthy Japanese (24-82 years old, 45 male and 64 female) by means of the 72-h recall method. We then used the ratio of apo/holo-activities of angiotensin converting enzyme (ACE ratio) that is a more sensitive index of zinc nutrition than zinc concentration in the serum and examined the correlation between their zinc intake and ACE ratio. Dietary zinc intake in healthy Japanese was maximal from rice and rice products. There were significant inverse correlations between the ACE ratio and dietary zinc intake from rice and rice products and shellfish, and a significant positive correlation between ACE ratio and dietary zinc intake from other beans and bean processed foods. On the other hand, there were no significant correlations between serum zinc concentrations and dietary zinc intake from any food group except processed fish. These findings suggested that rice is a major source of dietary zinc intake in healthy Japanese. It is also suggested that shellfish also has a major impact on zinc nutrition, although dietary zinc intake from this source is minimal. Since beans contain phytic acid, which inhibits the absorption of dietary zinc, it is suggested that intake of beans causes impairment of zinc nutrition. J. Med. Invest. 58 : 203-209, August, 2011

Keywords **:** *zinc nutrition, dietary zinc intake, zinc concentration in serum, angiotensin converting enzyme*

INTRODUCTION

Zinc is an essential trace element playing a role in several physiological functions in both humans and animals. In fact, zinc deficiency has been associated with growth disturbance (1-3), impairment of special senses including vision, taste and smell (4, 5), anorexia (6.7), dermatitis (8), sexual dysfunction as well as fetal and pregnancy complications (9-12). Especially, a major clinical manifestation of zinc deficiency is taste impairment, as shown by double-blind, placebo-controlled studies that have shown the efficacy of zinc supplementation in the taste recovery in patients with hypogeusia and low serum zinc concentration (13, 14).

But, in another double-blind, placebo-controlled

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study, Yoshida *et al.* reported the therapeutic effect of zinc picolinate in patients with idiopathic taste impairment with normal zinc levels in the serum (15). Therefore, it was suggested that zinc deficiency is a predominant factor underlying hypogeusia even when zinc concentrations are within normal ranges in the serum. It was also suggested that serum zinc concentration that has been widely used to assess zinc nutrition (16) is not a reliable indicator of the zinc nutritional status, probably because most zinc is associated with albumin and α-microglobulin in the blood, of which levels are influenced by stress and invasion, with the remaining less than 5% being the free form of zinc (17).

Recently, we developed a new test for the assessment of zinc deficiency taking the ratio of apo/holo-activities of angiotensin converting enzyme (ACE), a zinc dependent enzyme, as an index, suggesting that the ratio of apo/holo-ACE activities (ACE ratio) is a more sensitive index of zinc nutrition than measuring zinc concentration in the serum (18-20). In the present study, we first examined the daily zinc intake from each food group in healthy Japanese living in the central area of Japan (Nagano prefecture) by means of the 72-h recall method. We then used ACE ratio and zinc concentration in the serum as indices and examined the correlations between dietary zinc intake and zinc nutrition in healthy Japanese.

MATERIALS AND METHDS

Subjects

The present study included 109 healthy Japanese (24-82 years old, 45 male and 64 female) who participated in an annual mass health examination program of the Kita-mimaki area of Tomi city in Nagano prefecture (the central area of Japan) (21) in 2005. Their mean body height and weight was 158.6±7.8 cm and 58.9±9.0 kg, respectively. This study was performed in accordance with the declaration of Helsinki. The purpose and procedures of the study were explained to all subjects before obtaining informed consent. Dietary survey and blood sampling were performed in each subject in the morning.

Dietary zinc intake

Dietary survey was performed using the 72-h recall method (22). Dietitians interviewed each subject to identify foods and beverages ingested in the last three days. Subjects' dietary zinc intakes by food group were then calculated with the Standard Tables of Food Composition in Japan (5th revised and enlarged edition) (23). Foods recorded in the survey were grouped into 33 groups : rice and rice products, flour and flour products, other cereals, potato and potato products, other starch, sugar and sweetener, soybean and soybean processed foods, other beans and bean processed foods, seeds and nuts, green and yellow vegetables, other vegetables, vegetable juice, pickles, fruit, jam, fruit juice, mushrooms, seaweed, fish, processed fish, shellfish, beef, pork and other meat, poultry, organ meat, other meat, eggs, milk and dairy products, oils and fats, sweets, alcohol, other beverages, seasonings and spices.

Zinc concentration

Zinc concentration in the serum was measured by means of atomic absorption spectrometry (24) by SRL Co., Ltd. (Tokyo, Japan). Normal ranges of zinc concentrations in the serum are from 65 to 110 μg/dl.

ACE ratio

ACE activity in the serum was measured using a synthetic substrate, hippuryl-His-Leu (HHL), specifically designed for ACE (Peptide Institute Inc. Osaka, Japan). Twenty five μl of serum were incubated for 30 min at 37°C with 5 mmol/l HHL in 100 μl of 100 mmol/l phosphate buffer, pH 8.3, containing 600 mmol/l NaCl. The reaction was terminated by addition of 375 μl of 3% metaphosphoric acid, and then the mixture was centrifuged at 10,000 rpm for 10 min at 4°C. The supernatant was applied to a reverse-phase column (4 mm i.d.× 250 mm ; IRICA Instrument, Kyoto, Japan), which had been equilibrated with 10 mmol/l KH_2PO_4 and CH_3OH (1 : 1, pH3.0), and eluted with the same solution at a rate of 0.5 ml/min. Hippuric acid was detected by ultraviolet absorbance at 218 nm. One unit of ACE activity was defined as the amount of enzyme that cleaved 1 μmol hippuric acid/min (25).

Since ACE is a zinc-metallo enzyme, holo-ACE with zinc shows full ACE activity. After measuring ACE activity in the serum, ACE activity was then measured by the addition of zinc (150 μM in phosphate buffer at pH 8.3) to the serum *in vitro*. The increase of activity over the initial holo-ACE activity was determined as that of apo-ACE in the serum (26). The ratio of apo/holo-ACE activities was calculated as follows : ACE ratio (%) = apo-ACE

activity/holo-ACE activity × 100 (15-17).

Statistical analysis

The Pearson correlation analysis was used to evaluate the relationships among groups (Statistical Package for Social Science, version 16.0, Japan Inc., Tokyo, Japan). Results were considered statistically significant at p-values< 0.05.

RESULTS

The average total dietary zinc intake was 9.49± 3.11 mg/day (mean± S.D.) in 109 healthy Japanese (Table 1). Among food groups, subjects' dietary zinc intake was maximal from rice and rice products at 1.97± 0.90 mg/day, followed by milk and dairy products, soybeans and soybean processed foods, beef,

Table 1 Dietary zinc intake from food groups in healthy Japanese.

Food group	Dietary zinc intake (mg/day, mean± S.D., n=109)
Rice and rice products	1.97± 0.90
Milk and dairy products	0.91± 0.55
Soybean and soybean processed foods	0.83± 0.55
Beef, pork and other meat	0.81± 0.66
Fish	0.77± 0.71
Other vegetables	0.76± 0.53
Eggs	0.47± 0.28
Green and yellow vegetables	0.46± 0.33
Flour and Flour products	0.39± 0.30
Processed fish	0.36± 0.72
Other beverages	0.22± 0.29
Fruit	0.20± 0.19
Seasonings	0.20± 0.09
Sweets	0.16± 0.22
Potato and potato products	0.13± 0.11
Seeds and nuts	0.13± 0.19
Poultry	0.12± 0.22
Mushrooms	0.08± 0.08
Shellfish	0.07± 0.17
Other cereals	0.07± 0.16
Seaweed	0.06± 0.05
Other beans and bean processed foods	0.06± 0.17
Organ meats	0.04± 0.24
Alcohol	0.02± 0.05
Vegetable juice	0.01± 0.03
Spices	0.01± 0.01
Other meat	0.00± 0.04
Pickles	0.00± 0.01
Oils and fats	0.00± 0.00
Sugar and sweeteners	0.00± 0.01
Fruit Juice	0.00± 0.01
Jam	0.00± 0.00
Other starch	0.00± 0.00
Total	9.49± 3.11

pork and other meat, and fish. Although dietary zinc intake from flour and flour products was 0.39± 0.30 mg/day, the dietary zinc intake from rice and rice products correlated inversely with that from flour and flour products (R= -0.46, p< 0.001).

There were significant inverse correlations between the ACE ratio and dietary zinc intakes from rice and rice products (R= -0.190, p< 0.05), shellfish (R= -0.207, p< 0.05) and other starches (R= -0.268, P< 0.01) in healthy Japanese (Table 2). On the other hand, there were significant positive correlations between the ACE ratio and dietary zinc intakes from other beans and bean processed foods (R=0.333, p< 0.001) and poultry (R=0.265, p< 0.01). However, there was only a significant positive correlation between zinc concentration in the serum and dietary zinc intake from processed fish (R=0.192, p< 0.05) in healthy Japanese (Table 2).

There was an significant inverse correlation between the ACE ratio and zinc concentrations in

Table 2 Correlation between dietary zinc intake from food groups and ACE ratio or zinc concentration in the serum of healthy Japanese.

Food group	R	
	ACE ratio	Serum zinc concentration
Rice and rice products	-0.190*	0.093
Milk and dairy products	0.075	-0.086
Soybean and soybean processed foods	-0.001	0.022
Beef, pork and other meat	0.014	0.052
Fish	0.060	-0.011
Other vegetables	0.107	0.024
Eggs	-0.078	0.065
Green and yellow vegetables	0.141	-0.147
Flour and Flour products	0.156	-0.083
Processed fish	-0.095	0.192*
Other beverages	0.030	0.002
Fruit	0.107	-0.003
Seasonings	-0.127	0.057
Sweets	0.184	-0.144
Potato and potato products	0.074	-0.015
Seeds and nuts	0.079	0.047
Poultry	0.265**	-0.168
Mushrooms	0.023	0.007
Shellfish	-0.207*	0.161
Other cereals	-0.029	-0.050
Seaweed	-0.143	0.167
Other beans and bean processed foods	0.333***	-0.158
Organ meats	0.008	-0.004
Alcohol	0.119	-0.106
Vegetable juice	-0.122	0.091
Spices	-0.029	-0.011
Other meat	0.104	-0.132
Pickles	0.092	-0.080
Oils and fats	0.046	-0.074
Sugar and sweeteners	0.021	0.006
Fruit Juice	-0.075	0.057
Jam	0.062	-0.093
Other starch	-0.268**	0.045
Total	0.047	0.036

*$p< 0.05$, **$p< 0.01$, ***$p< 0.001$

the serum in healthy Japanese (R= -0.549, p< 0.001) (Fig. 1). But, there were no correlations between dietary zinc intake and the ACE ratio or zinc concentrations in the serum (Figs. 2 and 3).

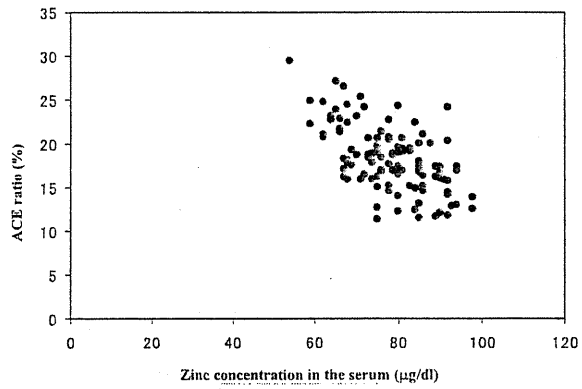

Fig. 1 : Inverse correlation between ACE ratio and zinc concentration in the serum in healthy Japanese living in Nagano prefecture (R= -0.549, p< 0.001).

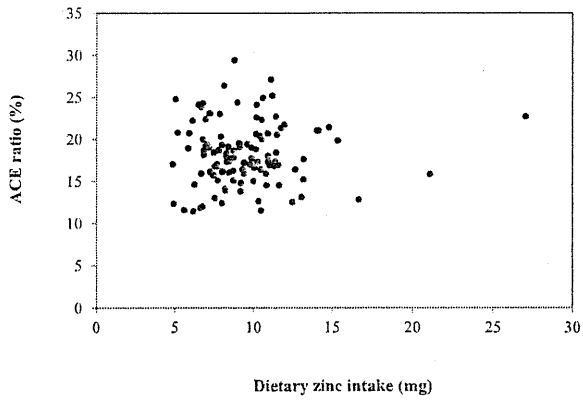

Fig. 2 : No correlation between dietary zinc intake and ACE ratio in healthy Japanese Nagano prefecture (R=0.047).

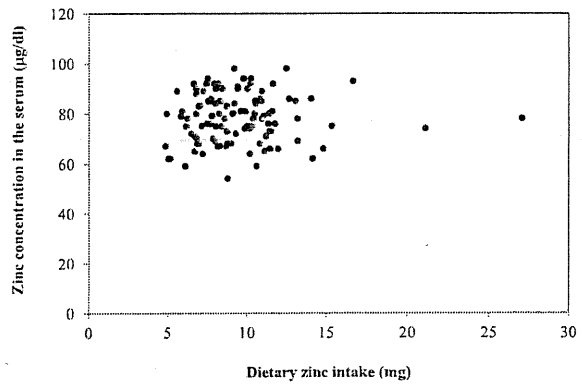

Fig. 3 : No correlation between dietary zinc intake and zinc concentration in the serum in healthy Japanese living in Nagano prefecture (R=0.036).

The average total dietary intake of energy, protein, fat and carbohydrate in 109 healthy Japanese were 1914± 448 kcal/day, 79.4± 20.8 g/day, 48.0± 15.3 g/day and 277.9± 75.9 g/day, respectively.

DISCUSSION

In the present study, the average total dietary zinc intake in healthy Japanese living in Nagano prefecture was 9.49± 3.11 mg/day, which is slightly more than the averaged Japanese value of dietary zinc intake (8.33 mg/day) from the National Nutrition Survey in Japan conducted by the Ministry of Health, Labor and Welfare, 2005 (27). Their dietary zinc intake was maximal from rice and rice products at 1.97± 0.90 mg/day, which was followed by milk and dairy products, soybeans and soybean processed foods, beef, pork and other meat, and fish. Although the concentration of zinc in rice is not so high (0.6 mg/100 g of well-milled rice from the Standard Tables of Food Composition in Japan) (23), it is suggested that rice and rice products are a major source of dietary zinc intake, probably because most Japanese eat large amounts of rice as a staple food. On the other hand, dietary zinc intake from flour and flour products, which are a minor source of zinc for most Japanese, was 0.39± 0.30 mg/day. However, the dietary zinc intake from rice and rice products correlated inversely with that from flour and flour products. This finding suggested that flour and flour products are another major source of dietary zinc intake in some Japanese who eat bread as a staple food.

There were inverse correlations between the ACE ratio and dietary zinc intake from rice and its products, and from shellfish. Since healthy Japanese receive zinc maximally from rice as a staple food, it is suggested that rice has a major impact on zinc nutrition as evaluated by ACE ratio, in addition to its being a major source of dietary zinc intake. On the other hand, dietary zinc intake from shellfish was minimal (0.07± 0.17 mg/day). However, the inverse correlation between the ACE ratio and dietary zinc intake from shellfish suggested that shellfish also has a major impact on zinc nutrition in healthy Japanese, probably because shellfish contains high concentrations of zinc (for example, 13.2 mg/100 g in oyster from Standard Tables of Food Composition in Japan) (23).

Although dietary zinc intake from other beans and bean processed foods was 0.06± 0.17 mg/day, there

was a positive correlation between the ACE ratio and dietary zinc intake from other beans and bean processed foods. Generally, healthy Japanese consume a considerable amount of beans, which contains a large amount of phytic acid (for example, 0.61-2.38 mg/100 g of kidney beans) (27, 28). Since phytic acid has been shown to inhibit the absorption of dietary zinc from the bowel (29), it is suggested that intake of beans causes impairment of zinc nutrition in healthy Japanese.

Although there was a significant inverse correlation between the ACE ratio and zinc concentration in the serum, there were no significant correlations between zinc concentration in the serum and dietary zinc intake from any food groups except processed fish. These findings were in line with our previous conclusion that the ACE ratio is a more sensitive indicator than the concentration of zinc in the serum in evaluating dietary zinc nutrition (18-20).

Although number of subjects tested in the present study was limited and their food intake may vary from day/season to day/season, their dietary intake of energy, protein fat and carbohydrate were in line with those reported from the National Nutrition Survey in Japan conducted by the Ministry of Health, Labor and Welfare, 2005 (27).

In conclusion, in the present study, the dairy zinc intake from food groups and the effects of food groups on zinc nutrition using the ACE ratio as an index were examined in healthy Japanese living in Nagano prefecture. It was suggested that rice and rice products are a major source of dietary zinc intake in healthy Japanese, probably because most Japanese eat large amounts of rice, which contains low concentrations of zinc, as a staple food. It was also suggested that shellfish, which contains high concentration of zinc, also has a major impact on zinc nutrition, although its dietary zinc intake was minimal. Since beans generally contain a large amount of phytic acid, which inhibits the absorption of dietary zinc from the bowel, it is suggested that intake of beans causes impairment of zinc nutrition.

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ORIGINAL

Study on the necessary survey days for energy intake in school children assessed by 7 day survey

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Abstract : Theoretically, the longer the period of a nutrition survey, the more reliable the results. However, a long survey can impose a burden on subjects and cause the results to become inaccurate. For adults, a 3 non-consecutive day survey is usually recommended ; however, for school children, at least in Japan, it has not been determined whether this is necessary. In this study we conducted a survey of 7 days and tried to find the minimum number of days necessary to determine the energy intake. The subjects were about 300 children aged from 6 to 7, 10 to 11 and 13 to 14 years old in a city in the western part of Japan. The weighing method was used for the school lunch and other meals were surveyed by 24-recalling method. For the 6-7 year-old school children, guardians were asked to keep dietary records. The final number of subjects who were able to complete the 7-day survey was 139. Energy intakes for each weekday were not statistically different ($p > 0.05$) and those for each weekend did not differ ($p > 0.05$). Average energy intakes on weekdays were higher than those on weekend days in 10-11 and 13-14 year-old children. The average intakes of energy in 10-11 and 13-14 year-old children were lower than Japanese estimated energy requirements (EER). However, body weight of more than 90% of subjects was within the normal range. The results suggest that a survey of one weekday is reliable for all weekdays and that of one week-end day is reliable for any weekend day and also indicate the necessity of further studies of EER in rapidly growing children. J. Med. Invest. 59 : 111-115, February, 2012

Keywords *: Japanese, school children, energy intake, necessary survey day*

INTRODUCTION

The dietary habits of Japanese school children have been changing in accordance with lifestyle. It is important to ensure a proper nutritional intake in children not only for physical and mental development, but also to establish good health habits to sustain them as adults. Energy is the most important factor in preparing meals, especially school meals, and also for body weight control. According to the 2007 National Health and Nutrition Survey in Japan (1), the ratios of obese and slightly obese in 12 to 14 year-old children changed from 2000 to 2006 : boys from 19.5% to 7.9%, girls from 25% to 20.3%.

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On the other hand, the ratios of thin and slightly thin children in the same age group changed as follows : boys from 29.2% to 37.7% and girls from 18.2% to 29.7%. In response to this situation, a licensing system for nutrition teachers was established to help Japanese school children acquire proper dietary habits (2). For guiding school children in proper eating habits, it is important to know their actual daily eating habits, especially their energy intake. In general, conducting surveys covering three non-consecutive days is recommended for surveys when the subjects are adults (3). However, the number of days available to estimate dietary habits of school children are often limited. In this study, we made a first attempt at establishing the number of survey day(s) necessary to determine energy intake.

METHODS

Study area and subjects

A census was conducted in a prefecture on three groups comprised of a total of about 300 schoolchildren aged from 6 to 7, from 10 to 11 (in elementary school), and from 13 to 14 (in junior high school). Each group consisted of about 100 children, made up more or less equally of boys and girls.

Survey period

The survey was conducted for seven consecutive days including either 4 weekdays and 3 weekend days or 5 weekdays and 2 weekend days in November 2009. An investigation of physical status was conducted before the commencement of the survey.

Cooperators

Requests for cooperation were made to elementary schools and junior high schools through the Board of Education of the city, and to research institutions such as universities, as well as to nutrition instructors and dietetic association members in the prefecture.

Survey of eating habits

Regarding the intake of school lunches, researchers measured the actual amount of each student's portion, while that of other meals, including in-between snacks, were surveyed by the 24-hour recalling method with the cooperation of the students' guardians. In the case of incomplete items or unclear descriptions on the form, the researchers confirmed details with the students directly or asked their guardians to fill out the items.

Calculation of nutrient intakes was made in accordance with the data listed in "Standard Tables of Food Composition in Japan, the Fifth Revised and Enlarged Edition"(4) (Food Composition Table). After the survey, the researchers converted estimated average requirements into gram mass, based on the "Standard Volume-to Weight Conversion Table"(5) included in the Food Composition Table (2001), which is used for the national health survey.

The nutritional intake was calculated based on data from the meal survey and the school lunch menu, obtained through the methods described above.

Degree of obesity

This study collected data on the height and weight of school children obtained at the time of the annual checkup conducted by each school in April, 2009 or the data collected on eating habits before commencing the survey. Using these data, the degree of obesity was derived by the following equation (6).

The degree of obesity=(Actual measured weight-Weight for height standards)/Weight for height standards x 100.

Children whose values were 20% greater than average were defined as obese, while children whose values were 20% lower than the average were defined as thin. Weight for height standards were derived from mean weights using the Annual Report of School Health Statistics Research 2009 (7)

Statistical analysis

Data were assessed by one-way ANOVA and then Tukey's multiple comparison test. The level of significance was set at $p< 0.05$.

Ethical considerations

This study was conducted with the approval of the Ethics Committee of Ochanomizu University, and in accordance with the "Helsinki Declaration : Ethical Principles for Research Involving Human Subjects" with special attention paid to the following : To prevent the identification of individuals, each subject's personal information was carefully coded and obtained data were strictly managed. We obtained consent that the participation in the research was by free will from the participants and their guardians by providing explanations about the objectives and details of the survey and the intention

to use the results for oral and written presentations. Even after commencement of the study, subjects were free to drop out, either of their own volition or at the guardian's behest, and no subjects were penalized in any way.

RESULTS

Subjects

The number of the subjects to be analyzed was 139 in total : boys and girls (6-7 year old 23 boys and 29 girls, 10-11 years old 16 boys and 12 girls and 13-14 year old 31 boys and 28 girls).

Table 1 shows the bodily features of the subjects to be analyzed. Obesity rates estimated by the equation shown in methods (%) for boys and girls were 3.5 ± 11.7 and -1.9 ± 8.0 (6-7 years old), -3.0 ± 11.9 and -3.3 ± 17.4 (10-11years old) and -3.3 ± 8.2 and 0.2 ± 17.4 (13-14 years old). When the distribution is normal, 95% of the subjects are between the range of mean-2SD and +2SD. When the sample size is over 30, the distribution is usually normal. Our subjects number was 139 in total. In fact among 139 subjects, 130 (93.5 %) had the normal body weight (from -20% to +20% of the standard weight for height reported by the Annual Report of School Health Statistics Research 2009 (7), only 9 (6.5%) were overweight and none was underweight.

Energy intake on weekdays and weekends

Figure 1 shows energy intake on weekdays and weekends by gender. The analysis was conducted for five weekdays with school lunch and two weekend days ; however, in the case of the school children aged from 10 to 11, due to a temporary school

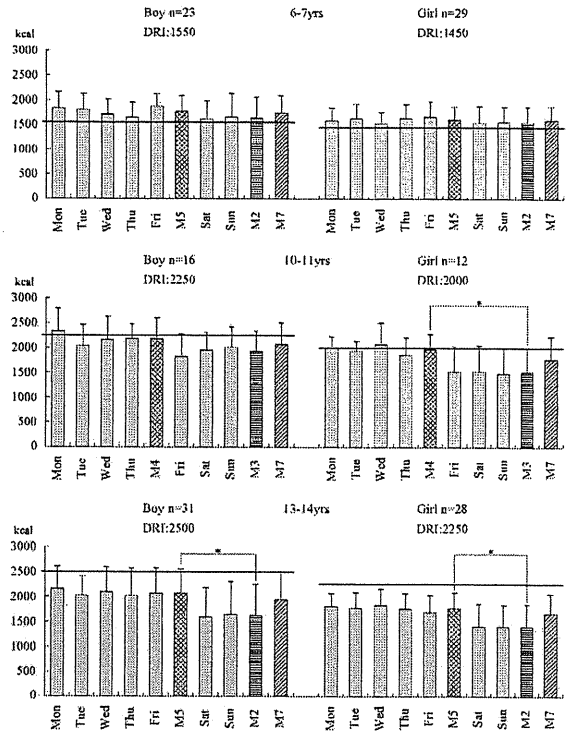

Fig. 1. Energy Intakes of 6-7, 10-11 and 13-14 yrs old children.
Data are means ± SD. Horizontal bar indicates the value of Japanese DRI 2010. M5 : Mean values of 5 weekdays (Monday to Friday), M2 : Mean values of 2 weekend days (Saturday and Sunday), M7 : Mean values of 7 days (Monday-Sunday), M4 : Mean values of 4 weekdays (Monday to Thursday), M3 : Mean values of 3 weekend days (Friday, Saturday and Sunday). For the DRI, estimated energy requirement for level-II physical activity based on dietary reference intakes for Japanese, 2010 was used. For 6-7 and 13-14 years old groups, the analysis was conducted for five weekdays with school lunch and two weekends ; however, for 10-11 years old group, due to a temporary school closing (on Friday), weekends were defined as Friday through Sunday and the analysis was conducted for four weekdays and three weekends. $*p < 0.05$

Table 1. Bodily features of the subjects

	Boy			Girl		
	6-7 yrs. (n=23)	10-11 yrs. (n=16)	13-14 yrs. (n=31)	6-7 yrs. (n=29)	10-11 yrs. (n=12)	13-14 yrs. (n=28)
Height (cm)	127.2 ± 6.1	139.7 ± 7.4	158.3 ± 9.5	124.7 ± 5.3	142.2 ± 6.9	154.1 ± 6.7
Weight (kg)	27.2 ± 4.0	33.7 ± 7.2	45.9 ± 7.1	24.5 ± 3.1	34.7 ± 7.8	47.0 ± 10.9
Degree of Obesity (%)	3.5 ± 11.7	-3.0 ± 11.9	-3.3 ± 8.2	-1.9 ± 8.0	-3.1 ± 17.4	0.2 ± 17.4

(mean value ± standard deviation)
Note 1 : The degree of obesity (Murata Method) = (actual measured weight - Weight-for-height standards/Weight-for-height standards) x 100
Weight-for-height standards were collected from the Annual Report of School Health StatisticsSports, Science and Technology). Research 2009 (the Ministry of Education, Culture, Sports, Science and Technology).

closing (on Friday), weekends were defined as Friday through Sunday and the analysis was conducted for four weekdays and three weekend days.

Subjects aged from 6 to 7

Energy intakes for each of the 7 days were statistically similar ($p > 0.05$) and the mean value of energy intake of boys was 1734 ± 351 kcal, while that of girls was 1,591 ± 288 kcal. It was found that both boys and girls satisfied the Estimated Energy Requirement (EER) 2010 (8).

Subjects aged from 10 to 11

Regarding the energy intakes on weekdays, there was no significant difference among four weekdays. The energy intake of boys for four weekdays was 2177 ± 423 kcal and that for girls 1964 ± 319 kcal. They were slightly lower than EER values (2,250 kcal for boys and 2,000 kcal for girls). The energy intakes on weekend days were 1,928 ± 409 kcal for boys and 1,516 ± 491 kcal for girls. The intakes against DRIs were 86% for boys and 76% for girls, respectively.

Subjects aged from 13 to 14

Energy intakes for each weekday were not statistically different ($p > 0.05$) and those for each weekend did not differ ($p > 0.05$). The mean energy intake of boys for five weekdays was 2,075 ± 476 kcal (EER : 2,500 kcal), while that of girls was 1,774 ± 311 kcal (EER : 2,250 kcal). The mean energy intake of weekend days was 1,617 ± 629 kcal for boys and that 1,404 ± 444 kcal for girls. The mean energy intake on weekend days was lower than that on weekdays ($p < 0.05$) in both gender. Boys satisfied about 65% of the EER, while girls satisfied only about 64% of the EER.

DISCUSSION

The results of this study suggest that for estimating the energy intake, a survey of one weekday is reliable for all weekdays, and that of one weekend day is reliable for all weekend days. According to Marr and Heady *et al.* (9), who conducted 7-day surveys of eating habits on males aged between 30 and 67 living in London, they reported 2-3 days is necessary to obtain reliable energy intake data. Basiotis *et al.* (10) reported that in American adults the minimum number of daily food records required to estimate energy intake is 3 days. Although the previous studies were conducted mostly in adults, Nelson *et al.* (11) conducted a survey in infants, school children, pregnant women and elderly people and reported that the survey period to estimate energy intakes can be less than seven days. we also tried an equation reported by Beaton *et al.* (12) to determine the necessary survey days required to estimate regular intakes of individuals. The equation was : $n = (1.96 \times CV \div D)^2$, where 1.96 is the 95% confidence range, CV is standard deviation (SD)/mean (%) and D is the deviation of the mean (%). Using this equation, we estimated the necessary survey days to estimate the energy intake in our subjects. The CVs was 7-9%. The results suggest that one weekday and one weekend day are enough, if 15% of deviation of the mean is used.

The energy intakes of 10-11 year-olds and 13-14 year-olds were similar in spite of different body weight and also lower than the values of Japanese EER (8). As one of the factors, we have to consider the accuracy of our survey. In our previous studies by the 24-hour recalling method for a 3 day survey in 900 children throughout the whole country also showed a similar tendency (report of the Japanese Ministry of Education, Science and Sports in 2009 unpublished). There are not other useful data for Japan, perhaps due to the difficulty of conducting surveys of school children. Another possible factor may be the strong desire to be thin among adolescents, especially girls as reported by Sano *et al.* (13). However, this is not an adequate explanation because among 139 subjects, none was underweight. If this is the case, there is a possibility that the Japanese estimated energy requirement (EER) for these age groups is too high. The basal metabolic rate (BMR) is the key to determining the EER, but BMR drops rapidly from children to young adults. For example, BMRs (kcal/day/kg body weight) at 10, 15 and 20 years old are : for boys 37.4, 27.0 and 24.0 respectively and for girls 34.8, 25.3 and 22.1 respectively. Basal metabolism rate (kcal/person) is obtained by multiplying BMR and body weight. The body weight (kg) of 10, 15 and 20 years old are : for boys 35.5, 58.4 and 63 respectively and for girls 34.5, 50.6 and 50.6 respectively. Considering the rapid changes in BMR and body weight in these age groups, our finding (a similar energy intake in 10-12 and 13-14 year-old children) may be possible. We need further studies about the energy requirements for school children.

The energy intake for weekend days was lower than that during weekdays except for the 6-7 year-old group. One of the factors contributing to the low energy intakes for these groups on weekends may be that there were many subjects who had just two meals a day on weekends because they skipped breakfast on weekend days. Missing breakfast can be correlated with age. However, further studies are required to reach a definite conclusion.

This study attempted to establish a survey period to ascertain energy intake focusing on school children, taking into account the daily eating habits of Japanese people, and suggested that it is possible to obtain reliable data by a survey conducted on one weekday and on one weekend day, thereby reducing the work load for subjects. Therefore, it can be said that the survey method described above has significant advantages.

CONFLICT OF INTEREST

None of the authors have any conflicts of interest to declare.

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Brief report

Self-reported fast eating is a potent predictor of development of impaired glucose tolerance in Japanese men and women: Tsukuba Medical Center Study

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ABSTRACT

We recorded self-reported eating patterns in 172 Japanese men and women who were subsequently followed for 3 years for the occurrence of impaired glucose tolerance (IGT).

Incidence of IGT was significantly higher in those who reported eating fast. Self-reported eating fast is a potent risk factor for development of IGT.

1. Introduction

Appropriate eating is essential in preventing IGT and T2DM. The association of dietary content, i.e., what to eat or how much to eat, with the development of IGT and T2DM has been well established. In contrast, to our knowledge the association between eating patterns and the risk of IGT and T2DM has not been investigated despite its importance in medical nutritional therapy.

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Abbreviations: IGT, impaired glucose tolerance; NGT, normal glucose tolerance; T2DM, type 2 diabetes; FPG, fasting plasma glucose; 2-hvalue, 2-h postprandial glucose; HDL, high-density lipoprotein; BMI, body mass index; CRP, C-reactive protein; OR, odds ratio; CI, confidence interval; y, year; SD, standard deviation; IU, international unit.

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Table 1 – ORs (95% CIs) for the 3-year incidence of IGT (logistic regression analyses).

	Model 1[a]		Model 2[b]		Model 3[c]	
	OR (95% CI)	P	OR (95% CI)	P	OR (95% CI)	P
Sex (female vs. male)	0.38 (0.13–1.14)	0.08	0.47 (0.14–1.59)	0.22	0.47 (0.14–1.62)	0.23
Age (years)	1.06 (0.98–1.15)	0.14	1.06 (0.97–1.14)	0.19	1.06 (0.97–1.15)	0.18
Family history of diabetes	1.86 (0.60–5.75)	0.28	1.70 (0.53–5.47)	0.38	1.91 (0.57–6.43)	0.29
Current smoking	0.56 (0.23–1.35)	0.20	0.56 (0.22–1.41)	0.22	0.48 (0.18–1.27)	0.14
Alcohol consumption (g/day)	1.02 (1.00–1.04)	0.11	1.02 (1.00–1.05)	0.05	1.02 (1.00–1.05)	0.05
Eating fast	2.47 (1.12–5.44)	0.03	2.67 (1.17–6.12)	0.02	2.43 (1.03–5.69)	0.04
Systolic blood pressure (mmHg)			1.00 (0.97–1.03)	0.93	0.99 (0.96–1.02)	0.56
Fasting plasma glucose (mmol/l)			2.15 (0.84–5.53)	0.11	1.88 (0.72–4.94)	0.20
Total cholesterol (mmol/l)			1.15 (0.72–1.84)	0.55	1.20 (0.73–1.96)	0.47
HDL cholesterol (mmol/l)			0.49 (0.11–2.20)	0.35	0.66 (0.14–3.13)	0.60
Triglyceride (mmol/l)			0.70 (0.36–1.39)	0.31	0.59 (0.28–1.24)	0.17
C-reactive protein (mg/dl)			0.13 (0.00–4.50)	0.26	0.08 (0.00–4.22)	0.21
Body mass index (kg/m^2)					1.20 (1.00–1.43)	0.04

[a] Model 1: adjustment for sex, age, family history of diabetes, current smoking, and alcohol consumption (g/day).
[b] Model 2: Model 1 plus adjustment for systolic blood pressure, fasting plasma glucose, total cholesterol, HDL cholesterol, triglycerides and C-reactive protein.
[c] Model 3: Model 2 plus adjustment for body mass index.

We therefore selected five eating patterns that are frequently reported as being unhealthy or related to obesity, insulin resistance, or deterioration of diabetes control, i.e., "eating fast" [1–9], "skipping meals" [8,10–12], "snacking frequently" [10,11,13], "late-night eating" [10,11,14], and "eating out frequently" [10,11,13], and attempted to clarify whether they are predictors of the development of IGT.

2. Research design and methods

We examined longitudinal data on 172 Japanese men (n = 120) and women (n = 52) aged 49.4 ± 5.0 y (mean ± SD, range 31–62) who received voluntary medical check-ups including a 75 g oral glucose tolerance test (OGTT) at the Tsukuba Medical Center, which is located at suburb of Tokyo. All had a follow-up 75 g OGTT 3 years after the first 75 g OGGT. Patients with IGT at baseline, as determined by medical history or results of the 75 g OGTT and who had liver cirrhosis, chronic hepatitis or renal dysfunction determined from biomarkers were excluded as study subjects. Mean values of baseline FPG, HbA_{1c}, and BMI of the subjects were 5.1 ± 0.4 mmol/l, 5.1 ± 0.3%, and 23.4 ± 2.9 kg/m^2, respectively.

IGT and T2DM were diagnosed according to the 1999 World Health Organization criteria based on the standard 75 g OGTT. IGT was diagnosed if the fasting plasma glucose (FPG) level was <7.0 mmol/l with a 2-h value ≥7.7 and <11.1 mmol/l. T2DM was diagnosed if the subjects reported a history of T2DM or if the FPG level was FPG ≥ 7.0 mmol/l or if the 2-h value was ≥11.1 mmol/l.

Whether subjects followed one or more of the eating patterns at baseline was determined by a survey that required a response of either "agree" or "disagree" to each of the following statements: (1) "I eat fast.", (2) "I often skip a meal.", (3) "I often eat between meals.", (4) "I eat supper late at night or eat something before going to bed.", and (5) "I often eat out." As a result, 75 (44%) agreed to "eating fast," 25 (15%) to "skipping meals," 33 (19%) to "snacking frequently," 73 (42%) to "late-night eating," and 37 (22%) to "eating out frequently."

2.1. *Laboratory procedures and clinical measurements*

Measurements of blood pressure and anthropometric parameters were done by registered nurses, and lifestyle characteristics such as smoking habits and alcohol consumption were determined by questionnaire. Blood samples were obtained the morning after an overnight fast. Blood glucose, serum lipid, and CRP values were measured using an autoanalyser.

2.2. *Statistical analysis*

Logistic regression analyses were used to estimate the association between an individual eating pattern and the incidence of IGT adjusted by their known risk factors and other potential confounders. All statistical analyses were performed by SPSS (version 16.0, Chicago, IL). Statistical significance was considered for $P < 0.05$.

This study was approved by the institutional review board of Ochanomizu University.

3. Results

Thirty-nine subjects (33 men and 6 women) developed IGT, including 2 cases of T2DM. After adjusting for confounders shown in Table 1, fast eating significantly increased the risk for development of IGT, as did alcohol consumption and baseline BMI. No other eating pattern was associated with an increased risk of development of IGT (data not shown). Even after adjustment for BMI (Model 3 in Table 1), the positive relationship between eating fast and development of IGT remained significant.

4. Conclusions

The eating rate was reported to be associated with obesity [2,4–7], hip-to-waist ratio [9], liver fat [9], and serum triglycerides [9]. Although associations with insulin resistance were also

reported in cross-sectional studies [3,8], the current study demonstrated for the first time, to our knowledge, that self-reported fast eating was an independent and potent risk factor for development of IGT.

One possible mechanism is that eating rapidly accelerates obesity, which is known to increase insulin resistance and become a risk factor for T2DM. We also found significant differences in baseline BMI, as shown in previous cross-sectional studies [2,4–7], but not in weight change during the observational period between those who did and did not report eating fast (data not shown). Otsuka et al. [3] reported that both self-reported eating fast and baseline BMI are significantly and independently associated with insulin resistance. Our study also demonstrated that eating fast and baseline BMI are both independent predictors of IGT. Therefore, it is unlikely that eating fast increased IGT mainly through obesity. Another hypothesis is that eating fast could increase postprandial blood glucose. Suzuki et al. [15] reported that thorough mastication elicited a significantly lower postprandial plasma glucose concentration compared to usual mastication.

This study has several limitations. Firstly, the number of subjects was relatively small. In addition, only two subjects newly developed T2DM, prohibiting an analysis regarding T2DM. Secondly, we used only self-reported eating patterns. Although the rate of eating is sometimes objectively evaluated by time spent eating a meal or using an eating monitor [4,9] (an electronic scale concealed in a table for automatically recording the weight of food removed (eaten) from a plate), these methods are not necessarily representative of ordinary life, making subjective assessment by questionnaire much more widely used [2,3,5–7], especially in epidemiological settings. Third, we did not obtain information on energy intake. However, we could speculate that energy intake might not differ largely between those who did and did not report eating fast because differences in weight change between the two groups were not significant. Further detailed studies are needed to determine whether eating fast increases the risk for IGT and T2DM via increasing energy intake.

In conclusion, though further prospective research is necessary in terms of T2DM, our study clarified that self-reported eating fast is a potent predictor of development of IGT.

Conflict of interest

There are no conflicts of interest.

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