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Assessment of Food Education by Urinalysis

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Abstract

"The Basic Law on Shokuiku (Food Education)" (Shokuiku Kihon Hou) was enacted in 2005 in Japan. First, we examined the relationship of Na/K ratio in the first morning spot urine (SU) with the ratio of 24-hour urine (24U) samples from 54 elementary school children, and confirmed Na/K ratios in the SU and the 24-U samples were correlated significantly. Next, we conducted 6 times the variety of Food Education Program (FEP), to encourage 34 boys and girls aged 7 to 12 to eat more Japanese traditional food items such as soy, sesame, seaweed, vegetable, fish, mushroom and potato, daily. And we checked the change of sodium (Na) / potassium (K) of SU samples collected 3 times before, after and in the midst of FEP to investigate the effect of FEP on Na/K of morning SU in elementary school children. The Na/K was decreased significantly in the 2nd and the 3rd SU from the baseline level. We confirmed the FEP's effect on the children's dietary behaviour not only from the questionnaire but also from the significant changes in Na/K of SU. In conclusions, the FEP collecting SU samples to check Na/K is expected to be a good method to estimate the effect of FEP on dietary behaviour.

Keywords: Food Education Program（FEP）, School children, Morning spot urine, 24-hour urine, Sodium to potassium ratio;

1. Introduction

Since 'The Base Low on Shokuiku (Food Education) (Shokuiku Kihon Hou) (Adachi M. 2008) was enacted in Japan in 2005, food education (FE) has been promoted nation-widely by schools, various groups and volunteers and FE appears to be contributing somewhat to the improvement of dietary custom in Japan (Yoshida T, et al. 2008, Nakamura T. 2008). Teachers for FE were newly appointed at elementary schools by the Ministry of Education, Culture, Sports, Science & Technology (2004). The guide line for nursery schools included additionally FE for training the ability to control diets by the Ministry of Education, Culture, Sports, Science & Technology in 2009. The final purpose of FE should be the promotion of health greatly depending on food intake. Therefore, if any objective estimation for what children are eating is available, the efficacy of FE and behavioural changes in dietary intake could be evaluated for the promotion of better FE. So far we applied the 24-hour urine (24U) collection for the estimation of various biomarkers of dietary intakes that were utilized world-widely for the analyses on the association of dietary intake with cardiovascular risks in WHO-coordinated Cardiovascular Diseases and Alimentary Comparison (CARDIAC) Study proposed by Yamori (Yamori Y, et al. 1984, Yamori Y. 1989 Yamori Y. et al, 2006).

Table 1. Japanese food recommended to eat daily by Food Education Program as "MA-GO-WA-YA-SA-SHI-I"

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	Ma	Go	Wa	Ya	Sa	Shi	I
Japanese name of food	Mame	Goma	Wakame	Yasai	Sakana	Shiitake	Imo
Meaning in English	Soy bean products	Sesame	Seaweeds	Vegetables	Fishery products	Japanese mushrooms	Potatoes
Practical food examples	Soy bean, Bean curd, Soybean flour, Natto, etc.	Nuts or Seeds, etc.	Laver, Mozuku, Brown alga, etc.		Fish, Octopus, Cuttlefish, Lobster, etc.	Enokidake mushroom, Shimeji mushroom	Potato, Sweet potato, Taro potato etc.

"Ma・Go・Wa・Ya・Sa・Shi・I" means "Grandchildren are kind hearted" in Japanese

This international cooperative study demonstrated salt intake estimated from 24U sodium (Na) excretion and Na/potassium (K) ratio in 24U were good biomarkers for the nutrition closely associated with cardiovascular risks, such as the mortality rates of stroke (Excerpts from the WHO CARDIAC Study Protocol. 1990, Yamori Y. 1989, Yamori Y, et al. 2001, Yamori Y. et al, 2006), and K excretion in 24U was proven to be a biomarker for the intake of vegetables and fruits (Smith-Warner SA, et al.2000, Sorof JM, et al. 1997). Moreover, 24U taurine and isoflavones were also proven to be good biomarkers for dietary intakes of seafood and soy bean products, respectively (Moriguchi EH, et al. 2004, M Mori, et al. 2004). Therefore, 24U collection could be regarded as a reliable method for the estimation of the association between diets and cardiovascular risks. The target populations were middle-aged males and females aged from 48 to 56, and 24U samples were collected from these adult populations (Yamori Y, et al. 1982). We thought these 24U samples, if collected successfully from children, would be useful for FE to estimate children's dietary intakes, and carried out in our preceding study (Mori M, et al. 2009) 24U collection from 177 school children of 3 elementary schools in Awaji Island, in the Inland Sea, Japan before and after the FE, which emphasised; 1) to eat various traditional food items once a day expressed as MA-GO-WA-YA-SA-SHI-I meaning "gland children are kind hearted" in Japanese and indicating MA (Mame=soy), GO (Goma=sesame), WA (Wakame=popular sea weed), YA (Yasai=Vegetables), SA (Sakana=Fish), SHI (Siitake=popular dietary fungi), and I (Imo=Potatoes), respectively (Table 1), 2) to take well balanced food containing nutrients grouped into 3 by colors; red (protein), yellow (carbohydrate and fat) and green (vitamins and minerals), and 3) to enjoy the natural taste of food with optional consumption of salt. The result of this FE showed 24U-estimated salt intake and 24U Na/K were significantly lower in the educated group compared with the control group, and 24U isoflavones and taurine were significantly higher or tended to be high in the former group than in the latter. This study confirmed the effect of FE by 24U data for the first time (Fig. 1).

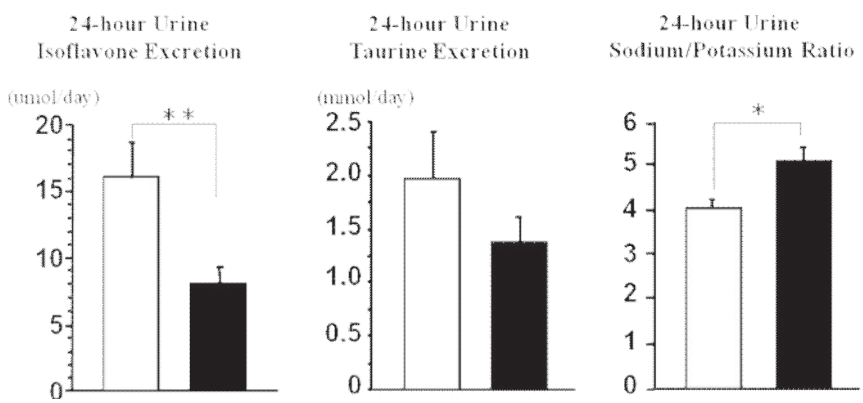

Figure 1: Comparison of 24-hour urine biomarkers in our preceding food education study in 2002

Participants of 83 male and female elementary school children aged 10 to 12 consisted of 41in food education program class (□) and 42 in the control class without food education program (■).: Food education in this study was the repeated lesson on the importance of Japanese food items abbreviated as "MA-GO-WA-YA-SA-SHI-I" (Table 1) at school lunch time every day. Mean ± standard error, *:$p<0.05$, **:$p<0.01$.

2. The Purpose of the Present Studies

These backgrounds led us to the new FE based on biomarkers on 24U checked before and after FE for the objective estimation of the efficacy of FE affecting dietary custom. Since the major target of FE is children and repetitive 24U collections is not an easy task. As more simplified urine collections instead of 24U collection, night urine collection and the 2nd morning urine collection were so far proposed for adults (Kawasaki T, et al. 1982, Kawasaki T, et al. 1993, Tanaka T, et al. 2002) but these methods are also not so easily applicable for children. Since spot urine (SU) collection is presently utilized for regular check of bacteria at nursery and elementary schools in Japan, we firstly tested the validity of SU collected from the first morning urine to be utilized alternatives for 24U and secondly tested whether or not the results of SU samples could be used for the efficacy estimation of FE.

3. Study Designs

3.1 Relationships between Morning Spot Urine (SU) and 24-hour Urine (24U) Samples .

Elementary school children aged 11.3±0.8, 54 in total, were invited to an explanation meeting after school at a school on Awaji Island Japan. After oral explanation of 24U and morning SU collections to the children and their parents, informed consent was signed and equipments for 24U and morning SU collection, which were urine boxes and SU container, were given to the volunteer participants. They were asked to discard the first morning urine and start to collect all urine of the day and the first morning urine of the next day in the urine boxes. The last morning urine was voided into a cup from which morning SU was sampled into a small SU container. The rest of the morning urine was sampled into a urine box, and both urine box and SU container were asked to bring to their school. The total urine in the urine box and SU container was weighted to calculate the total amount of 24U, and portions from both 24U and SU samples were sampled into 5 test tubes (2ml) and were submitted to a well-standardized analytical institute (SRL Co, Ltd. Tokyo, Japan) for the analyses of Na (mEq/L), K (mEq/L), creatinine (mg/dl) and molecular ratios of Na/K were calculated. Regression analysis was applied for the relationship of Na/K between 24U and morning SU. For confirming the reliability of 24U collection, volunteers were asked to record the time of urination and urinary volume was each time on the charts given to them. Additionally, when 24U volume is too small, creatinine was calculated from the participant's body weight to check the validity of 24U collection.

All data were expressed as the mean ± standard deviation. Correlation between 24U and SU Na/K ratio was tested by regression analysis and for statistical analysis SPSS ver.18.0 was used. P less than 5% was regarded significant.

Table 2. Schedule and contents of the Food Education Program

Step	Date	Theme and Contents	Material and Method
1	17th, Sep. 2004	Understanding nutrients by dividing food into 3 groups differently colored; red (protein), yellow (carbohydrates and lipid) and green (vitamins and minerals).	"Apron theater" for understanding food function and "Food cards" for understanding contained nutrients.
2	24th,Sep. 2004	Understanding the function of carbohydrates and lipid in the body.	Training to cook rice and understanding of the quantity of the sweeteners of drinks, and their energy contents.
3	8th,Oct. 2004	Understanding the function of protein in the body.	Try to make Tofu from soy bean. Knowledge of soy bean and soy products.
4	22th,Oct. 2004	Uunderstanding the function of vitamins and minerals in the body.	Fields visit and experience of harvest of vegetables. Try to cook harvested vegetables.
5	12nd,Nov. 2004	Planning on the Christmas recipe of good nutrition for their parents.	Making the nutritionally well balanced recipe from 3 colored groups of food
6	20th,Nov. 2004	Cooking training of the Christmas dinner and party.	Try to cook diets of nutritionally balanced recipe for enjoying food for Christmas party with parents.

Table 3. Participants for Food Educational Program

Grade	First	Second	Third	Forth	Fifth	Sixth	Total
Boys (n)	2	2	0	1	3	3	11
Girls (n)	8	3	3	2	5	2	23
Total	10	5	3	3	8	5	34

3.2 Effect of Food Education and Its Assessment by Spot Urine (SU) Samples.

FE for children was arranged in this study by COOP KOBE (Cooperative Association in KOBE City, Hyogo Prefecture), which was well known as a pioneer organization for consumer movements in Japan. After a long preparation with the leaders of COOP for FE program (FEP) a series of 6 classes for FEP (Table 2) was organized by emphasizing the catch phrase of "MA-GO-WA-YA-SA-SHI-I", 3 groups of nutrients in Japanese food and optimal salt intake, as already mentioned in the Background of this article. FE for children should be FE for their parents, so that the teaching materials and information given at the classes were also handed out for the parents with detailed explanation of the purpose of each class and home works were done by children together with their parents.

As for urine collection important for the evaluation of FE, detailed information was given before the classes to children and their parents, and folded urine cup and SU container (Fig.2) was sent to the participants after informed consents were obtained. The SU collection was similar to that for checking bacteria; the first morning urine voided into the cup was sampled into a SU container up to the level roughly corresponding to 10 ml. SU samples were collected 3 times in total; the first before the beginning of FEP in September, the 2nd after the 4th class of FEP in October and the 3rd at the end of the 6 times series of FEP, in November. The detailed explanation of FEP and urine collection was repeated at the first FE class. Questionnaires will asked to fill up twice before and after the FEP to analyze changes in the food custom, life style, altitude toward daily food intake and behavior related to food intake, if any. This study design was approved by the Ethics Committee of Institute for Health Restoration.

Participants, 34 in total, were elementary school children aged 6-12 (Table 3), recruited by public announcement through COOP. They were selected after the informed consents from the participants and their parents were obtained following the detailed explanation of the purpose and method of FE and of the procedure for SU collection (Fig.2). SU samples were brought to the FE class, and kept cool in a cold box, and then each urine was sampled into 5 test tubes (2 ml) at our laboratory for shipping to SRL on the same day for the measurement of Na, K and creatinine. The molar ratios of Na/K were calculated from the data retuned from SRL.

All data were expressed as the mean ± standard deviation. Differences in Na/K ratios from the base line of the 2nd and 3rd samples during the course of FEP were tested by paired *t*-test was applied to Na/K ratio before and after FEP, and for statistical analysis SPSS ver.18.0 was used. P less than 5% was regarded significant.

Figure 2. Folded paper cup for receiving voided urine (left) and spot urine container (right)

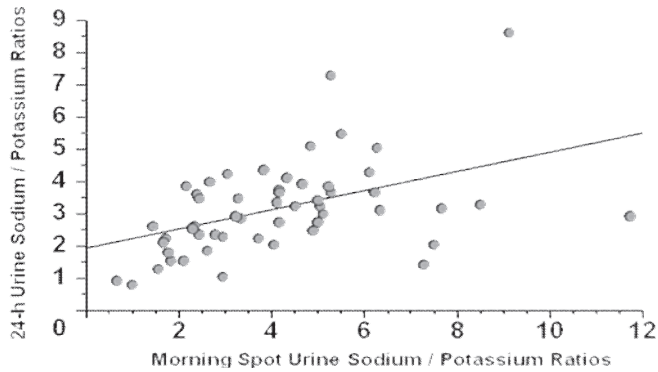

Figure 3. Correlation of 24-hour urine sodium / potassium ratios with morning spot urine sodium / potassium ratios
$y = 0.3x + 1.93$, $r^2 = 0.21$, $p<0.001$

4. Results

4.1. Relationships between morning SU and 24U samples

All 54 participants were proven to have collected 24U samples without any mistakes by their data of creatinine. As shown in Fig.3, significant association was noted in Na/K between SU (x) and 24U (y) data: $y=0.3x + 1.93$, $p<0.001$, $r^2<0.21$, indicating 24U Na/K could be estimated from SU Na/K. As for molar ratio of urinary Na/K SU data can be used instead of 24U data for the estimation of the effect of FE.

4.2. Effect of FE and Its Assessment by morning SU samples.

All 34 participants collected SU samples 3 times, before, after and in the middle in Fig4, the mean of Na/K gradually decreased from the initial level (4.8 ± 2.0) before FEP to the midst of FEP (4.2 ± 1.9) and further down to the end of FEP (3.8 ± 2.1). Statistical significances were observed in the difference between before and middle, and also in the difference before and after ($p<0.01$). The answers from participants in response to questionnaires were summarized in Fig.5. In addition, the influence of FEP was also noted in voluntary answers such as “became to eat fruit”, “came to eat Japanese vegetable-containing pancakes with a half amount of sauce”, “came to eat homemade French fried without salt”, etc.

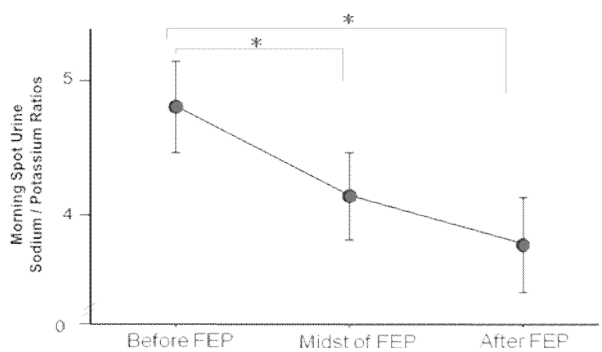

Figure 4. Change in sodium / potassium ratios during Food Education Program (FEP).
Mean ± standard error. *: $p<0.05$

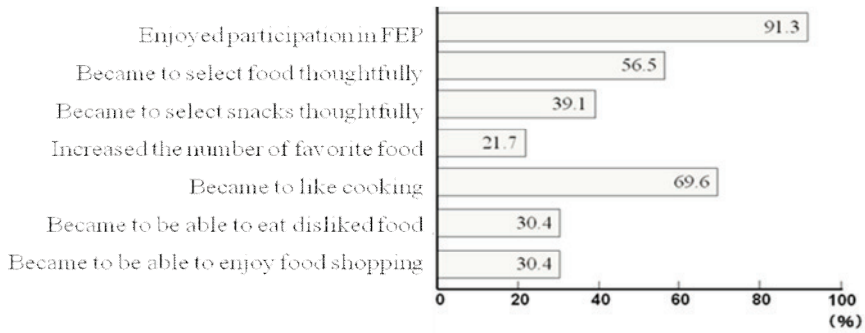

Figure 5. Subjective responses to Food education program (FEP) obtained by multiple answers system from participants.

5. Discussion

The first part of the present study confirmed Na/K ratios of morning SU was significantly associated with the ratios of 24U in school children and indicated the applicability of morning SU to the assessment of the FEP for children. So far for adults some simplified methods such as the collection of the 2nd morning urine were proposed (Kawasaki T, et al. 1982, Kawasaki T, et al. 1993, Tanaka T, et al. 2002) but morning SU sampling is much easier for children. Preceding studies showed significant positive associations of Na, K and Na/K in 24U with age and BMI in children (De Santo NG, et al. 1987, Moriyama M, et al. 1988). Although age and BMI should be considered about the 24U data of children, the influence of age and BMI may be negligible in short term FEP's. And repetitive collection of morning SU from the same participants should be useful for the estimation of the effect of FEP in individuals and also in the group. Although urinary biomarkers are variable day by day in individuals, the changes of the average are considered to correspond well to the changes in the diets (Shortt C, et al. 1988).

The effect of FE is usually assessed by questionnaire and also by food frequency questionnaire (FFQ) (Chiplonkar SA, et al. 2002, Ma J, et al. 2003, Dodd KW, et al. 2006), but the applicability of the present FFQ to children needs to be confirmed, so that we utilized morning SU sample to observe the effect of FEP for children and confirmed the Na/K data of morning SU was significantly influenced by the FEP. This study first demonstrated simple morning SU collection could be used for the evaluation of FEP for children and will be applied more easily to the objective assessment of FEP than the data collection of subjective answers to questionnaires commonly used up to the present.

This study, however, has some limitation. The data obtained from 24U and SU could be useful for the evaluation of food custom of the population (Shortt C, et al. 1988). When these methods are applied to the evaluation of individual nutrition intake such as Na, K and Na/K ratios, the dietary intake of the sampling day or the previous days usually affects the data. For example, if one takes festival food or take nothing to eat at all, Na, K or Na/K ratio should be greatly changed. Therefore, in our study, before starting FEP to be assessed by 24U or SU, participants should be instructed to continue to eat usual daily diets, Since urinary biomarkers are variable daily (Liu K, et al. 1979) repetitive sampling is needed also for the estimation of individual effects of FEP but, they may be more useful for the estimation of the group effect of FEP by comparing the data before and after FEP. In addition, our present study indicated significant changes in Na/K of morning SU during the period of FEP but we could not analyze what real dietary changes affected their Na/K in SU.

The present FEP simply emphasized traditional Japanese Food items (MA-GO-WA-YA-SA-SHI-I), nutritionally well balance among 3 categories of food colored differently (Nutrient balance) and the importance of natural taste of food with lees salt, and such a simple FEP was proven to be useful for increasing vegetable intake rich in K, decreasing salt intake and thus to reduce effectively Na/K ratios in morning SU.

6. Conclusion

Among various urinary biomarkers for dietary intakes Na/K ratios in morning SU were significantly positively associated with 24U Na/K ratios, which are well known to be related with cardiovascular risks. Na/K ratios in morning SU were decreased significantly during 2 months of our FEP, indicating Na/K in morning SU could be useful for the assessment of FEP for children.

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References

Adachi M. (2008). Theories of nutrition education and promotion in Japan: enactment of the “Food Education Basic Law”. *Asia Pac J Clin Nutr,* 17, Suppl 1:180-4.

Chiplonkar SA, Agte VV, Mengale SS, Tarwadi KV. (2002). Are lifestyle factors good predictors of retinol and vitamin C deficiency in apparently healthy adults? *Eur J Clin Nutr,* 56:96-104.

De Santo NG, Dilorio B, Capasso G, Russo F, Stamler J, Stamler R, Giordano C. (1987). The urinary sodium/potassium ratio in children from southern Italy living in Cimitile: a case for concern. *Int J Pediatr Nephrol*, 8:153-8.

Dodd KW, Guenther PM, Freedman LS, Subar AF, Kipnis V, Midthune D, Tooze JA, Krebs-Smith SM. (2006). Statistical methods for estimating usual intake of nutrients and foods: a review of the theory, *J Am Diet Assoc,* 106:1640-50.

Excerpts from the WHO CARDIAC Study Protocol. (1990). *J Cardiovasc Pharmacol,* Suppl 8 :S75-7,

Kawasaki T, Ueno M, Uezono K, Kawazoe N, Nakamura S, Ueda K, Omae T. (1982). Average urinary excretion of sodium in 24 hours can be estimated from a spot-urine specimen. *Jpn Circ J,* 46:948-53.

Kawasaki T, Itoh K, Uezono K, Sasaki H. (1993). A simple method for estimating 24 h urinary sodium and potassium excretion from second morning voiding urine specimen in adults. *Clin Exp Pharmacol Physiol,* 20:7-14.

Liu K, Cooper R, McKeever J, McKeever P, Byington R, Soltero I, Stamler R, Gosch F, Stevens E, Stamler J .(1979). Assessment of the association between habitual salt intake and high blood pressure: methodological problems. *Am J Epidemiol.*110(2):219-26.

Ma J, Betts NM, Horacek T, Georgiou C, White A. (2003). Assessing stages of change for fruit and vegetable intake in young adults: a combination of traditional staging algorithms and food-frequency questionnaires, *Health Educ Res,* 18:224-36.

Mori M, Xu JW, Mori H, Ling CF, Wei GH, Yamori Y. (2009). Comparative studies on 24-hour urinary taurine excretion in Japanese and Chinese adults and children – Need for food educational diets. *Advances in Experimental Medicine and Biology,* 643: 399-405.

Mori M, Sagara M, Ikeda K, Miki T, Yamori Y. (2004). Soy isoflavones improve bone metabolism in postmenopausal Japanese women, *Clin Exp Pharmacol Physiol,* 31 Suppl 2:S44-6.

Moriguchi EH, Moriguchi Y, Yamori Y, Impact of diet on the cardiovascular risk profile of Japanese immigrants living in Brazil: contributions of World Health Organization CARDIAC and MONALISA studies. (2004). *Clin Exp Pharmacol Physiol,* 31 Suppl 2:S5-7,

Moriyama M, Saito H. (1988). Twenty four-hour urinary excretion of sodium, potassium and urea in Japanese children, *Tohoku J Exp Med,* 154:381-8.

Nakamura T. (2008). The integration of school nutrition program into health promotion and prevention of lifestyle-related diseases in Japan, *Asia Pac J Clin Nutr,* 17, Suppl 1:349-51.

Shortt C, Flynn A, Morrissey PA. (1988). Assessment of sodium and potassium intakes. *Eur J Clin Nutr*, 42: 605-9.

Smith-Warner SA, Elmer PJ, Tharp TM, Fosdick L, Randall B, Gross M, Wood J, Potter JD. (2000). Increasing vegetable and fruit intake: randomized intervention and monitoring in an at-risk population, *Cancer Epidemiol Biomarkers Prev,* 9:307-17.

Sorof JM, Forman A, Cole N, Jemerin JM, Morris RC. (1997). Potassium intake and cardiovascular reactivity in children with risk factors for essential hypertension. *J Pediatr.* 131(1 Pt 1):87-94.

Tanaka T, Okamura T, Miura K, Kadowaki T, Ueshima H, Nakagawa H, Hashimoto T. (2002). A simple method to estimate population 24-h urinary sodium and potassium excretion using a casual urine specimen. *J Hum Hypertens,* 16:97-103.

The Ministry of Education, Culture, Sports, Science & Technology. (2004). The enforcement of laws about nutrition instructor system, from http://www.mext.go.jp/a_menu/shotou/eiyou/04111101/008.htm

The Ministry of Education, Culture, Sports, Science & Technology. (2009). A description in main reports about the cooperation of a day nursery, a kindergarten and the elementary school, from http://www.mext.go.jp/b_menu/shingi/chousa/shotou/057/shiryo/08111109/002.htm

Yamori Y, Kihara M, Fujikawa J, Soh Y, Nara Y, Ohtaka M, Horie R, Tsunematsu T, Note S, Fukase M. (1982). Dietary risk factors of stroke and hypertension in Japan -- Part 1: Methodological assessment of urinalysis for dietary salt and protein intakes, *Jpn Circ J,* 46:933-8.

Yamori Y, Nara Y, Kihara M, Mano M, Horie R. (1984). Simple method for sampling consecutive 24-hour urine for epidemiological and clinical studies, *Clin Exp Hypertens,* 6:1161-7.

Yamori Y. (1989). WHO CARDIAC Study--its experimental background and progress report, *J UOEH,* 20:11 Suppl: 30-8.

Yamori Y, Liu L, Ikeda K, Miura A, Mizushima S, Miki T, Nara Y; WHO-Cardiovascular Disease and Alimentary Comprarison (CARDIAC) Study Group. (2001). Distribution of twenty-four hour urinary taurine excretion and association with ischemic heart disease mortality in 24 populations of 16 countries: results from the WHO-CARDIAC study, *Hypertens Res.,* 24:453-7.

Yamori Y, Liu L, Mizushima S, Ikeda K, Nara Y; CARDIAC Study Group. (2006). Male cardiovascular mortality and dietary markers in 25 population samples of 16 countries. *J Hypertens*, 24(8):1499-505.

Yoshida T, Kouda K, Nakamura H, Nishio N. (2008). Taste development from health education among schoolchildren: A two-year intervention study, *J Physiol Anthropol,* 27: 1-5.