

## Ability of self-reported estimates of dietary sodium, potassium and protein to detect an association with general and abdominal obesity: comparison with the estimates derived from 24 h urinary excretion

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### Abstract

As under-reporting of dietary intake, particularly by overweight and obese subjects, is common in dietary surveys, biases inherent in the use of self-reported dietary information may distort true diet–obesity relationships or even create spurious ones. However, empirical evidence of this possibility is limited. The present cross-sectional study compared the relationships of 24 h urine-derived and self-reported intakes of Na, K and protein with obesity. A total of 1043 Japanese women aged 18–22 years completed a 24 h urine collection and a self-administered diet history questionnaire. After adjustment for potential confounders, 24 h urine-derived Na intake was associated with a higher risk of general obesity (BMI  $\geq 25$  kg/m<sup>2</sup>) and abdominal obesity (waist circumference  $\geq 80$  cm; both *P* for trend=0.04). For 24 h urine-derived protein intake, positive associations with general and abdominal obesity were observed (*P* for trend=0.02 and 0.053, respectively). For 24 h urine-derived K intake, there was an inverse association with abdominal obesity (*P* for trend=0.01). Conversely, when self-reported dietary information was used, only inverse associations between K intake and general and abdominal obesity were observed (*P* for trend=0.04 and 0.02, respectively), with no associations of Na or protein intake. In conclusion, we found positive associations of Na and protein intakes and inverse associations of K intake with obesity when using 24 h urinary excretion for estimating dietary intakes. However, no association was observed based on using self-reported dietary intakes, except for inverse association of K intake, suggesting that the ability of self-reported dietary information using the diet history questionnaire for investigating diet–obesity relationships is limited.

**Key words:** 24 h Urine: Sodium: Protein: Obesity

Although accurate assessment of habitual dietary intake is a prerequisite in studies of diet and health, the difficulty of obtaining dietary data that accurately represents what people usually eat is now generally acknowledged<sup>(1)</sup>. Misreporting, particularly under-reporting, of energy intake, a surrogate measure of total food intake, by a variety of dietary assessment methods relative to total energy expenditure measured by the doubly labelled water method, the gold standard for measuring free-living total energy expenditure, is common<sup>(2,3)</sup>. Additionally, misreporting of energy intake within a population might not be random, but might rather occur systematically within certain groups of the population<sup>(1–4)</sup>. In particular, overweight and obese subjects tend to under-report energy intake to a greater extent than lean subjects<sup>(1–5)</sup>.

Unfortunately, a potential solution to under-reporting, such as energy adjustment, is hindered by what appears to be a selective reporting of various nutrients and foods<sup>(4)</sup>. For example, protein is usually not under-reported to the same degree as carbohydrates and fats<sup>(2,4,6–8)</sup>, and between-meal snacks and foods considered to be unhealthy seem to be under-reported to a greater extent than those considered to be healthy<sup>(1,3,4,8)</sup>. Thus, biases inherent in the use of self-reported dietary information may distort or obscure the associations between diet and health, particularly obesity, or even create spurious ones. However, investigation of this possibility with the use of dietary biomarkers, such as 24 h urinary excretion of N (protein), Na and K<sup>(9–11)</sup>, is limited. In infants, for example, it has been found that a high intake of protein from

**Abbreviations:** DHQ, diet history questionnaire; PABA, *para*-aminobenzoic acid; WC, waist circumference.

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complementary feeding is associated with a higher risk of developing obesity, possibly because of the interplay with insulin-like growth factor-1<sup>(12)</sup>. Such mechanisms could contribute to gain in fat mass with greater habitual protein intake in adults<sup>(13)</sup>. In a small study in middle-aged men and women in Denmark, both protein intake estimated from 24 h urinary N excretion and self-reported protein intake were associated with greater weight gain after 6 years<sup>(13)</sup>. However, the authors did not examine other nutrients including Na and K<sup>(13)</sup>, whose effect on obesity are now being investigated<sup>(14–20)</sup>. For Na, a mechanism not triggered by increased energy intake has been proposed by an animal study, where a high consumption of salt contributed to development of obesity among rats<sup>(21)</sup>. The increase in adipose tissue mass is suggested to be caused by an increased capacity to incorporate glucose into lipids, and a higher lipogenic enzymatic activity may have promoted adipocyte hypertrophy and then excessive fat accumulation<sup>(21)</sup>. A higher intake of K may also be associated with a lower risk of obesity mainly due to higher intakes of fruits and vegetables, the major sources of K<sup>(22)</sup>, although the effect of fruits and vegetables on obesity is controversial<sup>(23)</sup>. A very limited number of studies have shown associations between either self-reported or 24 h urine-derived intakes of Na and K with body fatness measures; however, no investigation has been done on the basis of using both self-reported and 24 h urine-derived estimates simultaneously<sup>(14–20)</sup>.

Therefore, using 24 h urinary excretions of Na, K and N (protein) as established quantitative biomarkers of intakes of these nutrients<sup>(9–11)</sup>, we compared the relationships of 24 h urine-derived and self-reported intakes of Na, K and protein with general and abdominal obesity in a relatively large sample of young Japanese women. We hypothesised that the expected associations of Na, K and protein intakes with obesity are observed only when using 24 h urinary excretion for estimating dietary intakes, but not based on using self-reported dietary intakes, because of obesity-related biases in self-reported dietary information.

## Subjects and methods

### Study population

The present cross-sectional study was based on a survey conducted from February to March 2006 and from January to March 2007 among female dietetic students from fifteen higher-education institutions in Japan. Details of the study design and survey procedure have been described elsewhere<sup>(22,24,25)</sup>. Of the 1176 Japanese women who took part in the survey (response rate 56%), 1105 conducted the 24 h urine collection. For the analysis, we selected women aged 18–22 years ( $n$  1083). We then excluded those with missing information on the variables used ( $n$  5). We further excluded those whose 24 h urine collection was considered incomplete ( $n$  35), as assessed using the information on urinary creatinine excretion and body weight based on a strategy proposed by Knuiiman *et al.*<sup>(26)</sup>. This creatinine-based strategy has been validated against the *para*-aminobenzoic acid (PABA)

check method in a subsample ( $n$  654) of the present subjects (% of subjects having incomplete urine collection 5.5%; sensitivity 0.47; specificity 0.99; % of subjects misclassified 4%)<sup>(24)</sup>. Since exclusion of underweight subjects (BMI < 18.5 kg/m<sup>2</sup>,  $n$  118)<sup>(27)</sup> did not alter the findings of the present study (data not shown), these subjects were also included in the analysis, giving a final sample size of 1043 women.

The present study was conducted according to the guidelines laid down in the Declaration of Helsinki, and all procedures involving human subjects were approved by the ethics committee of the National Institute of Health and Nutrition, Japan. Written informed consent was obtained from each subject and also from a parent/guardian for subjects < 20 years old.

### Anthropometric measurements

Body height (to the nearest 0.1 cm) and weight (to the nearest 0.1 kg) were measured while subjects were wearing lightweight indoor clothes only, without shoes. BMI (kg/m<sup>2</sup>) was calculated as weight (in kg) divided by height (in m) squared. Waist circumference (WC) was measured at the level of the umbilicus (to the nearest 0.1 cm) at the end of a normal respiration while the subject was standing erect and with the arms at the side and the feet together. General obesity was defined as BMI  $\geq$  25 kg/m<sup>2</sup> while abdominal obesity was defined as WC  $\geq$  80 cm, based on cut-off points for Asian women according to the WHO<sup>(27)</sup>.

### 24 h Urine-derived dietary intake

A single 24 h urine sample was collected from each subject. A detailed description of the 24 h urine collection procedure has been published elsewhere<sup>(22,24,25)</sup>. Briefly, subjects were asked to collect all urine voided during a 24 h period, and to record the time of the start and end of the collection period, and the estimated volume of any missing urine specimens. The 24 h urine volume was adjusted by self-reported collection time (calculated from the self-reported time of the start and end of the collection period) and missing urine volume. This adjustment strategy has been validated using the PABA check method in a subsample ( $n$  654) of the present subjects<sup>(24)</sup>. All urine samples taken over the 24 h period were carefully mixed, and several aliquots were taken and transported at  $-20^{\circ}\text{C}$  to a laboratory (SRL, Inc. in 2006 and Mitsubishi Kagaku Bio-Clinical Laboratories, Inc. in 2007). In accordance with the standard procedure at each laboratory, urea N concentrations were measured using the enzymatic assay method, Na and K concentrations using the ion-selective electrode method and creatinine concentrations using the enzymatic assay method.

Total 24 h urinary excretion was calculated by multiplying the measured concentration by the (adjusted) volume of 24 h urine. To estimate 24 h urine-derived intake of protein, urea N content in urine was multiplied by 9.08, assuming that urea N is in constant proportion (85%) to total urinary N<sup>(9)</sup>, that 81% of ingested N is excreted through the urine<sup>(9)</sup> and that N constitutes 16% of protein. The 24 h urine-derived

intakes of Na and K were, respectively, estimated as Na content in urine divided by 0.86 (assuming that 86% of ingested Na is excreted through the urine)<sup>(10)</sup> and K content in urine divided by 0.77 (assuming that 77% of ingested K is excreted through the urine)<sup>(11)</sup>. Energy adjustment based on the density method (% of energy for protein and mg/4184 kJ for Na and K) was made using estimated energy requirement (i.e. total energy expenditure during weight stability), which was calculated with the use of sex- and age-specific equation published from the US Dietary Reference Intakes<sup>(28)</sup>, based on age, weight, height and self-reported physical activity<sup>(22)</sup>.

### Self-reported dietary intake

Self-reported information on dietary habits during the preceding month was obtained using a comprehensive self-administered diet history questionnaire (DHQ)<sup>(29–32)</sup>, which was conducted 1–3 d before conducting 24 h urine collection and anthropometric measurements. Details of the structure of DHQ and calculation method of dietary intake have been published elsewhere<sup>(29–32)</sup>. Briefly, the DHQ is a structured sixteen-page questionnaire that asks about the consumption frequency and portion size of selected foods commonly consumed in Japan, as well as general dietary behaviour and usual cooking methods. Estimates of daily intake for foods (150 items in total), energy, protein, Na and K were calculated using an *ad hoc* computer algorithm for the DHQ based on the Standard Tables of Food Composition in Japan<sup>(33)</sup>. Energy adjustment was made using self-reported energy intake based on the density method. Validity on the DHQ with respect to commonly studied nutritional factors has been investigated in several previous studies<sup>(29–32)</sup>. For example, Pearson's correlation coefficients were 0.52 for protein, 0.53 for K and 0.39 for Na between the DHQ and 16-d weighed dietary record in ninety-two women aged 31–69 years<sup>(31)</sup>, and 0.40 for K and 0.23 for Na between the DHQ and 24 h urinary excretion in sixty-nine female college students<sup>(29)</sup>. Further, Pearson's correlation coefficient between energy intake derived from the DHQ and total energy expenditure measured by the doubly labelled water was 0.22 in seventy-three women aged 20–59 years<sup>(30)</sup>.

### Other variables

Based on the reported home address, each subject was grouped into one of the three regions (north (Kanto, Hokkaido and Tohoku); central (Tokai, Hokuriku and Kinki); or south (Kyushu and Chugoku)) and into one of the three municipality levels (ward (i.e. metropolitan area), city or town and village). Residential status (living with family, living alone or living with others), current alcohol drinking (yes or no) and current smoking status (yes or no) were also self-reported. Physical activity was computed as the average metabolic equivalent hours score per day on the basis of the self-reported frequency and duration of five activities (sleeping, high- and moderate-intensity activities, walking and sedentary activities) over the preceding month<sup>(22)</sup>.

### Statistical analyses

All statistical analyses were performed using SAS statistical software (version 9.2; SAS Institute, Inc.). The 24 h urine-derived and self-reported intakes of Na, K and protein were categorised at quartile points based on the distribution. With the use of the PROC GLM procedure, multivariate-adjusted means (with standard errors) of BMI and WC were calculated according to the quartiles of dietary intakes. Further, using the PROC LOGISTIC procedure, multivariate-adjusted OR (95% CI) for general and abdominal obesity were calculated for each quartiles of dietary intakes, with the lowest quartile category used as the reference. We tested for linear trends with increasing levels of dietary intakes by assigning each subject the median value for the category and modelling this value as a continuous variable. The potential confounding factors considered were survey year, region, municipality level, residential status, current alcohol drinking, current smoking status, physical activity and 24 h urine-derived or self-reported intakes of other nutrients. All reported *P* values are two-tailed, and *P* values <0.05 were considered statistically significant.

### Results

The mean values of BMI and WC were 21.2 kg/m<sup>2</sup> and 72.7 cm, respectively (Table 1). The mean daily values of 24 h urine-derived and self-reported dietary intakes were 1962 and 2055 mg/4184 kJ for Na, 1144 and 1114 mg/4184 kJ for K and 14.0 and 13.5% of energy from protein, respectively. Pearson's correlation coefficients between 24 h urine-derived and self-reported intakes were 0.08 for Na, 0.28 for K and 0.20 for protein. The prevalence of general and abdominal obesity was 7.7 and 13.0%, respectively. Subjects with general obesity were less likely to live alone and more likely to live with others. There were more subjects defined as abdominally obese in the regions of north and south, with fewer in the region of central. No difference in self-reported energy intake was observed between obese (general or abdominal) subjects and their non-obese counterparts. Both general and abdominal obese subjects had a higher mean value of estimated energy requirement and 24 h urine-derived Na intake. Abdominal obese subjects also had a lower mean value of self-reported intakes of all the three nutrients.

There were positive associations among 24 h urine-derived intakes of all the three nutrients (Pearson's correlation coefficient: 0.35 for Na and K; 0.47 for Na and protein; 0.55 for K and protein; Table 2). Additionally, 24 h urine-derived intakes of all the three nutrients were associated with survey year, region and residential status. The higher quartile of intakes included more subjects in the 2007 survey; more subjects in the region of central and fewer in the region of north (except K); and more subjects living with family and fewer living alone (protein only). The 24 h urine-derived intakes also showed a positive association with current alcohol drinking and inverse associations with current smoking status (protein only) and physical activity.

As was the case in 24 h urine-derived intakes, there were positive associations between self-reported intakes of all the

**Table 1.** Basic characteristics of subjects\*  
(Mean values and standard deviations or percentages)

	All (n 1043)		General obesity				P†	Abdominal obesity				P†
			Yes (n 80; 7.7%)		No (n 963; 92.3%)			Yes (n 136; 13.0%)		No (n 907; 87.0%)		
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD		
Age (years)	19.6	1.0	19.6	1.1	19.6	1.0	0.73	19.4	1.0	19.6	1.0	0.01
Body height (cm)	158.4	5.4	158.8	5.5	158.3	5.4	0.52	160.2	5.1	158.1	5.4	<0.0001
Body weight (kg)	53.3	7.3	68.2	7.4	52.0	5.7	<0.0001	64.2	8.1	51.6	5.5	<0.0001
BMI (kg/m <sup>2</sup> )	21.2	2.5	27.0	1.9	20.7	1.9	<0.0001	25.0	2.7	20.7	1.9	<0.0001
Waist circumference (cm)	72.7	6.8	85.2	6.8	71.7	5.7	<0.0001	85.0	5.0	70.9	4.9	<0.0001
Survey year (%)							0.49					0.16
2006	38.6		35.0		38.9			44.1		37.8		
2007	61.4		65.0		61.1			55.9		62.2		
Region (%)							0.45					0.01
North	57.9		60.0		57.7			64.7		56.9		
Central	24.1		18.8		24.5			14.0		25.6		
South	18.0		21.3		17.8			21.3		17.5		
Municipality level (%)							0.70					0.47
Ward	16.1		17.5		16.0			12.5		16.7		
City	78.1		78.8		78.1			81.6		77.6		
Town and village	5.8		3.8		5.9			5.9		5.7		
Residential status (%)							0.002					0.18
Living with family	60.5		61.3		60.4			55.2		61.3		
Living alone	35.4		27.5		36.0			38.2		35.0		
Living with others	4.1		11.3		3.5			6.6		3.8		
Current alcohol drinking (%)	42.5		35.0		43.1		0.16	36.0		43.4		0.10
Current smoking (%)	2.5		0		2.7		0.14	1.5		2.7		0.41
Physical activity (total metabolic equivalents-h/d)	33.9	3.1	34.3	5.3	33.9	2.8	0.34	34.0	4.6	33.9	2.8	0.73
Self-reported energy intake (kJ/d)	7414	1896	7585	1889	7400	1897	0.40	7503	1920	7401	1893	0.56
Estimated energy requirement (kJ/d)	8275	766	8942	941	8220	723	<0.0001	8826	862	8193	715	<0.0001
24 h Urine-derived dietary intake‡												
Na (mg/4184 kJ)	1962	751	2143	728	1947	752	0.03	2087	782	1944	745	0.04
K (mg/4184 kJ)	1144	413	1103	337	1148	419	0.36	1116	416	1148	413	0.40
Protein (% of energy)	14.0	3.5	14.5	3.1	13.9	3.5	0.15	14.3	3.7	13.9	3.5	0.21
Self-reported dietary intake§												
Na (mg/4184 kJ)	2055	492	2036	503	2056	491	0.73	1955	522	2069	485	0.01
K (mg/4184 kJ)	1114	257	1067	288	1118	254	0.09	1038	256	1126	255	0.0002
Protein (% of energy)	13.5	1.9	13.2	2.0	13.5	1.8	0.14	13.0	1.8	13.6	1.9	0.0004

\* General obesity was defined as BMI  $\geq 25$  kg/m<sup>2</sup>; abdominal obesity was defined as waist circumference  $\geq 80$  cm<sup>(27)</sup>.

† P values for differences between (generally or centrally) obese and non-obese subjects based on the independent t test for continuous variables and the  $\chi^2$  test for categorical variables.

‡ Energy adjustment was made using estimated energy requirement.

§ Energy adjustment was made using self-reported energy intake.

three nutrients (Pearson's correlation coefficient: 0.31 for Na and K; 0.41 for Na and protein; 0.63 for K and protein; Table 3). Further, self-reported intakes of all the three nutrients were associated with survey year, region, municipality level and residential status. The higher quartiles of intakes included more subjects in the 2006 survey; more subjects in the region of north and fewer in the region of south; more subjects living in wards and fewer living in towns and villages (protein only); and more subjects living with family and fewer living alone. Self-reported protein intake was also inversely associated with current smoking status.

After adjustment for potential confounding variables, 24 h urine-derived Na intake was positively associated with BMI (*P* for trend=0.005) but not with WC (Table 4). The 24 h urine-derived protein intake also showed independent positive associations with both BMI (*P* for trend=0.0008) and WC (*P* for trend=0.02). Conversely, 24 h urine-derived K intake was independently and inversely associated with

both BMI (*P* for trend=0.03) and WC (*P* for trend=0.04). When self-reported dietary intakes were examined, intakes of Na and protein were not associated with BMI or WC. However, self-reported K intake showed independent and inverse associations with BMI (*P* for trend=0.009) and WC (*P* for trend=0.001).

Independent associations of Na, K and protein intakes with general and abdominal obesity are summarised in Table 5. The 24 h urine-derived Na intake was associated with a higher risk of both general (adjusted OR between extreme quartiles 2.49; 95% CI 1.15, 5.42; *P* for trend=0.04) and abdominal (adjusted OR 1.77; 95% CI 1.00, 3.16; *P* for trend=0.04) obesity. Similarly, 24 h urine-derived protein intake showed positive associations with both general (adjusted OR 2.95; 95% CI 1.21, 7.22; *P* for trend=0.02) and abdominal (adjusted OR 1.97; 95% CI 1.02, 3.83; *P* for trend=0.053) obesity. Conversely, 24 h urine-derived K intake was inversely associated with abdominal obesity only (adjusted OR 0.46; 95% CI 0.25, 0.87; *P* for trend=0.01).

**Table 2.** Selected characteristics of subjects according to the quartiles (Q) of 24 h urine-derived dietary intakes of sodium, potassium and protein (n 1043) (Mean values or percentages)

	24 h Urine-derived Na intake				P for trend*	24 h Urine-derived K intake				P for trend*	24 h Urine-derived protein intake				P for trend*
	Q1	Q2	Q3	Q4		Q1	Q2	Q3	Q4		Q1	Q2	Q3	Q4	
	(n 260)	(n 261)	(n 261)	(n 261)		(n 260)	(n 261)	(n 261)	(n 261)		(n 260)	(n 261)	(n 261)	(n 261)	
	Mean	Mean	Mean	Mean		Mean	Mean	Mean	Mean		Mean	Mean	Mean	Mean	
Survey year (%)					<0.0001					<0.0001					<0.0001
2006	49.6	38.3	36.4	30.3		46.9	44.1	33.7	29.9		51.2	42.5	33.0	28.0	
2007	50.4	61.7	63.6	69.7		53.1	55.9	66.3	70.1		48.9	57.5	67.1	72.0	
Region (%)					0.004					0.06					0.0004
North	66.2	60.9	53.6	51.0		59.6	60.5	59.0	52.5		65.8	62.8	60.2	42.9	
Central	16.5	23.8	26.1	29.9		22.7	24.9	22.6	26.1		15.4	20.7	21.8	38.3	
South	17.3	15.3	20.3	19.2		17.7	14.6	18.4	21.5		18.9	16.5	18.0	18.8	
Municipality level (%)					0.31					0.35					0.36
Ward	16.9	14.9	15.7	16.9		17.7	13.4	18.0	15.3		13.9	21.1	14.6	14.9	
City	79.2	80.1	78.2	75.1		77.3	82.0	75.9	77.4		82.3	72.4	79.7	78.2	
Town and village	3.9	5.0	6.1	8.1		5.0	4.6	6.1	7.3		3.9	6.5	5.8	6.9	
Residential status (%)					0.07					0.06					<0.0001
Living with family	53.9	60.5	64.8	62.8		54.2	57.9	63.6	66.3		47.7	63.6	61.3	69.4	
Living alone	41.9	35.6	31.4	32.6		42.7	38.3	33.3	27.2		46.9	33.3	34.9	26.4	
Living with others	4.2	3.8	3.8	4.6		3.1	3.8	3.1	6.5		5.4	3.1	3.8	4.2	
Current alcohol drinking (%)	37.3	42.9	41.8	47.9	0.02	36.2	38.7	47.1	47.9	0.002	37.7	41.4	44.8	46.0	0.04
Current smoking status (%)	2.7	1.5	2.7	3.1	0.59	3.1	2.3	1.9	2.7	0.80	3.9	2.7	2.3	1.2	0.049
Physical activity (total metabolic equivalents-h/d)	34.5	34.2	33.6	33.5	<0.0001	34.6	34.0	33.5	33.7	0.0005	34.7	34.1	33.6	33.3	<0.0001
24 h Urine-derived dietary intake†															
Na (mg/4184 kJ)	1109	1662	2119	2957	<0.0001	1652	1839	2025	2332	<0.0001	1561	1770	2105	2413	<0.0001
K (mg/4184 kJ)	984	1083	1166	1343	<0.0001	694	965	1203	1712	<0.0001	870	1049	1207	1450	<0.0001
Protein (% of energy)	12.0	13.4	14.2	16.3	<0.0001	11.5	13.2	14.7	16.5	<0.0001	10.0	12.6	14.6	18.6	<0.0001

\* For categorical variables, a Mantel-Haenszel  $\chi^2$  test was used; for continuous variables, a linear trend test was used with the median value in each quartile category of dietary intake as a continuous variable in linear regression.  
 † Energy adjustment was made using estimated energy requirement.

**Table 3.** Selected characteristics of subjects according to the quartiles (Q) of self-reported dietary intakes of sodium, potassium and protein (*n* 1043)  
(Mean values or percentages)

	Self-reported Na intake				<i>P</i> for trend*	Self-reported K intake				<i>P</i> for trend*	Self-reported protein intake				<i>P</i> for trend*
	Q1 ( <i>n</i> 260)	Q2 ( <i>n</i> 261)	Q3 ( <i>n</i> 261)	Q4 ( <i>n</i> 261)		Q1 ( <i>n</i> 260)	Q2 ( <i>n</i> 261)	Q3 ( <i>n</i> 261)	Q4 ( <i>n</i> 261)		Q1 ( <i>n</i> 260)	Q2 ( <i>n</i> 261)	Q3 ( <i>n</i> 261)	Q4 ( <i>n</i> 261)	
	Mean	Mean	Mean	Mean		Mean	Mean	Mean	Mean		Mean	Mean	Mean	Mean	
Survey year (%)					0.0007					0.009					<0.0001
2006	31.9	36.0	41.0	45.6		31.9	35.6	45.2	41.8		28.9	35.3	43.7	46.7	
2007	68.1	64.0	59.0	54.4		68.1	64.4	54.8	58.2		71.2	64.8	56.3	53.3	
Region (%)					0.02					0.0497					0.0009
North	51.5	58.6	59.8	61.7		55.8	55.6	57.9	62.5		51.5	56.7	58.6	64.8	
Central	27.3	21.1	26.1	21.8		23.9	24.9	24.9	22.6		25.4	24.9	24.5	21.5	
South	21.2	20.3	14.2	16.5		20.4	19.5	17.2	14.9		23.1	18.4	16.9	13.8	
Municipality level (%)					0.34					0.78					0.007
Ward	14.2	15.7	15.3	19.2		13.1	17.2	16.9	17.2		10.8	12.6	22.6	18.4	
City	80.4	78.9	78.5	74.7		83.1	77.4	75.5	76.6		83.5	81.6	70.1	77.4	
Town and village	2.4	2.4	6.1	6.1		3.9	5.4	7.7	6.1		5.8	5.8	7.3	4.2	
Residential status (%)					0.0001					<0.0001					<0.0001
Living with family	52.3	59.0	62.1	68.6		44.6	59.4	65.9	72.0		40.8	61.7	67.1	72.4	
Living alone	40.4	37.6	36.8	26.8		49.6	36.4	30.7	24.9		52.3	36.4	28.7	24.1	
Living with others	7.3	3.5	1.2	4.6		5.8	4.2	3.5	3.1		6.9	1.9	4.2	3.5	
Current alcohol drinking (%)	45.4	42.2	42.2	40.2	0.26	43.5	43.7	40.6	42.2	0.66	45.4	42.2	37.2	45.2	0.87
Current smoking status (%)	3.5	1.2	1.9	3.5	0.79	4.2	1.2	2.3	2.3	0.34	5.8	2.3	1.2	0.8	0.0003
Physical activity (total metabolic equivalents-h/d)	33.8	34.0	33.9	34.1	0.25	33.8	33.9	33.9	34.2	0.14	34.1	33.7	33.9	34.1	0.81
Self-reported dietary intake†															
Na (mg/4184 kJ)	1475	1883	2171	2686	<0.0001	1862	2016	2066	2274	<0.0001	1808	1988	2115	2307	<0.0001
K (mg/4184 kJ)	1001	1086	1137	1232	<0.0001	818	1015	1171	1452	<0.0001	927	1045	1160	1325	<0.0001
Protein (% of energy)	12.5	13.3	13.8	14.5	<0.0001	12.0	13.2	13.9	15.0	<0.0001	11.3	12.9	14.0	15.9	<0.0001

Sodium, potassium, protein intake and obesity

\* For categorical variables, a Mantel-Haenszel  $\chi^2$  test was used; for continuous variables, a linear trend test was used with the median value in each quartile category of dietary intake as a continuous variable in linear regression.  
† Energy adjustment was made using self-reported energy intake.

**Table 4.** BMI and waist circumference according to the quartiles (Q) of sodium, potassium and protein intakes (*n* 1043) (Mean values with their standard errors)

	Q1 ( <i>n</i> 260)		Q2 ( <i>n</i> 261)		Q3 ( <i>n</i> 261)		Q4 ( <i>n</i> 261)		<i>P</i> for trend*
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	
24 h Urine-derived dietary intake†									
Na (mg/4184 kJ; median)	1155		1659		2124		2766		
BMI (kg/m <sup>2</sup> )									
Crude	21.0	0.2	21.2	0.2	21.1	0.2	21.7	0.2	0.001
Adjusted‡	21.0	0.2	21.2	0.2	21.1	0.2	21.7	0.2	0.005
Waist circumference (cm)									
Crude	72.7	0.4	72.6	0.4	72.5	0.4	73.1	0.4	0.48
Adjusted‡	72.3	0.4	72.6	0.4	72.6	0.4	73.3	0.4	0.14
K (mg/4184 kJ; median)	708		968		1198		1620		
BMI (kg/m <sup>2</sup> )									
Crude	21.0	0.2	21.5	0.2	21.3	0.2	21.1	0.2	0.98
Adjusted‡	21.3	0.2	21.6	0.2	21.3	0.2	20.8	0.2	0.03
Waist circumference (cm)									
Crude	72.8	0.4	73.0	0.4	73.1	0.4	72.0	0.4	0.16
Adjusted‡	73.1	0.5	73.0	0.4	73.1	0.4	71.7	0.5	0.04
Protein (% of energy; median)	10.3		12.6		14.6		17.9		
BMI (kg/m <sup>2</sup> )									
Crude	20.9	0.2	21.1	0.2	21.6	0.2	21.4	0.2	0.005
Adjusted‡	20.7	0.2	21.1	0.2	21.5	0.2	21.6	0.2	0.0008
Waist circumference (cm)									
Crude	72.5	0.4	72.7	0.4	73.0	0.4	72.7	0.4	0.75
Adjusted‡	71.7	0.5	72.6	0.4	73.1	0.4	73.5	0.5	0.02
Self-reported dietary intake§									
Na (mg/4184 kJ; median)	1526		1882		2167		2580		
BMI (kg/m <sup>2</sup> )									
Crude	21.4	0.2	21.3	0.2	21.2	0.2	21.0	0.2	0.11
Adjusted‡	21.2	0.2	21.2	0.2	21.3	0.2	21.2	0.2	0.94
Waist circumference (cm)									
Crude	73.7	0.4	72.9	0.4	72.2	0.4	72.1	0.4	0.004
Adjusted‡	73.3	0.4	72.8	0.4	72.4	0.4	72.4	0.4	0.14
K (mg/4184 kJ; median)	837		1015		1173		1393		
BMI (kg/m <sup>2</sup> )									
Crude	21.7	0.2	21.2	0.2	21.3	0.2	20.7	0.2	<0.0001
Adjusted‡	21.6	0.2	21.2	0.2	21.3	0.2	20.8	0.2	0.009
Waist circumference (cm)									
Crude	74.2	0.4	72.9	0.4	72.8	0.4	71.1	0.4	<0.0001
Adjusted‡	73.8	0.5	72.9	0.4	72.8	0.4	71.4	0.5	0.001
Protein (% of energy; median)	11.5		12.9		14.0		15.6		
BMI (kg/m <sup>2</sup> )									
Crude	21.6	0.2	21.3	0.2	21.2	0.2	20.8	0.2	0.0002
Adjusted‡	21.4	0.2	21.3	0.2	21.2	0.2	20.9	0.2	0.08
Waist circumference (cm)									
Crude	73.9	0.4	73.0	0.4	72.5	0.4	71.6	0.4	<0.0001
Adjusted‡	73.2	0.5	72.8	0.4	72.7	0.4	72.3	0.5	0.20

\* A linear trend test was used with the median value in each quartile category of dietary intake as a continuous variable in linear regression.

† Energy adjustment was made using estimated energy requirement.

‡ Adjusted for survey year (2006 or 2007), region (north, central or south), municipality level (ward, city, or town and village), residential status (living with family, living alone or living with others), current alcohol drinking (yes or no), current smoking status (yes or no), physical activity (total metabolic equivalents-h/d, continuous), and 24 h urine-derived or self-reported intakes of the other two nutrients (continuous).

§ Energy adjustment was made using self-reported energy intake.

When self-reported information was used to estimate dietary intakes, Na and protein intakes were not associated with both general and abdominal obesity. However, self-reported K intake was associated with a lower risk of both general (adjusted OR 0.39; 95% CI 0.17, 0.92; *P* for trend=0.04) and abdominal (adjusted OR 0.48; 95% CI: 0.24, 0.93; *P* for trend=0.02) obesity.

## Discussion

To our knowledge, this is the first study to investigate the ability of self-reported estimates of dietary intakes of Na, K

and protein to detect an association with obesity, by comparison with the corresponding estimates derived from 24 h urinary excretion. When 24 h urinary excretion was used for estimating dietary intakes, we found the positive associations of Na and protein intakes with both general and abdominal obesity and the inverse association of K with abdominal obesity in a group of young Japanese women. However, no association was observed based on using self-reported dietary intakes, except for inverse associations between K intake and general and abdominal obesity. These results suggest that the ability of self-reported information using the DHQ, at least for

**Table 5.** Odds ratios for general and abdominal obesity according to the quartiles (Q) of sodium, potassium and protein intakes (*n* 1043)\* (Odds ratios and 95 % confidence intervals)

	Q1 ( <i>n</i> 260)		Q2 ( <i>n</i> 261)		Q3 ( <i>n</i> 261)		Q4 ( <i>n</i> 261)		<i>P</i> for trend†
	OR (reference)	OR	95 % CI	OR	95 % CI	OR	95 % CI		
<b>24 h Urine-derived dietary intake‡</b>									
Na (mg/4184 kJ; median)	1155		1659		2124		2766		
General obesity (%)	5.0		8.4		6.5		10.7		
Crude model	1	1.75	0.86, 3.55	1.32	0.63, 2.78	2.28	1.16, 4.52	0.03	
Adjusted model§	1	1.81	0.88, 3.74	1.43	0.66, 3.09	2.49	1.15, 5.42	0.04	
Abdominal obesity (%)	11.5		11.5		12.3		16.9		
Crude model	1	1.00	0.58, 1.71	1.07	0.63, 1.82	1.56	0.94, 2.56	0.06	
Adjusted model§	1	1.06	0.61, 1.83	1.20	0.69, 2.09	1.77	1.00, 3.16	0.04	
K (mg/4184 kJ; median)	708		968		1198		1620		
General obesity (%)	6.2		10.0		8.1		6.5		
Crude model	1	1.69	0.88, 3.23	1.33	0.68, 2.62	1.06	0.53, 2.15	0.80	
Adjusted model§	1	1.48	0.75, 2.90	1.03	0.49, 2.16	0.60	0.26, 1.40	0.09	
Abdominal obesity (%)	14.6		13.8		11.9		11.9		
Crude model	1	0.94	0.57, 1.53	0.79	0.47, 1.31	0.79	0.47, 1.31	0.30	
Adjusted model§	1	0.78	0.47, 1.31	0.59	0.33, 1.04	0.46	0.25, 0.87	0.01	
Protein (% of energy; median)	10.3		12.6		14.6		17.9		
General obesity (%)	4.6		7.7		9.6		8.8		
Crude model	1	1.72	0.82, 3.59	2.19	1.08, 4.46	2.00	0.97, 4.10	0.07	
Adjusted model§	1	2.02	0.94, 4.36	2.71	1.24, 5.94	2.95	1.21, 7.22	0.02	
Abdominal obesity (%)	11.2		13.0		13.8		14.2		
Crude model	1	1.19	0.70, 2.02	1.27	0.76, 2.15	1.32	0.78, 2.21	0.31	
Adjusted model§	1	1.46	0.84, 2.54	1.55	0.87, 2.77	1.97	1.02, 3.83	0.053	
<b>Self-reported dietary intake  </b>									
Na (mg/4184 kJ; median)	1526		1882		2167		2580		
General obesity (%)	8.5		8.4		5.8		8.1		
Crude model	1	1.00	0.54, 1.85	0.66	0.33, 1.30	0.95	0.51, 1.77	0.66	
Adjusted model§	1	1.13	0.59, 2.14	0.85	0.41, 1.74	1.23	0.61, 2.47	0.70	
Abdominal obesity (%)	17.7		14.2		9.6		10.7		
Crude model	1	0.77	0.48, 1.23	0.49	0.29, 0.83	0.60	0.34, 0.93	0.009	
Adjusted model§	1	0.84	0.51, 1.38	0.60	0.34, 1.03	0.77	0.44, 1.36	0.22	
K (mg/4184 kJ; median)	837		1015		1173		1393		
General obesity (%)	10.8		8.1		7.3		4.6		
Crude model	1	0.73	0.40, 1.31	0.65	0.35, 1.20	0.40	0.20, 0.80	0.01	
Adjusted model§	1	0.71	0.38, 1.33	0.67	0.34, 1.34	0.39	0.17, 0.92	0.04	
Abdominal obesity (%)	19.2		14.2		11.1		7.7		
Crude model	1	0.69	0.44, 1.10	0.53	0.32, 0.86	0.35	0.20, 0.60	<0.0001	
Adjusted model§	1	0.79	0.48, 1.29	0.62	0.36, 1.07	0.48	0.24, 0.93	0.02	
Protein (% of energy; median)	11.5		12.9		14.0		15.6		
General obesity (%)	10.8		8.1		5.8		6.1		
Crude model	1	0.73	0.40, 1.31	0.51	0.26, 0.97	0.54	0.29, 1.03	0.03	
Adjusted model§	1	0.72	0.38, 1.36	0.47	0.23, 0.99	0.53	0.23, 1.22	0.09	
Abdominal obesity (%)	18.9		14.9		9.2		9.2		
Crude model	1	0.76	0.48, 1.20	0.44	0.26, 0.74	0.44	0.26, 0.74	0.0003	
Adjusted model§	1	0.86	0.52, 1.41	0.54	0.30, 0.98	0.64	0.33, 1.27	0.11	

\* General obesity was defined as BMI  $\geq 25$  kg/m<sup>2</sup>; abdominal obesity was defined as waist circumference  $\geq 80$  cm<sup>(27)</sup>.

† Logistic regression models were used with the median value in each quartile category of dietary intake as a continuous variable in logistic regression.

‡ Energy adjustment was made using estimated energy requirement.

§ Adjusted for survey year (2006 or 2007), region (north, central or south), municipality level (ward, city, or town and village), residential status (living with family, living alone or living with others), current alcohol drinking (yes or no), current smoking status (yes or no), physical activity (total metabolic equivalents-h/d, continuous) and 24 h urine-derived or self-reported intakes of other two nutrients (continuous).

|| Energy adjustment was made using self-reported energy intake.

estimating Na and protein intakes in terms of investigating diet–obesity relationships, is limited.

Only a limited number of studies have investigated the dietary intakes of protein, Na and K in relation to body fatness. Self-reported protein intake was positively associated with subsequent weight gain in two very large prospective studies in European countries<sup>(34,35)</sup>. Very recently, a small study in middle-aged Danish men and women has shown that both self-reported and 24 h urine-derived protein intakes are associated with greater weight gain<sup>(13)</sup>. Consistent with these

studies, we found positive associations between 24 h urine-derived protein intake and general and abdominal obesity. For Na, self-reported intake was cross-sectionally associated with a higher risk of obesity in Korean children and adults, independent of energy intake<sup>(14)</sup>. The 24 h urinary excretion was cross-sectionally associated with higher BMI in young Swedish men<sup>(15)</sup> and higher BMI and WC in middle-aged men and women in Venezuela<sup>(16)</sup>, although no adjustment was made in these two studies. In a study in German children and adolescents, higher 24 h Na excretion was associated with

subsequent increase in body fat percentage after adjustment for sugar-sweetened beverages or energy intake<sup>(17)</sup>. In middle-aged Danish men and women, 24 h urinary excretion of Na was associated positively with change in fat mass and inversely with change in fat-free mass, independently of energy intake<sup>(18)</sup>. We also found that 24 h urine-derived Na intake was associated with a higher risk of general and abdominal obesity with energy adjustment. For K, we are not aware of studies where 24 h urinary K is investigated in relation to body fatness; however, self-reported K intake was inversely associated with abdominal obesity independent of energy and macronutrient intakes in a Korean national representative cross-sectional study<sup>(19)</sup>. Additionally, the ratio of urinary Na to K in a first-void morning urinary sample was cross-sectionally associated with lower percentage body fat in a multiethnic cohort in the USA<sup>(20)</sup>. We also found inverse associations between 24 h urine-derived and self-reported K intake and general (self-report only) and abdominal obesity, although the present study found no association between the ratio of Na to K and obesity (data not shown).

While all the three nutrients were associated with obesity when dietary intake was derived from 24 h urinary excretion, only K, but not Na and protein, was associated with obesity when self-reported dietary intake was used. This seems reasonable given the modest (although still low) correlation between 24 h urine-derived and self-reported intakes for K compared with very low correlations for Na and protein. Additionally, as the validity of the DHQ has been examined mainly in adults<sup>(29–32)</sup>, the validity of the instrument for assessing dietary intakes in young women is largely unknown. Further, the DHQ is not suitable for assessing Na intakes<sup>(29)</sup>, as are other dietary assessment questionnaires. In any case, the present findings highlight the difficulty in obtaining valid estimates of dietary intake (at least of some nutrients such as Na and protein) from self-reported dietary information using the DHQ as a basis for investigating diet–obesity relationships.

The strengths of the present study include the use of objective biomarkers for dietary intakes of Na, K and protein and measured anthropometric data in a relatively large sample. However, there are also several limitations. First, the cross-sectional nature of the study does not permit the assessment of causality owing to the uncertain temporality of the association. Only a prospective study with the use of biomarkers or carefully taking into account dietary misreporting would provide better understanding of the relationship between dietary intakes of Na, K and protein and obesity. Second, the present results were based on a highly selected population of female dietetic students, who probably had a higher education level and greater knowledge of diet and nutrition than the general population. It is not clear how such characteristics affected the associations we observed here. Thus, the present findings may only be specific to the present population and may not be extrapolatable to the Japanese population in general. Third, obtaining a valid dietary intake derived from urine excretion requires that 24 h urine collection be complete. We, therefore, excluded participants whose 24 h urine collection was considered incomplete, as assessed based on a strategy<sup>(26)</sup> validated against the PABA check method<sup>(24)</sup>, as described

above. Also, a repeated analysis in 601 women having complete urine assessed by PABA (PABA recovery  $\geq 85\%$ ) in a subsample ( $n$  654) of the present participants provided similar results (data not shown). Although our use of only a single 24 h urine sample is not an optimal way to characterise individual habitual dietary intake and introduces random errors<sup>(36)</sup>, this kind of error would nevertheless tend to result in bias towards attenuating rather than enhancing the relationship, and multiple 24 h urine collections would have only provided more precise results. Fourth, concerns have been expressed regarding the precision of the correction factors used to estimate the dietary intakes from 24 h urine. Many factors may influence the percentage of dietary protein (N), K and Na excreted in the urine, including the absolute level of dietary intake, season during which the balance studies is conducted, race and cooking methods<sup>(37)</sup>. Here, we used correction factors observed in previous carefully designed balance studies<sup>(9–11)</sup>. Fifth, 24 h urine-derived dietary intake was energy-adjusted using estimated energy requirement calculated based on an equation from the US Dietary Reference intakes<sup>(28)</sup> in addition to non-validated information on self-reported physical activity. Although the equation was developed based on a large number of measurements of total energy expenditure by the doubly labelled water method and is highly accurate, it is predominantly based on data from white populations<sup>(28)</sup>, and might, therefore, be inappropriate for the present Japanese population. Nonetheless, as we found the similar results when the associations between 24 h urine-derived intakes and obesity were examined without energy-adjustment (data not shown), any measurement error of estimated energy requirement would not have a major impact on the present findings. Sixth, self-reported dietary data were collected using a dietary assessment questionnaire (i.e. DHQ). Thus, the present findings might be specific to this dietary assessment questionnaire and should be interpreted in this context, and different dietary assessment methods may perform differently. It should also be emphasised that the DHQ and a 24 h urinary collection assess dietary intake over different time periods, namely in the previous month for the former and in the previous 1 d for the latter. Ideally, the associations between obesity and self-reported and biomarker-based intakes should be investigated over similar time frames for comparing them. Finally, although we adjusted for a variety of potential confounding variables, residual confounding could not be ruled out.

In conclusion, in this cross-sectional study in a group of young Japanese women, we showed positive associations between Na and protein intakes and general and abdominal obesity, and an inverse association between K intake and abdominal obesity when dietary intake was estimated on the basis of 24 h urinary excretion. Conversely, when self-reported information was used to estimate dietary intake, only associations between K and general and abdominal obesity were observed, with no association for Na and protein intakes, suggesting only limited ability of self-reported dietary intakes of, at least, Na and protein using the DHQ to detect diet–obesity associations. The present study highlights the need to critically evaluate self-reported dietary data in diet–obesity research as well as potential usefulness

of even a single 24 h urine collection. Further research using a self-reported instrument and a biomarker with the same reference period (e.g. multiple 24 h recalls and multiple 24 h urinary collections) would be of interest.

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The authors' contributions are as follows: K. M. contributed to the concept and design of the survey, coordination of the fieldwork, data collection and management, hypothesis formulation, statistical analysis, data interpretation and manuscript writing; M. B. E. L. helped in writing of the manuscript; S. S. contributed to the concept and design of the survey, data collection and manuscript editing; K. U. contributed to the concept and design of the survey and data collection. All authors read and approved the final manuscript.

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