FEASIBILITY OF OVERNIGHT URINE FOR ASSESSING DIETARY INTAKES OF SODIUM, POTASSIUM, PROTEIN AND SULFUR AMINO ACIDS IN FIELD STUDIES

MASASHI OGAWA, M.D.

The feasibility of using overnight urine as an alternative to 24-hr urine was examined on measures of dietary intake of sodium (Na), potassium (K) and protein as well as the sulfur amino acids, which are contained mainly in animal protein. It was also of interest whether urinary excretions of taurine (Tau: final metabolite of sulfur amino acids, contained mainly in animal protein) and excretions of 3-methylhistidine (3-MHis: the product of breakdown of skeletal muscle protein, quantitatively excreted into urine) were appropriate in assessing the dietary intake of animal protein and total protein, respectively.

Overnight urine specimens were collected from 16 subjects (19 to 60 years old) with normotension or borderline hypertension without complications. Creatinine (Cr) ratios to Na, K, urea nitrogen (UN) and inorganic sulfate (SO_4) derived from overnight urine and from 24-hr urine specimens showed significant correlations. Similar correlations were also found for the Na/K and SO_4 /UN ratios between overnight and 24-hr urine specimens. Concentrations of Tau and 3-MHis (mmol per g Cr) of overnight urine specimens were strongly correlated with 24-hr urinary excretions of Tau and 3-MHis (μmol per day), respectively. Furthermore, significant correlations were found between 24-hr urinary excretions of UN and 3-MHis and between those of SO_4 and Tau. These results indicate that an overnight urine specimens are available for assessing dietary intakes of Na and K, as well as protein and sulfur amino acids in field studies.

EXCESS sodium intake causes hypertension in laboratory animal models¹ and humans^{2,3}. Increased potassium intake may protect against this effect^{4,5}. Current studies on experimental animal models⁶ and epidemiological evidence⁷ suggest that supplementary protein (particularly animal protein) might reduce high blood pressure

and reverse hypertensive complications. Attempts to determine the influence of these nutritional factors on the development of hypertension in man have yielded contradictory results. This may be, in part, due to the practical problems of assessing sodium (Na), potassium (K) and protein intake or excretion, or both, in free-living populations. Dietary recall histories are unreliable in predicting dietary intake of these nutritional factors. One quantitative way to assess dietary intake in free-living populations is by 24-hr urine collection. However, it is difficult to obtain 24-hr urine collections, even though simple portable devices are available to do so^{8,9}.

Key Words:

Dietary intake
Protein
Sodium
Potassium
Taurine
3-methylhistidine

(Received December 18, 1985; accepted February 21, 1986)

Department of Internal Medicine, National Kawatana Hospital, Kawatana-cho, Higashisonogi-gun, Nagasaki, Japan

Mailing address: Masashi Ogawa M.D., at the Department of Internal Medicine, National Kawatana Hospital, Kawatana-cho, Higashisonogi-gun, Nagasaki 859-36, Japan

Most Na and K are excreted into the urine, in the absence of excess perspiration.¹⁰ Protein or nitrogen is also excreted into the urine, mainly in the form of urea nitrogen (UN), except for a small loss into the feces and from the skin.¹¹

Sulfur enters the body mainly as a constituent of the amino acids, taurine, cystine and methionine.¹² Food also contains inorganic and organic sulfate, but these sources provide only a small part of the body's requirement for sulfur.¹² Practically speaking, the human body is dependent on methionine and cystine for its sulfur supplies. Sulfur is excreted into urine mainly as inorganic sulfate (SO_4).^{12,13} Thus, urinalysis may well be a useful quantitative method for assessment of nutritional status, at least with regard to cations, protein and sulfur amino acids.¹³

Because 24-hr urine collections are difficult to obtain in population studies, the reliability of partial urine samples used as substitutes for 24-hr urine specimens was of interest. Thus, this report compares 24-hr Na and K excretions as well as UN and SO_4 excretions with those obtained in single specimens voided at the time of awakening in the morning (overnight urine) with 24-hr urine.

Taurine (Tau), the final metabolite of methionine, is distributed widely in mammalian tissues, particularly in skeletal muscle, heart retina, spleen, and brain.¹² Although the physiological and biochemical roles of Tau have not yet been specified, urinary excretion of Tau is used as an indicator of dietary intake of animal protein, which contains a large amount of Tau.¹²

3-Methylhistidine (3-MHis) is an amino acid found mainly in myofibrillar protein.¹⁴ When the protein is degraded, 3-MHis is released but cannot be re-utilized; it is subsequently rapidly and quantitatively excreted in urine.^{15,16} In the light of suggestions^{15,16} that the rate of urinary excretion of 3-MHis should reflect the break-down of protein containing this amino acid, 3-MHis has been used as another biochemical marker for evaluation of somatic protein mass.¹⁷ Because muscle protein is conserved through a reduction in protein break-down during periods of inadequate protein intake, protein restriction tends to diminish urinary excretion of 3-MHis and protein supplements tend to increase it.¹⁸

Therefore, this report compares 24-hr urinary excretions of UN and SO_4 with those of 3-MHis and Tau, respectively, and examines the feasibility of using overnight urine samples as substitutes for 24-hr urine specimens to estimate 24-hr

urinary excretions of Tau and 3-MHis.

SUBJECTS AND METHODS

Twenty-one subjects with normotension or borderline hypertension (11 men and 10 women, 19 to 65 years old) were initially selected for this study. Borderline hypertension was defined as blood pressure intermittently above 141 mmHg systolic, or 91 mmHg diastolic, as measured with a sphygmomanometer with the subject in the sitting position. Patients with renal function impairment, liver disease, malignant tumor and diabetes mellitus were excluded. The subjects had received neither diuretics nor antihypertensive agents for at least two weeks prior to the study. Because our basic interest is in finding a method for subdividing free-living populations into subgroups based on their blood pressure levels and dietary intake of nutrients, borderline hypertensive patients were included in this study. Kohashi and Katori¹⁹ reported a decrease of urinary Tau excretion in essential hypertension and recently Ogawa et al.²⁰ found a decrease of plasma Tau and methionine in essential hypertension. Therefore, the relationship between 24-hr urinary excretions of Tau and SO_4 was examined in the group including borderline hypertensive patients.

Both 24-hr urine and overnight urine were collected from all subjects as follows: the first urine passed in the morning was discarded and then subsequent urine was collected until the next morning, with a simple portable device (Memorette, Sumitomo Bakelite, Japan) which could accurately collect 1/50 of 24-hr total urine.⁸ The first urine voided in the next morning was collected as "overnight urine" in this study.

The completeness of each 24-hr urine collection was confirmed by examining the 24-hr urinary excretion of creatinine (Cr). If the 24-hr urinary excretion of Cr was less than a reasonable value according to the standard curves of urinary Cr reported by Joossens et al., the 24-hr urine sample was excluded from this study as an incomplete collection. Sixteen subjects (8 men and 8 women, 19 to 60 years old) were finally included in this study. The participants were composed of 5 normotensive and 11 borderline hypertensive patients. Blood pressure of normotensive patients were 126 ± 10 mmHg in systole and 62 ± 4 mmHg in diastole, and those of borderline hypertensive patients 150 ± 4 mmHg

in systole and 92 ± 2 mmHg in diastole. Of the normotensive patients, 3 had cardiac arrhythmia (paroxysmal atrial tachycardia with no basic heart disease) and 2 had transient ischemic attack. In all cases, the general physical and nutritional states of the patients were normal when they were entered into this study. Serum Cr was less than 1.02 mg/dl, and Cr clearance higher than 82 l/day in all patients. Their 24-hr urinary excretions of Cr were higher than 900 mg.

All samples were stored at -20°C until assayed. Concentrations of Na and K were determined by flame photometry (IL-643, Instrument Laboratory, USA), UN by the urease method, Cr by Jaffe's procedure²¹ and SO_4 by the turbidimetric method described by Berglund and Sörbo²²

Dietary Na and K ingestion relative to body size was then assessed from the Na/Cr and K/Cr ratios, respectively. Protein intake from SO_4/Cr (a possible index of the amount of dietary sulfur-containing amino acid derived mainly from animal protein)¹³ UN/Cr (a possible index of total protein intake)¹¹ and SO_4/UN (an index related to the "net dietary protein calorie per cent" or sulfur amino acid score in the diet as previously reported)²³ were evaluated.

Amino acids analyses were performed for 11 subjects including 3 normotensives and 8 borderline hypertensives. Concentrations of amino acids, including Tau and 3-MHis, in urine samples were measured by high-performance liquid chromatography (Shimadzu LC-3A, Japan). Amino acids were separated on a Shimadzu ISC-07/S1504 (stainless steel column, 150×4.0 mm I.D). Amino acids were detected with a fluorescence monitor (Shimadzu RF-530, Japan) after post-column derivatization of amino acids using O-phthalaldehyde and sodium hypochlorite²⁴. The concentrations were represented as mmol per g Cr.

No information on the purpose of the study was given to the participants before the examination in order to avoid intentional change in their usual dietary habits.

The data are represented as mean $\pm$ S.D. The statistical significance of differences was assessed by means of Student's t-test, and regression lines and correlation coefficients were calculated by the least-squares method. Differences and correlation coefficients were considered to be statistically significant at $p < 0.05$.

TABLE I AGE OF SUBJECTS AND CONSTITUENTS OF 24-hr URINE

Mean $\pm$ SD			
Age	51 ± 16	years old	(16)
Urine volume	1539 ± 539	ml/day	(16)
Na excretion	168 ± 68	mEq/day	(16)
K excretion	40 ± 6	mEq/day	(16)
UN excretion	8431 ± 2239	mg/day	(16)
SO_4 excretion	1495 ± 812	mg/day	(16)
Tau excretion	948 ± 31	$\mu\text{mol/day}$	(11)
3-MHis excretion	204 ± 14	$\mu\text{mol/day}$	(11)
Cr excretion	1153 ± 34	mg/day	(16)

Number of subjects studied is presented in parentheses.

TABLE II CORRELATION COEFFICIENTS AND REGRESSION EQUATIONS BETWEEN OVERNIGHT URINE AND 24-hr URINE

	R	Slope	Constance
Na/Cr	0.837**	0.830	0.041
K/Cr	0.753**	0.820	0.117
UN/Cr	0.772**	0.820	1.716
SO_4/Cr	0.840**	0.676	0.463
Na/K	0.901**	0.764	0.961
SO_4/UN	0.938**	0.973	0.014

Correlation coefficients and regression equations were calculated from 16 pairs.

** $p < 0.01$

TABLE III CORRELATION COEFFICIENTS AND REGRESSION EQUATIONS OF Tau AND 3-MHis BETWEEN OVERNIGHT URINE AND 24-hr URINARY EXCRETION

	R	Slope	Constance
Tau	0.875**	1210.5	112.2
3-MHis	0.762**	871.2	47.2

Correlation coefficients and regression equations were calculated from 11 pairs. Concentrations of Tau and 3-MHis in overnight urine were represented as mmol per g Cr.

** $p < 0.01$

RESULTS

Complete 24-hr urine samples and overnight urine samples were obtained from 16 subjects (8 men and 8 women). The data from analysis of

Fig.1. Correlations between 24-hr urinary excretion of Tau (μmol/day) and 24-hr urinary excretion of SO₄ (mg/day) (left graph), and between 24-hr urinary excretion of 3-MHIs (μmol/day) and 24-hr urinary excretion of UN (mg/day) (right graph).

Fig.2. Correlations between 24-hr urinary excretion of cystine (μmol/day) and 24-hr urinary excretion of SO₄ (mg/day) (left graph), and between 24-hr urinary excretion of methionine (μmol/day) and 24-hr urinary excretion of SO₄ (mg/day) (right graph).

24-hr urine specimens are presented in Table I. The 24-hr urinary excretion of Na was 168 ± 68 mEq/day, which corresponds to 9.8 g NaCl/day. The importance of complete urine collection has

been stressed by Joossens et al²⁵ and, on the basis of their standard curves of urinary Cr, all the examinees finally included in our study provided complete collections of 24-hr voided

urine. Table II shows that the ratios of Na/Cr, K/Cr, UN/Cr, SO_4 /Cr, Na/K and SO_4 /UN of the overnight urine samples were highly correlated with those in the 24-hr urine specimens ($p < 0.01$ for all case), the highest correlation occurring for Na/K and the next highest for SO_4 /UN.

As shown in Table III, the concentrations of Tau and 3-MHis (mmol per g Cr) of overnight urine samples were highly correlated with 24-hr urinary excretions of Tau and 3-MHis, respectively ($p < 0.01$ for each case).

Furthermore, the correlations between 24-hr urinary excretions of UN and 3-MHis (a possible index of total protein intake) as well as between those of SO_4 and Tau (a possible index of animal protein intake) were examined, and high correlations were found ($p < 0.01$ in each case), as shown in Fig. 1. In the case of other sulfur amino acids such as methionine and cystine, such correlations were not observed, as shown in Fig. 2.

DISCUSSION

The method most frequently used to measure urinary excretions of Na and K is the collection of 24-hr urine specimens. This is also in the case for evaluating urinary excretions of UN and SO_4 .

This method has two major disadvantages for use in free-living population groups. The first of these is the difficulty in collecting such specimens, even if a convenient portable device is used. This is both logistically difficult for and embarrassing to persons who are employed, or who for any other reason spend a portion of the day away from home. In addition, if such collections are acceded to, many subjects prefer to make these collections on weekends to avoid the above complications. This could lead to a selective bias away from normal values, if the subject's activity and meal intake during the week-end are different from those during the week,⁹ as is likely. The second disadvantage is the inability to assess the completeness of the 24-hr collection once collected if a portion of the collection was missed and not recorded.

Investigators have therefore examined the utility of overnight urine collections. Such specimens are far easier and more convenient to obtain than 24-hr collections.

Several studies have considered the use of overnight urine specimens for estimating 24-hr urinary excretions of Na, or both Na and K^{26,27} There has, however, been no report on the utility

of overnight urine collections for estimating 24-hr urinary excretions of Na, K, UN, SO_4 , Tau and 3-MHis in the same samples, though Yamori et al.²⁸ reported the utility of partial urine samples including overnight urine samples for assessment of 24-hr urinary excretions of Na, K, UN and SO_4 . In the present study, we corroborated the findings of previous investigators that a good correlation between Na excretions calculated from overnight urine and from 24-hr urine could be obtained.

In order to assess 24-hr excretion of various constituents with partial urine, the partial urine sample should be representative of the 24-hr urine sample. Therefore, the ratios of Na, K, UN and SO_4 to Cr were selected as parameters, since Cr excretion has been considered to be proportional to lean body mass,¹⁰ and evaluation of the ratio of any component to Cr has been regarded as a useful means for correcting individual differences in urinary nutritional surveys.^{11,12} Good correlations of the ratios of Na, K, UN and SO_4 to Cr between overnight urine and 24-hr urine specimens were obtained. In addition, there were good correlations of Na/K as well as SO_4 /UN between overnight urine and 24-hr urine specimens. Other biochemical indicators of protein intake are Tau and 3-MHis, whose excretions in urine reflect the dietary intakes of animal protein²⁹ and total protein,¹⁵ respectively. When the concentration of amino acids was represented as mmol per g Cr, good correlations were also observed between overnight urinary Tau and 3-MHis (mmol per g Cr) and 24-hr urinary excretions of Tau and 3-MHis (μmol per day), respectively.

Dietary protein is finally broken-down to UN, which is excreted in urine.¹¹ The present study demonstrated a good correlation between 24-hr urinary excretions of UN and 3-MHis.

Animal protein contains large amounts of sulfur amino acids such as methionine, cystine and Tau.¹² Animal protein ingested is metabolized and excreted mainly in the urine as SO_4 or Tau.¹² Therefore, it is expected theoretically that 24-hr urinary excretions of SO_4 and Tau would be related with each other. Indeed, this study demonstrated a good correlation between 24-hr urinary excretions of SO_4 and Tau. On the other hand, no correlation was observed between 24-hr urinary excretions of cystine or methionine and those of SO_4 . The difference between the correlation for 24-hr urinary excretions of SO_4 and Tau, and that for 24-hr urinary excretions

of SO_4 and other sulfur amino acids such as methionine and cystine, may be explained by differences in a rate of renal clearance of these amino acids in the kidney.³⁰ Thus, the validity of assessment of dietary intake by measurements of 24-hr urinary excretions of UN and SO_4 is confirmed by the determinations of 24-hr urinary excretion of amino acids such as Tau and 3-MHis.

These results indicate that overnight urine samples can be used as reliable substitutes for 24-hr urine specimens in field studies of nutritional status.

REFERENCE

1. DAHL LK: Effects of chronic excess salt feeding: induction of salt sustaining hypertension in rats. *J Exp Med* 114: 231, 1961
2. SASAKI N: High blood pressure and the salt intake of the Japanese. *Jpn Heart J* 3: 313, 1962
3. MENEELY GR, BALL COT: Experimental epidemiology of chronic sodium toxicity and the protective effect of potassium chloride. *Am J Med* 25: 713, 1958
4. DAHL LK, LEITL G, HEINE M: Influence of dietary potassium and sodium/potassium molar ratios on the development of salt hypertension. *J Exp Med* 136: 318, 1972
5. PRIDDLE WW: Hypertension-sodium and potassium studies. *Can Med Assoc J* 86: 1, 1962
6. YAMORI Y, HORIE R, IKEDA K, NARA Y, LOVENBERG W: Prophylactic effect of dietary protein on stroke and its mechanism. In *Prophylactic Approach to Hypertensive Disease*, ed by YAMORI Y, LOVENBERG W, FREIS ED, Raven Press, New York, 1979, pp 497
7. REED D, McGEE D, YANO K: Biological and social correlates of blood pressure among Japanese men in Hawaii. *Hypertension* 4: 406, 1982
8. TOCHIKUBO O, UEDA S, KANEKO Y: Simple portable device for sampling a whole day's urine and its application to hypertensive out patients. *Hypertension* 5: 270, 1983
9. YAMORI Y, NARA Y, KIHARA M, HORIE R: Simple method for sampling consecutive 24-hour urine for epidemiological and clinical studies. *Clin Exper Hyper* 6: 1161, 1984
10. DAHL LK: Salt intake and salt need. *New Engl J Med* 258: 1152, 1958
11. SIMMONS WK: Urinary urea nitrogen/creatinine ratio as indicator of recent protein intake in field studies. *Am J Clin Nutr* 25: 539, 1972
12. JACOBSEN JG, SMITH LH: Biochemistry and physiology of taurine and taurine derivatives. *Physiol Rev* 43: 424, 1968
13. SIMMONS WK: Use of inorganic sulfate sulfur/creatinine ratio in field studies. *Am J Clin Nutr* 26: 72, 1973
14. YOUNG VR, MUNRO HN: N -methylhistidine (3-methylhistidine) and muscle protein turnover: an overview. *Federation Proc* 37: 2291, 1978
15. YOUNG VR, ALEXIS SD, BALIGE BS, MUNRO HN, MUECKE W: Metabolism of administered 3-methylhistidine. Lack of muscle transfer ribonucleic acid charging and quantitative excretion as 3-methylhistidine and its N-acetyl derivatives. *J Biol Chem* 247: 3592, 1972
16. LONG CL, HAVERBERG LN, YOUNG VR, KINNEY M, MUNRO HN, GEIGER JW: Metabolism of 3-methylhistidine in man. *Metabolism* 24: 929, 1975
17. HAIDEN M, HAIDEN SQ: Assessment of protein-calorie malnutrition. *Clin Chem* 30: 1286, 1984
18. BISTORIAN BR, WINTERER JC, BLACKBURN GL, YOUNG VR, SHERMAN M: Effect of a protein-sparing diet and brief fast on nitrogen metabolism in mildly obese subjects. *J Lab Clin Med* 89: 1030, 1977
19. KOHASHI N, KATORI R: Decrease of urinary taurine in essential hypertension. *Jpn Heart J* 24: 91, 1983
20. OGAWA M, TAKAHARA A, ISHIJIMA M, TAZAKI S: Decrease of plasma sulfur amino acids in essential hypertension. *Jpn Circ J* 49: 1217, 1985
21. JAFFE M: Ueber der Niederschlag, welchen Pikrinsäure in normalem Harn erzeugt und Über eine neue Reaction des kreatinins. *Z Physiol Chem* 10: 391, 1886
22. BERGLUND F, SORBO B: Turbidimetric analysis of inorganic sulfate in serum, plasma and urine. *Scand J Clin Lab Invest* 12: 147, 1965
23. PELLETT PL: Urinary sulfate sulfur as a measure of the protein value of diets. *Pro Nutr Soc* 24: xxxvii, 1965
24. ISHIDA Y, FUJITA T, ASAI K: New detection and separation method for amino acids by high-performance liquid chromatography. *Chromatography* 204: 143, 1981
25. JOOSSENS JV, CLAESSENS J, GEBOERS J, CLAES JH: Electrolytes and creatinine in multiple 24-hr urine collections (1970-1974). In *Epidemiology of Arterial Blood Pressure*, ed by KESTELOVT H, JOOSSENS JV, Martius Nijhoff Medical Division, Hague, 1980
26. WATSON RL, LANGFORD HG: Usefulness of overnight urines in population groups: Pilot studies of sodium, potassium, and calcium excretion. *Am J Clin Nutr* 23: 290, 1970
27. LUFT FC, FINEBERG NS, SLOAN RS: Overnight urine collections to estimate sodium intake. *Hypertension* 4: 494, 1982
28. YAMORI Y, KIHARA M, FUJIKAWA J, et al.: 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, 1982
29. NARA Y, KIHARA M, MANO M, et al.: Urinary sulfoamino acids as a indicator of animal protein intake. *Sulfur Amino Acids* 7: 279, 1984 (in Japanese)
30. CUSWORTH DC, DENTO CE, et al.: Renal clearance of amino acids in normal adults and in patients with aminoaciduria. *Biochem J* 74: 550, 1961