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Concentration Adjustment of Spot Samples in Analysis of Urinary Xenobiotic Metabolites

Andrea Trevisan, MD

The reliability of the adjustment for the reduction of concentration-dilution effects in urinalysis of spot specimens was evaluated; 95.4 % of spot specimens showed a creatinine concentration between 0.5 and 3 g/liter, whereas 59.8% showed a specific gravity between 1.010 and 1.030. Only 6% of the specimens exceeded the specific gravity of 1.035 corresponding to a creatinine concentration of 3 g/liter. Therefore, an acceptable value for specific gravity in spot specimens of 1.035 is proposed. Although either specific gravity or creatinine provides a reliable mean of adjustment, creatinine is preferred because it has fewer physiological confounding factors.

Key words: urinary concentration adjustment, creatinine, specific gravity

INTRODUCTION

The collection and analysis of spot urine specimens is frequently used for biological monitoring of occupational exposure to xenobiotics for the following reasons: 1) some analytes are excreted during or immediately after exposure; 2) difficulty in collecting 24-hour specimens in nonhospitalized subjects; 3) the possibility that 24-hour collection dilutes analytes such as those listed in number 1. Short-term period of collection is another means of urine sampling, but even here hydration-dehydration and perspiration might cause evaluation errors. For all of these reasons, spot specimens are often used, although the problem of concentration-dilution effects of urine remains. Adjustment of urinary values is usually performed to a standard specific gravity of 1.024 or to creatinine concentration. It is common practice [Alessio et al., 1985] to discard samples that are too dilute (specific gravity lower than 1.010, creatinine lower than 0.5 g/liter) or too concentrated (specific gravity higher than 1.030; creatinine higher than 3 g/liter). Some authors [Berlin et al., 1985] claim that the concentration adjustment offers no practical advantages for cadmium in urine, inasmuch as urinary creatinine shows marked intraindividual and interindividual variations [Alessio et al., 1985]. This wide variation of urine concentration in spot samples makes it unacceptable for expression of urinary values per unit volume without adjustment [Pryde, 1982].

Istituto di Medicina del Lavoro, Universita' di Padova, Padova, Italy
Address reprint requests to Andrea Trevisan, M.D., Istituto di Medicina del Lavoro, Universita' di Padova, Via Facciolati 71, I-35127 Padova, Italy.
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TABLE I. Methods Employed for the Assessment of the Urinary Analytes in Various Studies, 1962–1978

Analyte	Method	Reference
Metals		
Cd	AAS	Ross and Gonzales [1974]
F	Electrode	NIOSH [1973]
Mn	AAS	Watanabe et al. [1978]
Zn	AAS	Willis [1962]
Metabolites		
ALA	UV-Vis	Davis and Andelman [1967]
CP	Fluorimeter	Soulsby and Smith [1974]
HA	UV-Vis	Ogata et al. [1969]
Solutes		
PR	UV-Vis	Piscator [1962]
B2	RIA	Kit Phadebas (Sweden)
Enzymes		
ACE	UV-Vis	Summary [1976]
GGT	UV-Vis[a]	Kit Boehringer (FRG)
LDH	UV-Vis[a]	Kit Boehringer (FRG)

[a]Kinetic method at 30°C.

The adjustment of urinary values to creatinine is, on the other hand, a common practice in biological monitoring of occupational exposure [Lauwerys, 1983]. The present work reports a study on spot and 24-hour specimens and the relationship among some urinary analytes—unadjusted and adjusted—to creatinine, specific gravity, or 24-hour volume.

METHODS

Specific gravity using an AT 315 URICON (Japan) diffractometer and creatinine with a commercial kit (Boehringer, Mannheim, FRG) based on Jaffe's method [1886] were determined in 1,079 spot urine specimens from 850 males and 229 females and in 333 24-hour samples from 250 males and 83 females, aged 20–60 years. The specimens were obtained from the laboratory of Industrial Toxicology and Clinical Chemistry of the Institute of Occupational Health from 1985 to 1987. Spot specimens were collected from 8 to 9 A.M.

In addition, 24-hour urine samples from 20 male subjects aged 20–40 years, admitted to the Institute of Occupational Health for ascertainment of occupational disease, were collected from 8 A.M. to 8 A.M. of the next day. Volume, creatinine, and specific gravity were determined in each 24-hour specimen. The urinary analytes cadmium (Cd), fluorine (F), manganese (Mn), zinc (Zn), delta aminolevulinic acid (ALA), coproporphyrin (CP), hippuric acid (HA), total proteins (PR), B2-microglobulin (B2), gammaglutamyltranspeptidase (GGT), angiotensin-converting enzyme (ACE), and lactate dehydrogenase (LDH) were determined according to the methods reported in Table I.

The analytes then were used unadjusted, adjusted to 24-hour volume, adjusted to standard specific gravity of 1.024, or adjusted to grams of creatinine. Statistical evaluation of the results utilized Student's *t*-test and linear regression.

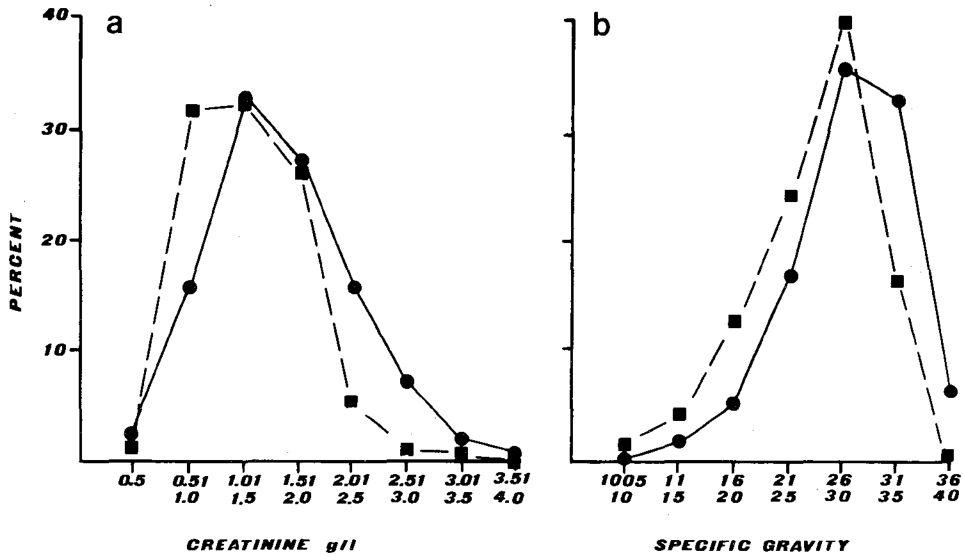

Fig. 1. Percent distributions of (a) creatinine and (b) specific gravity of 1,079 spot (●) and 333 24-hour specimens (■).

RESULTS

There were 1,029 (95.4%) spot specimens whose creatinine concentration ranged from 0.5 to 3 g/liter; only 59.8% (N = 645) showed a specific gravity between 1.010 and 1.030. In the 24-hour specimens, 97.3% (N = 324) had a creatinine concentration between 0.5 and 3 g/liter, and 81.7% (N = 272) showed a specific gravity between 1.010 and 1.030.

Figure 1 reports the percent distribution of (a) creatinine and (b) specific gravity of spot and 24-hour specimens. Spot and 24-hour samples show the highest proportion in the creatinine range between 1 and 1.5 g/liter and in the specific gravity range between 1.026 and 1.030. The majority of the samples has creatinine values lower than 2 g/liter, and specific gravity higher than 1.025 for spot and for 24-hour specimens.

A specific gravity of 1.024 in spot samples corresponds to a creatinine around 1.1 g/liter. Specific gravity and creatinine showed a good correlation in spot and 24-hour specimens ($y = 0.095x - 96.44$, $r = 0.870$ and $y = 0.070x - 70.80$, $r = 0.878$, respectively).

Table II reports the ranges regarding volume, specific gravity, creatinine, and analyte concentration of 20 hospitalized subjects included in the study.

Table III shows the relationship (r regression coefficient and slope) between 24-hour volume adjusted (reference value) and unadjusted or adjusted (creatinine or specific gravity) values of 20 hospitalized subjects. The relationship was evaluated among values determined in the same 24-hour urine sample. Generally, the adjustment to specific gravity shows the best correlation with the adjustment to 24-hour volume; the creatinine-adjusted values are always lower than those adjusted to 24-hour volume and specific gravity or unadjusted. The adjustment to creatinine or specific gravity does not substantially improve the correlation with respect to unad-

TABLE II. Urinary Data of 20 Hospitalized Subjects Included in the Study

	Range
Volume (liters)	0.96–1.78
Specific gravity	1.019–1.029
Creatinine g/liter	0.95–1.51
Cd mcg/24 hour	1.0–54.5
F mg/24 hour	0.4–5.6
Mn mcg/24 hour	2.0–14.3
Zn mcg/24 hour	793–3,954
ALA mg/24 hour	1.8–8.6
CP mcg/24 hour	30–570
HA g/24 hour	0.6–5.2
PR mg/24 hour	50–1,462
B2 mcg/24 hour	19–1,546
ACE IU/24 hour	27.8–261.5
GGT IU/24 hour	24.0–92.7
LDH IU/24 hour	5.2–28.0

TABLE III. Linear Regression Coefficient "r" and Slope Between 24-Hour Volume Adjusted and Unadjusted/Adjusted to Creatinine and Specific Gravity Values of 20 Hospitalized Subjects

	24-Hour specimens vs.					
	Unadjusted		Creatinine		Specific gravity	
Analyte	r	Slope	r	Slope	r	Slope
Cd	0.882	0.528	0.981	0.652[a]	0.984	0.647[a]
F	0.961	0.854	0.934	0.679[a]	0.952	0.959[a,b]
Mn	0.930	0.402	0.759	0.252[a]	0.865	0.324[a,b]
Zn	0.863	0.502	0.651	0.175[a]	0.826	0.319[a,b]
ALA	0.647	0.613	0.542	0.396[a]	0.769	0.600[b]
CP	0.972	0.955	0.874	0.642[a]	0.953	0.767[a,b]
HA	0.936	0.768	0.923	0.767	0.974	0.928[a,b]
PR	0.886	0.556	0.970	0.661[a]	0.950	0.585[b]
B2	0.978	0.720	0.939	0.447[a]	0.959	0.591[a,b]
ACE	0.894	0.809	0.937	0.578[a]	0.965	0.785[b]
GGT	0.536	0.366	0.579	0.263[a]	0.683	0.350[b]
LDH	0.855	0.728	0.900	0.622[a]	0.956	0.869[a,b]

[a]$p < 0.05$ between the slopes of adjusted to creatine or specific gravity vs. unadjusted.
[b]$p < 0.05$ between the slopes of specific gravity vs. creatine adjusted values.

justed values. Moreover, the 24-hour volume adjusted values show levels higher than unadjusted and creatinine or specific gravity adjusted values.

DISCUSSION

A significant number of spot and 24-hour specimens show a similar distribution of creatinine and specific gravity, even if spot samples on the average have specific gravity and creatinine concentrations (1.027 and 1.42 g/liter, respectively) higher than ($p < 0.001$) the 24-hour specimens (1.024 and 1.25 g/liter, respectively).

The spot specimens display acceptable creatinine values (between 0.5 and 3 g/liter); a high percentage (around 40%) show specific gravity values higher than

1.030. It seems reasonable to suggest range limitations for specific gravity in spot specimens between 1.010 (only 0.6% of samples lower than this value) and 1.035 (6% higher than this value). Creatinine about 3 g/liter corresponds to a specific gravity of 1.035.

The present study shows a wide interindividual variation of urine concentration of spot specimens, corroborating the necessity of the adjustment. In reality, two spot samples with the same value per unit volume have different meanings if the creatinine concentration shows a value of 0.5 or 3.0 g/liter; the relative value may vary six fold. Pryde [1982] holds that concentration in a single urine sample, without adjustment for concentration-dilution effects, is unacceptable because of the wide intrainterindividual variation in urine output. The author suggests adjustment for creatinine because it is more suitable for concentrated and diluted urines, and it is not sensitive to toxic effects on the renal tubule.

Other authors [Berlin et al., 1985; Alessio et al., 1985] raise some doubts; the adjustment does not bring substantial advantages in reliability or accuracy; moreover, creatinine and specific gravity show only a slight correlation. The results reported here, in agreement with Elkins et al. [1974] and Pryde [1982], point out, on the contrary, a good relationship between specific gravity and creatinine in both spot and 24-hour specimens.

Specific gravity reflects the urinary concentration of salts, urea, sulfates, and phosphates. If tubular alterations are present, the reabsorption of solutes decreases, and then the adjustment to specific gravity is unreliable. Moreover, specific gravity is affected by diet and environmental conditions. Creatinine is filtered by glomerulus, partially secreted by the tubule, and not reabsorbed. The influence of diet and muscular stress, which can cause an increase of creatinine excretion, is marginal. Hourly variations in creatinine output are lower than those in volume and solutes [Pryde, 1982].

No substantial difference between adjusted or unadjusted vs. 24-hour volume adjusted values was observed. A reasonable explanation could be that the analytes, creatinine, and specific gravity were determined in the same 24-hour urine sample. The 24-hour collection may mask the variation in concentrations and in urine output. Another explanation might be that the 24-hour volume affected creatinine concentration more than total solutes and some analytes more than others [Araki, 1978].

In conclusion, our findings suggest that the adjustment of urinary analytes in spot samples needs correction for concentration-dilution effects, particularly when single samples and nonhomogeneous groups of persons are analyzed. Our opinion is that the expression of urinary values per unit volume without adjustment is unacceptable, in agreement with other authors [Pryde, 1982; Lauwerys, 1983]. In our study adjustment for either specific gravity or creatinine in 24-hour samples gave results comparable to 24-hour volume adjustment. However, in spot samples, creatinine adjustment is preferable in order to avoid problems with specific gravity adjustment, such as urinary sugar and/or protein and primitive or toxic tubulopathies [Pryde, 1982].

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REFERENCES

Alessio L, Berlin A, Dell' Orto A, Toffoletto F, Ghezzi I (1985): Reliability of urinary creatinine as a parameter used to adjust values of urinary biological indicators. Int Arch Occup Environ Health 55:99–106.

Araki S (1978): The effects of water restriction and water loading on urinary excretion of lead, delta-aminolevulinic acid and coproporphyrin. Br J Ind Med 35:312–317.

Berlin A, Alessio L, Sesana G, Dell' Orto A, Ghezzi I (1985): Problems concerning the usefulness of adjustment of urinary cadmium for creatinine and specific gravity. Int Arch Occup Environ Health 55:107–111.

Davis JR, Andelman SL (1967): Urinary delta-aminolevulinic acid (ALA) levels in lead poisoning. I. A modified method for the rapid determination of urinary delta-aminolevulinic acid using disposable ion-exchange chromatography columns. Arch Environ Health 15:53–59.

Elkins HB, Pagnotto LD, Smith HL (1974): Concentration adjustments in urinalysis. Am Ind Hyg Assoc J 35:559–565.

Jaffé M (1886): Uber den Niederschlag, welchen Pikriksaure in normalem Harn erzengt und uber eine neue Reaktion des Kreatininis. Z Physiol Chem 10:391–400.

Lauwerys R (1983): ''Industrial Chemicals Exposure: Guidelines for Biological Monitoring.'' Davis; CA: Biochemical Publications.

NIOSH (1973): Fluoride in urine. Method n 114. Manual of analytical methods. Vol I. DHMS Publication.

Ogata M, Tomokuni K, Takatsuka Y (1969): Quantitative determination in urine of hippuric acid and m- or p-methylhippuric acid metabolites of toluene and m- or p-xylene. Br J Ind Med 26:330–334.

Piscator M (1962): Proteinuria in chronic cadmium poisoning. 2. The applicability of quantitative and qualitative methods of protein determination for the demonstration of cadmium proteinuria. Arch Environ Health 5:55–62.

Pryde DE (1982): Is it appropriate to adjust the results of urine analyses for concentration-dilution effects? International Workshop on Biological Indicators of Cadmium Exposure. ''Diagnostic and Analytical Reliability.'' CEC-IUPAC, Luxembourg, 7th–9th July.

Ross RT, Gonzales JG (1974): Direct determination of cadmium in biological samples by selective volatilization and graphite tube reservoir atomic absorption spectrophotometer. Anal Chem 70: 443–447.

Soulsby J, Smith RL (1974): A simplified method for the quantitative determination of urinary coproporphyrin in lead workers. Br J Ind Med 31:72–74.

Summary JJ (1976): The spectrophotometric determination of human serum carboxypolypeptidase with angiotensin converting enzyme like-activity. Clin Sci Mol Med 50:312–327.

Watanabe T, Tokunaga R, Iwahana T, Tati M, Ikeda M (1978): Determination of urinary manganese by the direct chelation-extraction method and flameless atomic absorption spectrophotometry. Br J Ind Med 35:73–77.

Willis JB (1962): Determination of lead and other heavy metals in urine by atomic absorption spectroscopy. Anal Chem 34:614–617.