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The Validity of Spot Urine Samples for Low-Level Occupational Mercury Exposure Assessment and Relationship to Porphyrin and Creatinine Excretion Rates

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ABSTRACT

Hg and porphyrin levels in single void urine specimens (spot samples) were compared with calculated 24-hr urine levels in 35 (25 male and 15 female) practicing dentists who had been occupationally exposed to low levels of elemental Hg. The study aimed to: 1) determine the individual variability for Hg and porphyrin concentrations in spot samples over a 24-hr period; 2) test for the presence of diurnal variation in urinary Hg and porphyrin concentrations; and 3) determine the time of day at which a spot sample would give a Hg concentration closest to the 24-hr average concentration. Results confirmed previous reports of a first-order diurnal pattern with a mid-morning peak for Hg concentration ($P < .0001$). A second-order model best described creatinine excretion ($P = .0089$), with peaks at about 5:00 and 19:00. The use of creatinine adjustment for Hg concentration significantly reduced the intraindividual variation around the diurnal curve. No diurnal patterns were found for any of the porphyrins examined. We recommend that, for small clinical studies using urinary Hg concentration, 24-hr sampling would be ideal, but that for mass screenings and cross-sectional studies, spot samples may be useful because they correlate fairly well with 24-hr averages (creatinine adjusted, $r = 0.61$; unadjusted, $r = 0.74$). Because of the existence of diurnal variation, for all cases using serial sampling attention should be paid to time of day.

Single-void urine samples ("spot" samples) are used universally for the assessment of occupational exposure to Hg. The spot sample is widely used because it is often impractical to collect urine samples over longer periods of time, especially when testing of large numbers of individuals is required, such as in industrial settings. The implicit assumption is made that the concentration of Hg in the spot sample is proportional to the 24-hr urinary Hg concentration. Yet, little is known about the validity of spot samples for the determination of Hg exposure. Although it has been known for decades that significant random variation exists within individuals for Hg levels determined from temporally proximal spot urine samples, few attempts have been made to characterize this variation.

Those few studies which have examined the variability of Hg excretion in urine have shown that, in addition to random intraindividual variation, there appears to exist a circadian or diurnal variability as well (Araki *et al.* 1983; Calder *et al.*, 1984; Mason and Calder, 1994; Piotrowski *et al.*, 1975; Vokac *et al.*, 1980; Wallis and Barber, 1982). This cycle is characterized by a peak during the morning period. Although this diurnal variation has been shown to exist in highly Hg-exposed workers, it has been demonstrated in nonoccupationally exposed individuals as well (Araki *et al.*, 1983). No studies have been reported in which low-level occupational exposures, such as those found among dentists, have been examined for intraindividual spot sample variation, or for diurnal variation.

Many studies of the effects of Hg exposure have relied on the use of spot urine samples as a measure of Hg exposure. Because significant intraindividual variation in spot urine Hg levels exists, the validity of all Hg studies based upon this measure must be questioned. The presence of a diurnal variation further brings into question the validity of studies which have used the spot sample as a measure of Hg exposure. This includes many of the studies which have been done for dental exposures, both occupational and clinical. Several studies have attempted to "correct" for this variation by stan-

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ABBREVIATIONS: sin, sine; cos, cosine.

dardizing against some other parameter of urinary excretion, such as creatinine or specific gravity (Piotrowski *et al.*, 1975; Araki *et al.*, 1986; Barber and Wallis, 1986; Clarkson *et al.*, 1986; Mason and Calder, 1994). These attempts have not yet resulted in a satisfactory method of correction.

Changes in the urinary porphyrin excretion pattern have been suggested as an alternative biomarker measure of Hg exposure. Hg alters porphyrin metabolism in kidney epithelial cells, resulting in a Hg-specific change in the normal pattern of porphyrin excretion (Woods *et al.*, 1991). This change, characterized by excess concentrations of 4- and 5-carboxyl porphyrins and by the appearance of an atypical porphyrin ("precoproporphyrin"), which is not detected in normal urine, has been studied in a group of dentists using single spot urine samples (Woods *et al.*, 1993). However, no studies have been reported which explore the intraindividual variation of porphyrin profiles in spot samples, or which examine the diurnal variation.

The present study addressed these issues by comparing Hg and porphyrin levels in single void urine specimens (spot urine samples) with calculated 24-hr urine levels in practicing dentists. Specifically, the study aimed to: 1) determine the individual variability for Hg and porphyrin concentrations in spot urine samples over a 24-hr period; 2) test for the presence of diurnal variation in urinary Hg and porphyrin concentrations in practicing dentists; and 3) determine the time of day at which a spot urine sample would give a Hg concentration closest to the 24-hr average concentration.

Methods

Subject selection. Dentists were selected for this study because they: 1) have prolonged occupational Hg exposure at a range known to be above that generated by Hg-containing dental fillings in the mouth, but below that of other more highly exposed occupational groups; 2) are demographically very similar in terms of socioeconomic status, educational level, work practices and other factors which might otherwise confound the evaluation of the parameters under evaluation; 3) are highly compliant in terms of their participation as subjects in research projects such as this one; and 4) are readily accessible to us through the University of Washington School of Dentistry research activities. Dentists were selected from the membership of the Seattle-King County Dental Society. Because there is no known relationship between Hg exposure and location of dental practice, male dentists were recruited randomly from a four zipcode area in close proximity to the University of Washington campus. The next three geographically nearest zip code areas were additionally needed to recruit sufficient female dentists. Eligible subjects were defined as general practitioners over 25 years of age who have full-time dental practices (practice more than 20 hr/week) in which Hg amalgam is used. Dentists were excluded if they reported significant illness or disability which could affect renal function, creatinine excretion or porphyrin metabolism, pregnancy or lactation, a part-time dental practice (less than or equal to 20 hr/week), the use of medications known to be porphyrinogenic or total abstention from use of Hg amalgam. Dentists were not asked to restrict their seafood before test date. The principal concern with respect to seafood would be consumption of organic Hg, principally methylmercury, of which less than 0.1% is excreted by way of the urine. Moreover, there is no reason to believe that any organic Hg which is excreted in the urine would not display the same diurnal pattern as a Hg consumer as inorganic Hg or Hg vapor. Therefore, we do not expect that the results obtained would be influenced by the source of Hg exposure.

Sample collection. Each dentist was asked to provide spot urine samples at every void for a continuous 24-hr period. Urine collection took place on a day in the middle-to-end of the work week (Thursday-Sunday) not after a break longer than a 2-day standard weekend. Spot collections were made regardless of location (office or home). Subjects were instructed to begin collection at the first morning void and to continue until the next morning. At each void, the urine specimen was first captured in a calibrated container (male or female urinal). An aliquot of the sample was poured into a polyethylene 50-ml, screw-top container which was then labeled with the total original volume and the time of collection. The urine specimens were kept refrigerated during the 24-hr collection period. Upon completion of the 24-hr urine collection, all materials were picked up from the dental office and subsequently transported to the laboratory *via* insulated coolers. Urine specimens were logged in and frozen immediately for preservation until analysis.

Sample Analyses

Urinary Hg analysis. For Hg analyses, all urine samples were shipped frozen to Dr. Naleway's laboratory at the American Dental Association Health Foundation for analysis by flameless atomic absorption spectrometry (Chou and Naleway, 1983). A complete series of quality control test samples derived from standard reference material inorganic Hg solutions, including both water and spiked urine samples containing total Hg concentrations in the range of 0 to 100 ppb, was run with each set of 20 test samples. Four replicate analyses were performed for each sample, and the mean of these four was used for each sample value. The replicability of the urinary Hg analysis was very high (S.D. between replicate analyses = 0.1 ug/l).

Urinary porphyrin and creatinine analyses. Urinary porphyrin concentrations in urine samples were analyzed using high-pressure liquid chromatography separation and quantitation with a spectrofluorometric detection method, as described by Woods *et al.* (1991). This procedure permits quantitation of urinary porphyrins with a detection sensitivity of 0.5 pmol. The accuracy and precision of porphyrin recovery from urine samples using the procedure used herein has been described previously in detail (Bowers *et al*, 1992). The accuracy of porphyrin recovery has been established in terms of recovery of added porphyrin standards from control human urine samples, reproducibility of recovery of individual porphyrins from urine and concordance of porphyrin measurements with published values and ranges (Bowers *et al.*, 1992; Woods *et al.*, 1993). Creatinine levels were determined using a standard colorimetric method (Sigma Chemical Co. (St. Louis, MO). Confirmatory creatinine levels were assessed for all samples by Dr. Bollen (AMB Laboratory) at the University of Washington School of Dentistry, also using a standard colorimetric method (Sigma).

Statistical analyses. Two creatinine values were obtained for each sample from two different laboratories. Although the values obtained in one laboratory (AMB) were about 8% higher on average, the two values were similar in most cases (within 25 for 70% of the samples), and were highly correlated (r = 0.91 on the logarithmic scale). They were averaged (on the log scale) to give a single creatinine value, which was used in all subsequent analyses. A small number of missing values of one of the porphyrin values (precoproporphyrin) was filled in using a predictive model based on the other five porphyrin values (the r-squared value was 0.45).

A transformation of the values for the Hg, creatinine and porphyrin concentrations using the natural logarithm was applied to stabilize the variance and give values with an approximately normal distribution, for the purposes of estimating diurnal variation. This transformation also allowed the estimation of diurnal variation as a percentage of change from the 24-hr average value. Hg and porphyrin concentration values were analyzed using both unadjusted values and adjustments for creatinine.

Diurnal variation in concentration values was analyzed using the

following model to describe changes over time (Anderson, 1971), (the value of pi is 3.14159):

$$\text{Log (Concentration)} = B_0 + B_1 \sin(2 \times \text{pi} \times T/24) + B_2 \cos(2 \times \text{pi} \times T/24).$$

This formula describes the variation of the log-transformed concentration with the sampling time, T, recorded in hours using a 24-hr clock. The magnitude of the diurnal variation (peak variation) is given by the amplitude B, where $B^2 = B_1^2 + B_2^2$.

A value of $B = 0.20$, for example, can be interpreted as approximately 20% variation of the 24-hr average (the exact variation would be 100 [exp (0.20) − 1]% = 22% above average at the highest point and 100 [1 − experiment (−0.20)] = 18% below average at the lowest point). The phase angle *Theta* = Arctan (B_2/B_1) in radians determines the time T_0 of the peak concentration by the formula *Theta* = $2 \times \text{pi} \times T_0/24$. Higher order sinusoidal terms also were estimated; these took the form: sin $(4 \times \text{pi} \times T/24)$ and cos $(4 \times \text{pi} \times T/24)$ for second-order terms and sin $(6 \times \text{pi} \times T/24)$ and cos $(6 \times \text{pi} \times T/24)$ for third-order terms. Adjustment for creatinine was done in two different ways: by analyzing the log of the adjusted concentration (ratio to creatinine) using the above models and also by including the log-transformed creatinine value in the regression model. These two methods yielded very similar results and only results based on the latter method are reported here.

The diurnal variation models were fit by the generalized least-squares method with an exchangeable correlation structure between multiple values for an individual subject using the procedure "mixed" of the SAS statistical software. Similar results were obtained by using alternative correlation structures, including autoregressive structures of order 1 and 2 and a general stationary structure which allows different correlation values for pairs of observations at different distances apart. The models were refit by using the method of generalized estimating equations to calculate robust S.E. estimates of the coefficients which are not dependent on knowing the correct correlation structure. This gave very similar results, and confirmed that the results were not dependent on an assumption about the intrasubject correlation. Tests of the null hypothesis of "no diurnal variation" (*i.e.*, H_0:$B_1 = B_2 = 0$ or H_0:$C_1 = C_2 = 0$) were performed using the likelihood ratio test based on the increase in the log-likelihood due to the diurnal variation terms in the model. Tests of higher order sinusoidal terms and differences between sexes were performed similarly using the likelihood ratio test method.

Results

Subjects. A total of 35 dentists participated in the study (20 males and 15 females). Body mass is a consideration when examining for creatinine excretion rates, and males tend to exhibit greater mass. Table 1 shows mean values for age, weight and height by gender for the participants. All subjects were healthy. None worked at night, or had recently travelled across several time zones (*i.e.*, none was on an unusual diurnal schedule). None was currently taking or had recently taken medications known to be porphyrinogenic.

TABLE 2

Twenty-four hour volume weighted average concentrations for males and females

	Mean	S.D.	Minimum	Maximum
Males: (n = 20)				
Creatinine (g/l)	1.20	0.43	0.60	1.90
Hg (μg/l)	3.03	2.10	0.78	8.26
Pentaporphyrin (μg/l)	2.73	1.53	0.27	6.38
Precoproporphyrin (μg/l)	1.72	1.34	0.11	5.03
Coproporphyrin (μg/l)	9.92	10.56	0.55	37.13
Uroporphyrin (μg/l)	4.81	2.54	0.22	11.61
Heptaporphyrin (μg/l)	2.59	1.56	0.32	5.49
Hexaporphyrin (μg/l)	1.88	1.41	0.14	4.60
Total porphyrin (μg/l)	23.64	15.38	3.13	58.79
Females: (n = 15)				
Creatinine (g/l)	0.89	0.46	0.25	1.82
Hg (μg/l)	2.05	1.27	0.51	4.67
Pentaporphyrin (μg/l)	1.75	1.47	0.15	5.11
Precoproporphyrin (μg/l)	0.50	0.34	0.08	1.32
Coproporphyrin (μg/l)	9.66	7.21	0.00	23.88
Uroporphyrin (μg/l)	3.05	1.77	0.05	6.44
Heptaporphyrin (μg/l)	1.99	2.36	0.35	10.05
Hexaporphyrin (μg/l)	1.07	0.92	0.14	2.84
Total porphyrin (μg/l)	18.02	10.79	3.41	47.52

24-Hour average concentrations. Table 2 shows the 24-hr average concentrations of Hg, creatinine and porphyrins for males and females. As indicated in table 2, males in this study appear to have higher levels than females of total secreted creatinine, Hg and total porphyrins, although none of the individual porphyrin values differed significantly among men and women subjects. Differences between genders observed in 24-hr volume-weighted average concentrations, however, were reduced or eliminated in the creatinine-adjusted values.

Diurnal variation. The results of the analyses performed for first- and second-order diurnal variation for creatinine, Hg (adjusted and unadjusted for creatinine) and all porphyrins (adjusted and unadjusted for creatinine) are seen in table 3. Significant first-order (a single peak and a single low during a 24-hr period) diurnal variation was found in creatinine concentration and in Hg values adjusted for creatinine. Near-significance was seen (P = .056) for first-order effects for Hg unadjusted for creatinine. No significant first-order diurnal variation was found for any of the individual porphyrins or for total porphyrins. Note that the estimated amplitudes of the diurnal variation for some of the porphyrins were as large as that of Hg, but these were not statistically significant for the porphyrins because of the large within-subject variation in the porphyrin values.

The second-order terms were not significant for either adjusted or unadjusted Hg, but were significant for creatinine

TABLE 1

Descriptive statistics of the study population

Gender	n	Variable	Mean	S.D.	Minimum	Maximum
Female	15	Age	37.0	5.7	26.9	45.5
		Height (inches)	65.0	2.6	61.0	70.0
		Weight (lbs)	133.3	13.0	115.0	160.0
Male	20	Age	47.1	12.4	31.4	71.2
		Height (inches)	71.2	2.6	66.0	76.0
		Weight (lbs)	186.0	28.8	140.0	255.0

TABLE 3
Results of analysis of first-order and second-order diurnal variation

Variable	First-Order Model				Second-Order Terms	
	Amplitude[a]	Time of Peak	Chi-Square[b]	P	Chi-Square[b]	P
Creatinine	28	23:54	21.54	<.0001	9.44	.0089
Hg	19	3:02	5.76	.056	4.71	.095
Hg[c]	21	9:05	31.72	<.0001	4.06	.13
Pentaporphyrin	8	1:57	0.19	.91	0.59	.74
Pentaporphyrin[c]	8	9:12	0.23	.89	0.96	.62
Precoproporyrin	14	17:21	0.73	.69	1.42	.49
Precoproporyrin[c]	18	16:49	1.13	.57	2.06	.36
Coproporphyrin	22	17:35	2.64	.27	2.61	.27
Coproporphyrin[c]	22	18:10	2.76	.25	2.23	.33
Uroporphyrin	23	0:38	1.86	.39	3.27	.19
Uroporphyrin[c]	13	0:36	0.54	.76	5.12	.077
Heptaporphyrin	13	3:20	0.59	.74	0.43	.81
Heptaporphyrin[c]	6	6:44	0.17	.92	0.65	.72
Hexaporphyrin	19	10:16	1.48	.48	2.81	.25
Hexaporphyrin[c]	21	11:15	1.56	.46	3.95	.14
Total porphyrin	11	20:54	1.66	.44	2.52	.28
Total porphyrin[c]	9	19:42	1.09	.58	3.02	.22

[a] The definition of amplitude and its relationship to the percentage of variation above and below the 24-hr average is given under "Methods."
[b] All chi-square test statistics have 2 dF.
[c] Adjusted for creatinine.

(P = .0089). No second-order terms were significant for any individual or combined porphyrin values. Third-order sinusoidal terms were tested for creatinine and Hg. None of these was close to being significant.

Differences in diurnal variation between genders were examined for each of the variables listed in table 3. Significant differences were found only for precoproporphyrin (P = .04). Given the large number of tests performed, this finding must be interpreted as providing at most weak evidence for a difference between the sexes. We must note, however, that the statistical test to detect differences in diurnal variation between sexes is quite insensitive, and further study of possible gender differences is warranted.

Figure 1 shows time plots of the percentage of change in creatinine concentration relative to the 24-hr mean concentration for each subject. The first- and second-order models are relatively similar, with only a slight second "low" from the second-order model superimposed over the single peak of the single-order curve. In the second-order model, there are two equal peaks at approximately 5:00 and 19:00 and a deep trough (more than 40% below average) at 12:00. Note that the first-order model does not provide an adequate fit because of the significance found for the second-order terms (P = .0089). The intraindividual variability around the fitted diurnal variation curves is quite high.

Figure 2 shows time plots of the percentage of change in Hg concentration as well as first- and second-order diurnal variation curves for Hg unadjusted for creatinine. In the first-order model, a peak is seen at about 3:00, and a low at about 15:00. The fit of this model is adequate, although it should be noted that the second-order terms are near-significant (P = .095). The effect of adding the second-order terms is to reveal greater variation within the waking hours than for the single order curve, indicated by the presence of a dominant peak at approximately 7:00, a second minor peak at approximately 20:00 and a deep trough (≅ 27% below the mean) at 13:00. Similarly to the creatinine results seen in figure 1, the intraindividual variability around the diurnal curves is very high.

The effect on Hg concentration of the adjustment by creatinine may be seen in the time plot presented in figure 3. Creatinine adjustment results in first- and second-order curves which appear visually to be very similar. The first-

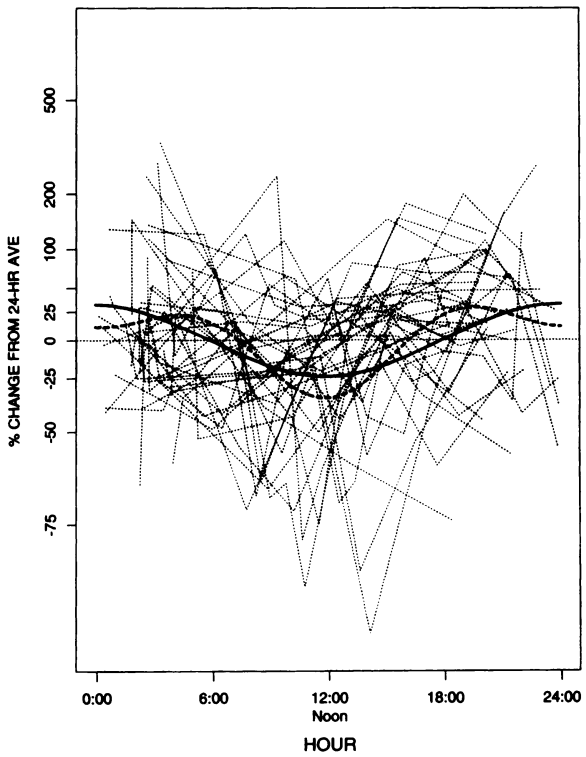

Fig. 1. Percentage of change in creatinine concentration relative to the 24-hr mean concentration for each subject plotted on a logarithmic scale. The solid curve is the first-order diurnal variation curve given by log (creatinine) = −.0076 sin (2 pi T/24) + .2762 cos (2 piT/24). The dashed curve is the second-order diurnal variation curve given by log (creatinine) −.0321 sin (2 pi T/24) + .2749 cos (2 pi T/24) + .0008 sin (4 pi T/24) − .1674 cos (4 pi T/24).

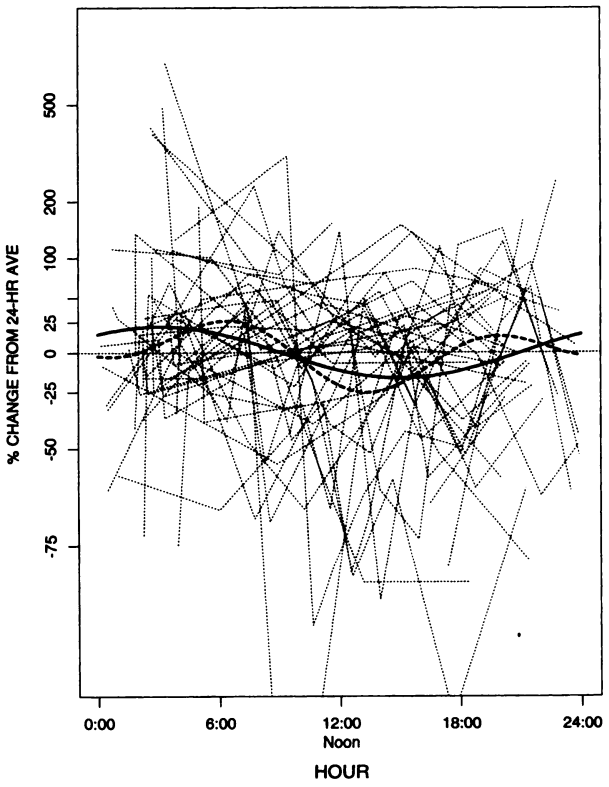

Fig. 2. Percentage of change in Hg concentration relative to the 24-hr mean concentration for each subject plotted on a logarithmic scale. The solid curve is the first-order diurnal variation curve given by log (Hg) = .1344 sin (2 pi *T*/24) + .1316 cos (2 pi *T*/24). The dashed curve is the second-order diurnal variation curve given by log (Hg) = .0929 sin (2 pi *T*/24) + .1098 cos (2 pi *T*/24) − .0864 sin (4 pi *T*/24) − .1348 cos (4 pi *T*/24).

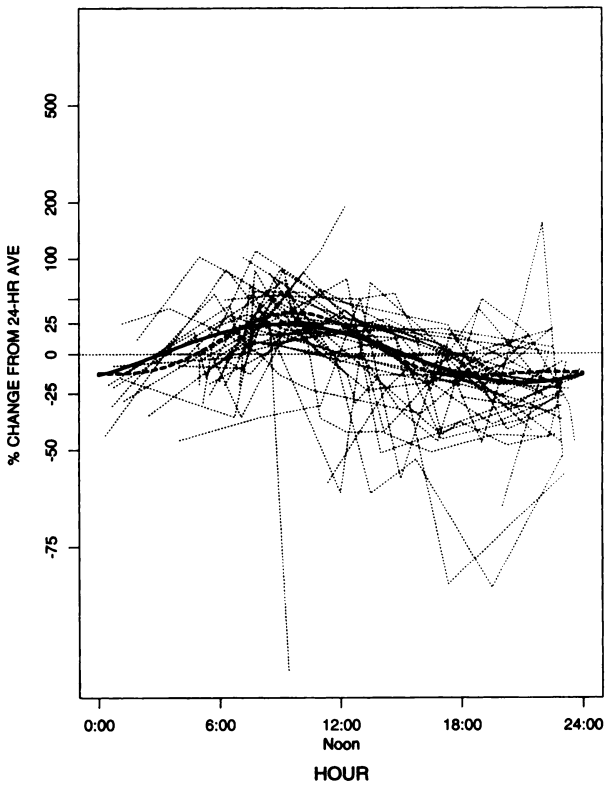

Fig. 3. Percentage of change in creatinine-adjusted Hg concentration relative to the 24-hr mean concentration for each subject plotted on a logarithmic scale. The solid curve is the first-order diurnal variation curve given by log (Hg/creatinine) = .1482 sin (2 pi *T*/24) − .1547 cos (2 pi *T*/24). The dashed curve is the second-order diurnal variation curve given by log (Hg/creatinine) + .1326 sin (2 pi *T*/24) − .1753 cos (2 pi *T*/24) − .0757 sin (4 pi *T*/24) + .0349 cos (4 pi *T*/24).

order model does provide an adequate fit in this case, because the second-order terms are not significant (P = .13). The effect of creatinine adjustment on the first-order model is to shift the peak from about 3:00 to 9:00, when the creatinine concentration has dropped toward its lowest value at just before 12:00. In addition, the intraindividual variation around the curves is much lower than for unadjusted Hg, although the deviations from the 24-hr means are still quite large for several individual spot samples.

Spot *vs.* 24-hr sample. From figure 1 it may be seen that, for creatinine, if the first-order model is considered, a spot sample taken near a usual first-morning void time period of 5:00 to 8:00 will typically result in less than 10% variation from the 24-hr average. A sample taken during the 16:00 to 20:00 period would result in a similar variation from the 24-hr average.

In the case of unadjusted Hg values (see fig. 2), the significant first-order model would suggest taking a sample at about 9:00 or 21:00 to minimize variance from the 24-hr average. For Hg adjusted by creatinine (see fig. 3), samples taken between 3:00 and 5:00 or between 14:00 and 16:00 will best match the 24-hr average.

The diurnal variation curves indicate the bias in spot sample results taken at specific times, *i.e.*, the average deviation from the 24-hr mean concentration. However, despite this bias, spot sample results were reasonably highly correlated with 24-hr averages across the sample. The correlation between unadjusted Hg concentration values in spot samples and 24-hr means was 0.74. Creatinine-adjusted spot sample concentrations had a correlation of 0.61 with 24-hr average mercury concentrations, and a correlation of 0.83 with creatinine-adjusted 24-hr concentrations.

Discussion

All 35 subjects, by self report, provided complete 24-hr urine samples. In two cases, the total volume was less than 500 ml, and were considered to possibly have been missing samples from the 24-hr period. Because of the statistical methods used for this study, a small number of missing samples would not have had any substantial effect on the estimation of diurnal variation. Consequently, all available data were included in the statistical analyses. This study confirmed previous reports of a peak for urinary Hg which occurs in the morning. Additionally, a significant diurnal pattern was found for creatinine excretion which appears to be at a minimum around noon and a peak late at night. A second-order model for creatinine provides a better description of the variation, and indicates the presence of two peaks at about 7 hr on either side of noon. In both first- and second-order models, the low remains at about noon.

It appears from this study that the use of creatinine adjustment for Hg concentration in spot urine samples does not affect the magnitude of the diurnal variation, but significantly reduces intraindividual variation around the diurnal curve. It has been reported that total intraindividual variation in urinary Hg concentration is reduced by creatinine adjustment (Mason and Calder, 1994); however, Mason and Calder (1994) did not distinguish between diurnal variation and variation around the diurnal curve. When this adjustment is performed, both first- and second-order models appear to similarly indicate a peak concentration in mid-morning, and a low concentration in mid-to-late evening.

This study was unable to establish the existence of diurnal patterns for any of the porphyrins tested. These findings are consistent with those from other studies (Boynton and Roth, 1994), in which no underlying diurnal pattern of urinary porphyrin excretion was found among normal adult subjects. However, prior studies, including the present one, have been limited by small numbers of subjects. A larger study may provide evidence of diurnal pattern for urinary porphyrin excretion. Additionally, although the effects of Hg exposure on the excretion patterns of certain porphyrins is well-established (Woods *et al.*, 1991), the levels of Hg exposure seen in this study population have not been highly correlated with significant alterations in these porphyrin values.

This study provides an indication of the appropriate times to collect spot urine samples, depending on the purpose of the sampling. If, for example, the interest is in obtaining the maximum urinary Hg concentration value for the individual, a mid-to-late morning spot sample would be indicated. If the need is to approximate the 24-hr average, and if creatinine adjustment is to be performed, the samples should be taken between 3:00 and 5:00 or between 14:00 and 16:00 hr. If unadjusted Hg values are to be used, the proper time for collection will depend somewhat more on whether the first- or second-order model is being considered. To determine when the best time would be for urinary creatinine concentration values, the curves in figure 1 may be consulted for peak, low or 24-hr average "best" times.

In terms of the impact on study design in which urinary Hg or creatinine concentrations are to be utilized, for small clinical studies, 24-hr sampling would be ideal. If this is not practical, and spot samples are to be used, they should be collected at the same time each day (for serial sampling). For mass screenings and cross-sectional studies, spot samples may be useful because they correlate fairly well with 24-hr averages. Again, for serial measurements, attention should be paid to sampling at the same time each day.

This study found no evidence for diurnal variation in porphyrin concentrations in spot urine samples. Therefore, no specific time of urine collection is recommended.

Finally, it is important to realize that the number of subjects in this study is relatively small, and larger studies are needed to better characterize the diurnal variation which is found for both Hg and creatinine in individuals. Additionally, the form of Hg to which the subjects in this study were exposed was elemental Hg, and at relatively low levels of exposure. The results of this study, therefore, are most likely appropriate for consideration with similarly exposed populations.

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