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# Variability of lead in urine and blood in healthy individuals

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ABSTRACT

*Background:* Lead is a non-essential toxic trace element. Lead in blood (BPb) is the most common biomarker of lead exposure but lead in urine (UPb) has also been used. There is, however, limited data on the variability of UPb in the general population and the association with BPb.
*Objectives:* Our aims were to assess variability of lead in repeated blood and urine samples. The diurnal variation of UPb was also examined as well as associations with BPb.
*Methods:* We established an openly available biobank including 60 healthy non-smoking individuals, 29 men and 31 women, 21–64 years of age (median 31 years), with repeated sampling of blood and urine. Timed urine samples were collected at six fixed time points in two 24 h periods, about one week apart, and adjusted for creatinine and specific gravity (SG). BPb and UPb were analyzed by inductively coupled plasma mass spectrometry. The within- and between-individual variabilities and intra-class correlation coefficients (ICCs; ratios of the between-individual to total observed variances) were calculated using mixed-effects models.
*Results:* The ICCs for UPb samples were mostly above 0.5, when adjusted for creatinine or SG, and higher for overnight samples compared with daytime samples. The highest ICCs were obtained for BPb (ICC = 0.97) and for urine samples corrected for dilution by SG or creatinine. The ICC was 0.66 for overnight samples adjusted for creatinine. High correlations with BPb were found for 24 h UPb ($r_s = 0.77$) and overnight samples, e.g. $r_s = 0.74$ when adjusted for SG. There was diurnal variation of UPb with lowest excretion rate in overnight samples. There was also a significant association between the Pb excretion rate and urinary flow rate.
*Conclusions:* In addition to BPb, UPb adjusted for creatinine or SG seems to be a useful biomarker for exposure assessment in epidemiological studies.

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## Ethical approval

The study was approved by the Ethics Review Board at the University of Gothenburg and, all participants signed a written informed consent to participate in the study.

## 1. Introduction

Lead (Pb) is a non-essential trace element and a toxic metal of human health concern. Although exposure has decreased over time, adverse health effects have been shown in children and adults at low exposure levels as reviewed by The National Toxicology Program, US (NTP, 2012) and Bergdahl and Skerfving, 2022. In children lead affects the central nervous system also at moderate exposure levels as shown by associations between lead in blood (BPb) and IQ in prospective studies (Bergdahl and Skerfving, 2022; Lanphear et al., 2019). In adults, causal associations have been demonstrated between lead exposure and both hypertension and chronic kidney disease in the general population (EFSA, 2010; NTP, 2012), also in populations with low-level BPb in

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Sweden (Gambelunghe et al., 2016; Harari et al., 2018).

Exposure to Pb in the general population occurs mainly via diet, dust, or drinking water, but inhalation of cigarette smoke and Pb-contaminated ambient air may also contribute (Bergdahl and Skerfving, 2022). Lead is mainly stored in bone (half-life 5–20 years) while BPb reflects both recent exposure and the body-burden (Barbosa et al., 2005; Bergdahl and Skerfving, 2022). BPb is the most commonly used biomarker of lead exposure both in the general population and at occupational exposure. In blood, about 99% of Pb is found in erythrocytes, while levels in plasma are very low. Excretion of lead occurs through feces and urine, and lead in urine (UPb) is sometimes used as a biomarker of exposure. It is likely that UPb mirrors the filterable fraction of lead in plasma (Tsaih et al., 1999; Barbosa et al., 2005, Sommar et al., 2014), which at constant exposure is in equilibrium with Pb in erythrocytes and bone. If exposure changes, UPb will change more rapidly than BPb. In addition, UPb concentrations may vary with diuresis, and to correct for this, UPb is often adjusted using specific gravity (SG) or creatinine (crea). There are limited data on the associations between BPb and UPb in the general population (Bergdahl and Skerfving, 2022; Sommar et al., 2014).

For epidemiological studies and for risk assessment solid exposure data are needed. In many studies only a single sample per individual is collected; for urine sampling a first morning sample or a daytime spot sample. This may introduce misclassification bias (Rappaport and Kupper, 2008; Wang et al., 2019) if concentrations vary over time due to change in external exposure, daily activities or physiological status. For a single sample to be representative of long-term exposure, the within-individual variability should be low and when used in epidemiologic studies, the ratio between the within-individual variability and between-individual variability should be small. For biomarkers, the intra-class correlation coefficient (ICC), the ratio of the between-individual variance to the total variance, is often calculated. ICC values exceeding 0.75 are considered having good to excellent reliability in exposure classification, while an ICC below 0.4 indicates that a single sample will provide poor reliability (Rosner, 2015). The variability of lead in blood and urine in the general population remains understudied (Sommar et al., 2014; Wang et al., 2016). BPb is most often used in epidemiological studies but sometimes blood samples are lacking. There is also limited information on diurnal variation of lead in urine and influence of flow rate.

In the present report, we approach this lack of information using a "variability biobank" based on 60 healthy adult non-smoking Swedish individuals (Sallsten and Barregard, 2021; Barregard et al., 2021). Our aims were to assess variability of lead in repeated blood and spot urine samples, and clarify if UPb could be of any use in epidemiological studies. Timed urine samples were collected at fixed time points and corrected using both creatinine and SG. Thus, the diurnal variation for different urine sample types could also be examined as well as the associations between BPb and various measures of UPb.

## 2. Methods

### 2.1. Study subjects, blood and urine sampling

We have established an openly available biobank including 60 healthy individuals, 29 men and 31 women, with repeated sampling of blood and urine. None had diabetes, hypertension, kidney disease or self-reported medication for any other chronic disease. The biobank has been described in detail elsewhere (Sallsten and Barregard, 2021). The study subjects were 21–64 years of age (median 31 years), all were non-smokers (87% never-smokers). Most participants were born in Sweden but 37% were born in other countries. BMI was calculated from body weight and height. The median consumption of meat and fish was 4.5 melas/week and 2 meals/week, respectively, similar among men and women. All individuals had serum creatinine and estimated glomerular filtration rate within normal limits.

Non-fasting blood samples were collected twice in Lithium heparin metal free tubes (Vacuette 454,056, Greiner Bio-One, US for whole blood and BD 368886, Becton Dickson, US for erythrocytes) with an interval of approximately one week. Two samples of whole blood and one erythrocyte sample were missing out of 120 potential samples. Urine sampling was also performed twice; at six times points during two 24 h periods: at 9.30, 12.00, 14.30, 17:30, 22:00 and overnight (ON) leading to a total of 12 samples per subject. On the morning of the starting day subjects were instructed to discharge the first void of the day and record the time and date in the protocol as the starting time for the 24 h sampling. Overnight samples were collected in the next morning (from approximately 22:00 until the first morning void). They were instructed to collect each void, at the specific time, using a separate bottle and to record the time of each void. Polypropylene bottles were used for urine collection and polypropylene microtubes for aliquots as detailed by Sallsten and Barregard (2021). For each urine sampling period the total volume and sampling time were recorded. Two urine samples out of 720 potential samples were not complete. Creatinine (Crea) and specific gravity (SG) were measured in all urine samples (Sallsten and Barregard, 2021).

The study was approved by the Ethics committee at the University of Gothenburg (Dnr: 213–12).

### 2.2. Analyses of lead and data transformation

Concentrations of lead in urine and blood were analyzed at the National Institute of Occupational Health in Oslo, Norway (NIOH). The laboratory regularly takes part in external quality control programs. Methods for metal analyses in urine, as well as levels and variability of 22 elements in 24-h urine, have been described in detail elsewhere (Barregard et al., 2021). Urine samples were analyzed by inductively coupled plasma sector-field mass spectrometry (ICP-SF-MS). The analysis of whole blood, plasma and red blood cells samples was performed by inductively coupled plasma quadrupole mass spectrometry (ICP-QMS) using an Agilent 8800 Triple Quadrupole ICP-MS instrument (Agilent Technologies, Santa Clara, CA, USA) calibrated with matrix matched standard solutions. An internal standard solution (100 μL) containing 2.0 μg $mL^{-1}$ of gallium, germanium, indium and thallium, and 100 μL of an $^{129}I$ solution containing approximately 1.7 μg $mL^{-1}$ $^{129}I$, were added to 0.5 mL of whole blood, plasma or red blood cells in a 15 mL polypropylene tube before dilution to 5 mL with a solution containing 2% (w/w) ammonium hydroxide ($NH_4OH$), 4% (w/w) n-butanol, 0.1% (w/V) ethylenediaminetetraacetic acid (EDTA) and 0.1% (w/w) Triton X-100. The dilution solution was prepared from $NH_4OH$ solution (≥25% $NH_3$ in $H_2O$, TraceSELECT® Ultra, for trace analysis) n-butanol (CHROMASOLV® PLUS, for HPLC, ≥99.7%), EDTA (99.995% trace metal basis) purchased from Sigma-Aldrich (St. Louis, MO, USA) and Triton X-100 (pro analysi) purchased from Merck (Darmstadt, Germany). Seronorm™ Trace Elements (Seronorm™, Sero AS, Billingstad, Norway) human whole blood and urine quality control materials were used for quality assurance. For whole blood two quality control samples were used Seronorm L1 (lot 1,406,263) and Seronorm L2 (lot 1,406,264); the results (mean, ± standard deviation) versus recommended values were 10.9 μg/L ± 0.16 (N = 14) *versus* 9.9 μg/L, 320 μg/L ± 6.5 (N = 14) *versus* 337 μg/L. For urine two quality control samples were used Seronorm L1 (lot 1,403,080) and Seronorm L2 (lot 1, 403,081); the results (mean, ± standard deviation) versus recommended values were 0.45 μg/L ± 0.03 (N = 16) *versus* 0.72 μg/L, 84.5 μg/L ± 2.9 (N = 16) *versus* 80.1 μg/L.

The detection limit (DL, 3*SD of the blank samples, calculated on each day of analysis) for lead in urine varied between 0.03 and 0.08 μg/L and concentrations in 12 samples (1.7%) were below the DL. For blood and erythrocytes (EryPb) none of the samples were below DL (whole blood 0.09–0.11 μg/L, erythrocytes 0.09–0.19 μg/L). Urine concentrations below the DL were replaced by DL divided by the square root of 2 in the statistical calculations (Hornung and Reed, 1990). For plasma,

however, nearly all samples (96%) had levels below the DL (0.10–0.16 μg/L), and results are not commented further.

Lead excretion rate per hour (UPbrate) and per 24 h (24hUPb) were calculated from UPb concentration, volume, and sampling time. Urinary flow (UF) rate was calculated from sampling time and urinary volume. The urinary concentration of lead was adjusted for diuresis using creatinine and SG (UPbcrea, UPbSG). SGstandard = 1.015 was used in the SG adjustment (Suwazono et al., 2005). For UPbcrea, 45 samples were excluded due to too diluted or concentrated urine (Crea <0.3 g/L or >3 g/L) (Cocker et al., 2011). For UPbSG, 54 samples were excluded because they were too concentrated (SG > 1.030) (DHHS, 2004; Smolders et al., 2014).

Urine samples from the "variability biobank" are kept in a low temperature freezer and open for researchers examining normal variability of their biomarkers (Sallsten and Barregard., 2021; Barregard et al., 2021).

### 2.3. Statistics

Since multiple samples were available for each study participant, the within ($\sigma^2_{wY}$) and between ($\sigma^2_{bY}$) individual variabilities were calculated using mixed-effects models, separately for lead concentration, UPbcrea, UPbSG, UPbrate and 24hUPb. Natural log-transformation was used since the data were highly skewed. To determine if common variances could be used for men and women (i.e., if $\sigma^2_{bY(men)} = \sigma^2_{bY(women)}$, and $\sigma^2_{wY(men)} = \sigma^2_{wY(women)}$), we used mixed-effects models containing an intercept only. Three different variance structures were compared: common between- and within-individual variances for men and women, distinct between-individual but common within-individual variances, and distinct between- and within-individual variances, using a likelihood ratio test (significance level; $p < 0.05$) where the difference in -2 log likelihood follows a chi square distribution (Rappaport and Kupper, 2008). Then the estimated ratio of the between-individual variance to total observed variance, the ICC, was calculated. In some models (using the variance structure determined above) sex, age, BMI, collection time or urinary flow rate (log-transformed) was also added as a fixed effect to evaluate the significance of these parameters ($p < 0.05$), see below.

Geometric means (least squares means) and 95% confidence intervals (CI) were calculated for the different lead variables using the variance structure determined above (with or without sex as fixed effect in the models). Geometric means at different time points were calculated using time and sex as fixed effects in the models.

The associations between lead in blood and urinary lead in ON or 24 h urine samples were estimated using the Spearman correlation coefficient. The mean levels of the two days were used in these calculations. The relationships were also examined in linear regression models.

Associations between urinary flow rate and lead excretion rate were assessed within individuals by calculation of the Spearman correlation coefficient ($r_s$) for each participant. The overall mean of the correlation coefficients was then calculated. In a mixed effect model also sex, age and BMI were included as fixed effects in the model together with log-transformed urinary flow rate.

All calculations were performed with version 9.4 of the SAS software package (SAS Institute, Cary, NC, USA). Statistical significance was set at $p < 0.05$ for all tests and two-sided confidence intervals were used.

## 3. Results

### 3.1. Lead levels and associations between lead in blood and urine

The geometric means of lead biomarkers were significantly higher in men than in women, 11.2 μg/L and 8.2 μg/L in whole blood and 0.59 μg and 0.42 μg in 24-h urine (Table 1). The Pb excretion rates (μg/h) and the unadjusted UPb concentrations were also higher in men, while the difference by sex was not statistically significant when daytime or ON spot urine Pb concentrations were adjusted for creatinine or SG (Table 1).

The Spearman correlation coefficients between lead in blood and 24 h urine samples were high ($r_s = 0.77$ for whole blood and 0.72 for erythrocytes, $p < 0.0001$), Table 2 and Fig. 1. The correlations were only slightly lower for ON urine samples and the highest correlation was found between blood and SG corrected urine samples ($r_s = 0.74$). The Spearman correlations between 24 h urine samples and overnight urine

**Table 2**
Spearman correlation coefficients between lead biomarkers in blood, 24 h urine (μg) and overnight urine samples (calculated using mean level of day 1 and 2 for each individual).

| | EryPb μg/L | Urine overnight samples | | | | 24 h urine |
|---|---|---|---|---|---|---|
| | | UPb μg/L | UPb μg/g crea | UPbSG μg/L | UPbrate μg/h | UPb μg/24 h |
| BPb | 0.97 | 0.60 | 0.63 | 0.74 | 0.73 | 0.77 |
| EryPb | | 0.60 | 0.58 | 0.74 | 0.71 | 0.72 |
| UPb μg/L | | | 0.47 | 0.71 | 0.64 | 0.58 |
| UPb μg/g crea | | | | 0.82 | 0.69 | 0.69 |
| UPbSG μg/L | | | | | 0.74 | 0.68 |
| UPbrate μg/h | | | | | | 0.83 |

**Table 1**
Geometric means (GM) and 95% confidence intervals (95% CI) for lead in urine and blood samples as calculated from the mixed effects models.

| | N | All | Men (N = 29) | Women (N = 31) | p–value |
|---|---|---|---|---|---|
| | | GM (95% CI) | GM (95% CI) | GM (95% CI) | sex |
| Urine | | | | | |
| 24h UPb μg | 119 | 0.50 (0.44–0.56) | 0.59 (0.51–0.69) | 0.42 (0.36–0.49) | 0.0024 |
| 24h UPb μg/L | 119 | 0.31 (0.28–0.36) | 0.38 (0.32–0.45) | 0.26 (0.22–0.31) | 0.0038 |
| All spot samples | | | | | |
| UPb μg/L | 718 | 0.31 (0.27–0.36) | 0.37 (0.31–0.45) | 0.27 (0.22–0.32) | 0.01 |
| UPbcrea μg/g crea | 673 | 0.33 (0.29–0.36) | 0.31 (0.26–0.36) | 0.34 (0.30–0.39) | 0.307 |
| UPbSG μg/L | 664 | 0.28 (0.25–0.31) | 0.29 (0.24–0.35) | 0.27 (0.24–0.31) | 0.663 |
| UPbrate μg/h | 718 | 0.020 (0.018–0.022) | 0.024 (0.021–0.029) | 0.018 (0.015–0.020) | 0.004 |
| Overnight (ON) samples | | | | | |
| UPb μg/L | 119 | 0.37 (0.32–0.43) | 0.44 (0.36–0.94) | 0.31 (0.26–0.38) | 0.016 |
| UPbcrea μg/g crea | 117 | 0.29 (0.26–0.33) | 0.28 (0.24–0.34) | 0.30 (0.25–0.35) | 0.684 |
| UPbSG μg/L | 108 | 0.28 (0.25–0.31) | 0.30 (0.24–0.38) | 0.27 (0.24–0.31) | 0.479 |
| UPbrate μg/h | 119 | 0.016 (0.014–0.018) | 0.020 (0.017–0.025) | 0.013 (0.011–0.015) | <0.001 |
| Blood | | | | | |
| Whole blood μg/L | 118 | 9.5 (8.6–10.5) | 11.2 (9.8–12.7) | 8.2 (7.2–9.3) | 0.0015 |
| Erythrocytes μg/L | 119 | 19.2 (17.6–21.0) | 21.9 (19.4–24.7) | 17.0 (15.1–19.1) | 0.0045 |

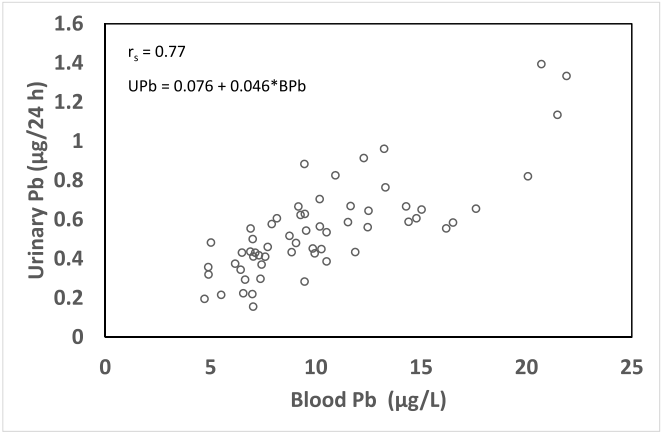


**Fig. 1.** Association between lead in blood and 24 h lead in urine ($r_s$ = Spearman correlation coefficient). Mean values of day 1 and day 2 are shown for each individual.

samples were around 0.7 ($p < 0.0001$), lowest for unadjusted lead concentration. The slope and intercept in a regression model between 24hUPb (µg) versus BPb were 0.046 and 0.076, respectively. Inclusion of sex, age, BMI and flowrate caused only minor changes of the slope (0.044). The median ratio between 24hUPb (in µg) and BPb was 0.054. The median ratio for ON urine UPbcrea (µg/g crea) and BPb was 0.027 for men and 0.039 for women.

### 3.2. Variability of lead in blood and urine

For both whole blood and erythrocytes as well as for 24 h urine samples a model with common variances could be used for both women and men (likelihood ratio test). The between-individual variance dominated with ICCs above 0.8, Table 3A. For spot urine samples a model with common variance could only be used for some of the urinary lead variables (for UPb all spot samples, daytime and ON samples, for UPbcrea ON samples and for UPbrate daytime and ON samples), while in some cases distinct between- and within-individual variances had to be used for men and women (for UPbcrea all spot samples and daytime samples, for UPbSG daytime and ON samples and for UPbrate all spot samples), Table 3B. Common within-individual variance and different between-individual variances were found for UPbSG for all spot samples. In these models higher ICCs were generally obtained for men. The ICCs for UPb were higher for ON samples compared to spot samples regardless of sampling time. The highest ICC for ON samples was obtained for UPbrate (0.71, men and women) and for UPbSG (0.88, men). Twelve out of 18 ICC values (67%) were estimated to be above 0.5.

### 3.3. Circadian rhythm and influence of flow rate

Fig. 2 shows the variation in geometric mean levels of UPb, UPbcrea, UPbSG and UPbrate over the six sampling times. The excretion rate was lowest for ON, with statistically significant differences versus all other

**Table 3**

A. Variances of log-transformed lead in blood and 24 h urine samples and ICC (intra-class correlation).

| | Whole blood | Erythrocytes | 24 h urine |
|---|---|---|---|
| Number | 118 | 119 | 119 |
| ICC (common for men and women) | 0.974 | 0.961 | 0.806 |
| Between–individual variance, (95% CI) | 0.15 (0.10–0.22) | 0.12 (0.08–0.18) | 0.18 (0.12–0.28) |
| Within–individual variance (95%CI) | 0.004 (0.002–0.006) | 0.004 (0.003–0.007) | 0.04 (0.03–0.06) |

B. Variances of log-transformed lead in urine spot samples and ICC (intra-class correlation).

| Type of sample | UPb µg/L | UPb µg/g creatinine | UPbSG µg/L | UPbrate µg/h |
|---|---|---|---|---|
| All spot samples | | | | |
| Number of samples | 718 | 673 | 664 | 718 |
| ICC (common for men and women) | 0.417 | | | |
| Between–individual variance, (95% CI) | 0.24 (0.17–0.38) | | | |
| Within–individual variance/between days, (95%CI) | 0.34 (0.30–0.38) | | | |
| ICC Men | see above | 0.717 | 0.675 | 0.574 |
| Between–individual variance, (95% CI) | | 0.20 (0.09–0.31) | 0.25 (0.15–0.47) | 0.23 (0.09–0.37) |
| Within–individual variance/between days, (95%CI) | | 0.08 (0.07–0.09) | 0.12 (0.11–0.14) | 0.17 (0.15–0.20) |
| ICC Women | see above | 0.532 | 0.494 | 0.405 |
| Between–individual variance, (95% CI) | | 0.13 (0.06–0.20) | 0.12 (0.07–0.22) | 0.15 (0.06–0.25) |
| Within–individual variance/between days, (95%CI) | | 0.11 (0.10–0.14) | 0.12 (0.11–0.14) | 0.22 (0.19–0.26) |
| **All daytime spot samples (all minus ON)** | | | | |
| Number of samples | 599 | 556 | 556 | 599 |
| ICC (common for men and women) | 0.408 | | | 0.515 |
| Between–individual variance, (95% CI) | 0.27 (0.19–0.42) | | | 0.19 (0.14–0.30) |
| Within–individual variance/between days, (95%CI) | 0.34 (0.30–0.38) | | | 0.18 (0.16–0.21) |
| ICC Men | see above | 0.738 | 0.693 | see above |
| Between–individual variance, (95% CI) | | 0.21 (0.10–0.32) | 0.24 (0.11–0.36) | |
| Within–individual variance/between days, (95%CI) | | 0.07 (0.06–0.09) | 0.10 (0.09–0.13) | |
| ICC Women | see above | 0.533 | 0.467 | see above |
| Between–individual variance, (95% CI) | | 0.14 (0.06–0.21) | 0.12 (0.05–0.19) | |
| Within–individual variance/between days, (95%CI) | | 0.12 (0.10–0.14) | 0.14 (0.12–0.16) | |
| **Overnight (ON) samples** | | | | |
| Number of samples | 119 | 117 | 108 | 119 |
| ICC (common for men and women) | 0.531 | 0.664 | | 0.709 |
| Between–individual variance, (95% CI) | 0.23 (0.14–0.43) | 0.15 (0.10–0.26) | | 0.27 (0.18–0.44) |
| Within–individual variance/between days, (95%CI) | 0.20 (0.14–0.30) | 0.08 (0.06–0.12) | | 0.11 (0.08–0.16) |
| ICC Men | see above | see above | 0.876 | see above |
| Between–individual variance, (95% CI) | | | 0.34 (0.14–0.53) | |
| Within–individual variance/between days, (95%CI) | | | 0.05 (0.03–0.10) | |
| ICC Women | see above | see above | 0.431 | see above |
| Between–individual variance, (95% CI) | | | 0.09 (0.003–0.17) | |
| Within–individual variance/between days, (95%CI) | | | 0.12 (0.07–0.21) | |

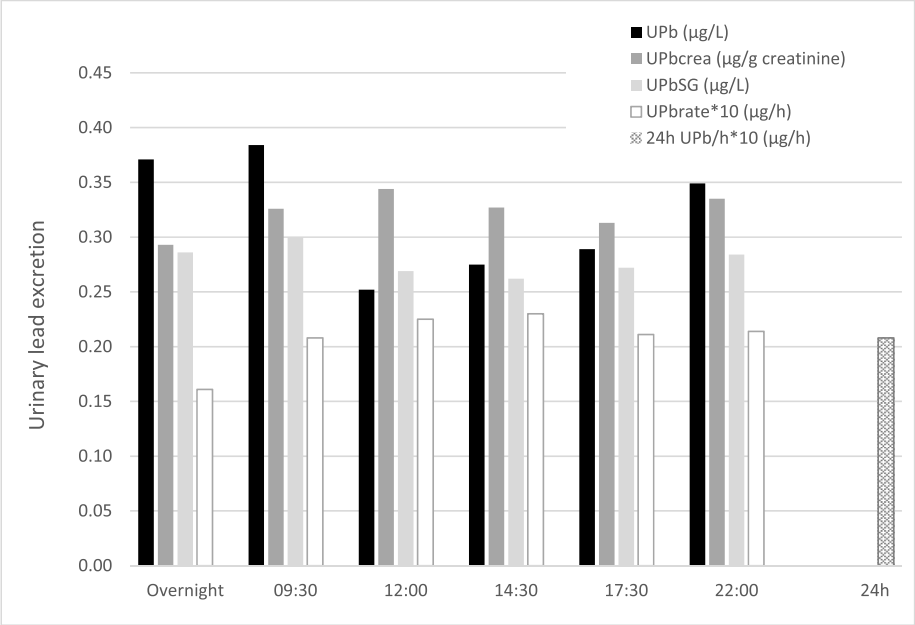


**Fig. 2.** Geometric means of urinary lead for different sampling time points.

sampling time points. Similar results were obtained for UPbcrea except for time point 17:30. Unadjusted lead concentrations in ON urine and at 9.30 were both significantly higher than at time points 12:00, 14:30 and 17:30. For UPbSG the highest level was found at 9:30, with statistically significant differences versus 12:00, 14.30 and 17.30.

There was a significant positive association between urinary flow rate and lead excretion rate within individuals. The mean of the 60 individual Spearman correlation coefficients was 0.34 ($p < 0.0001$, *t*-test) but somewhat lower when ON samples were excluded (mean $r_s = 0.26$, $p < 0.0001$). The slope of a log-log regression of lead excretion rate versus UF was for all samples 0.30 ($n = 718$, $p < 0.0001$), and 0.26 ($n = 599$, $p < 0.0001$) when ON samples were excluded. As mentioned in the method section, these results were obtained in mixed model analyses, adjusted for age, sex and BMI. When also time was included in the models, time was not a significant factor when overnight samples were excluded. This indicates that in daytime the variation in UPbrate is mainly explained by the variation in UF.

## 4. Discussion

The most interesting findings in the present study are that urinary lead in 24 h urine and ON urine is highly correlated with BPb and that the ICCs for UPbcrea, UPbSG and UPbrate are high. In addition, the results show that the UPb excretion rate is lowest overnight and increases with urinary flow rate.

### 4.1. Associations between lead in urine and blood

UPb has been used in biological monitoring of lead only to a limited extent (Bergdahl and Skerfving, 2022). More than 99% of BPb is found in erythrocytes, but UPb originates from Pb in plasma and is filtrated in the glomeruli. There is also some tubular reabsorption. At occupational exposure and high BPb, the ratio between PPb and BPb increases due to saturation of Pb-binding sites in the erythrocytes (Bergdahl and Skerfving, 2022), and thereby also the ratio between UPb and BPb increases (Bergdahl et al., 1997; Gulson et al., 1998; Fukui et al., 1999). At low exposure levels in individuals with only environmental exposure, Sommar et al. (2014) found a linear relationship between UPb in ON urine adjusted for creatinine and BPb, in agreement with the results in our study. We found high correlations between BPb and ON urine samples, both for unadjusted ($r_s = 0.60$) and dilution-adjusted ($r_s = 0.63–0.73$) UPb. Therefore, when BPb is not available or is biased due to a large change in erythrocyte volume fraction, UPb may be a useful alternative in epidemiological studies, especially since the intra-individual variability is modest, as discussed below. Lead in urine might also be useful as a proxy for plasma Pb levels in studies of lead toxicity (Tsaih et al., 1999), while on the individual level the variability is too large to use UPb to predict the very low PPb levels (Bergdahl and Skerfving, 2022; Tsaih et al., 1999; Barbosa et al., 2005; Sommar et al., 2014).

### 4.2. Variability of lead in urine and blood

Few previous studies have examined variability of lead in urine in the general adult population. Findings in 20 Swedish men were reported by Sommar et al. (2014) and in 11 Chinese men by Wang et al. (2016). Those two studies showed quite different results. In the Swedish men the ICCs for ON urine were relatively similar to those of the present study; UPb 0.34 vs 0.53 in our study, UPbcrea 0.87 vs 0.66, UPbSG 0.78 vs 0.88, UPbrate 0.52 vs 0.71 despite the fact that urine samples were collected months apart, and in our study only a week apart. In the Chinese men, samples were adjusted for creatinine and the ICCs for UPbcrea were very low both for spot samples and 24 h samples (≤0.10). In ON samples collected days apart the ICC for UPbcrea was 0.36 and in samples collected months apart it was 0.11. The UPb levels in the Chinese men were, however, about 10 times higher than in our study and the study by Sommar et al. (2014). The ICC for 24 h samples was high in the present study but collection of this type of sample in large cohorts is difficult.

For blood an extremely high ICC was obtained in our study (ICC = 0.97), consistent with ICC = 0.95 in non-exposed subjects in the study by Sommar et al. (2014) but higher than in another study conducted in women only (ICC = 0.83) (Smith et al., 2002). In lead exposed workers a slightly lower ICC (0.91) was found by Sommar et al. (2014). A high ICC for BPb (0.81) was also obtained when blood samples were collected over six years period in elderly Korean subjects (Lee et al., 2017). In epidemiological studies lead in erythrocytes could as well be used since the ICC for lead in erythrocytes was almost identical to that for lead in whole blood (0.96 vs 0.97).

In our study the within-individual variance for Pb in urine tended to

be higher for women compared with men resulting in lower ICCs. Different Pb kinetics have been reported between women and men (Barbosa et al., 2005, Popovic et al., 2005). Premenopausal women appeared to retain Pb more avidly or release Pb more slowly compared to postmenopausal women and to men. To our knowledge investigation of different variance structure by gender in the general population for metals has previously been performed only for cadmium (Akerstrom et al., 2014). For UPbcrea a common variance structure was found for ON samples but distinct variances for daytime as well as for all spot samples. It is easier to find distinct variances in all spot samples since the number of observations are much larger than for ON samples.

ICC values exceeding 0.75 are considered desirable for excellent reliability in exposure classification, while values below 0.4 indicates that a single sample will not be acceptable (Rosner, 2015). In conclusion lead in blood, erythrocytes and urine corrected for dilution by creatinine or SG seem appropriate for exposure assessment in the general population in epidemiological studies. For Pb in urine adjusted for dilution the ICC is acceptable both for ON and daytime samples.

### 4.3. Diurnal variation and influence of flow rate on the excretion of lead in urine

The excretion of Pb was lowest in overnight samples. Diurnal variation of PBrate in urine has also been shown in 19 occupationally exposed male metal workers (Yokoyama et al., 2000, Aono and Araki, 1988). In these workers the Pbrate was lowest in ON urine and the circadian rhythm of Pb in urine was parallel to the plasma and erythrocytes rhythms (Yokoyama et al., 2000). These workers had, however, about 100 times higher Pbrate than the subjects of our study.

The diurnal variation of UPbrate is likely to be affected by the variation in urinary flow rate. We found significant correlations between UPbrate and UF for all urine samples as well as for daytime samples. The association was still significant when time was included in the mixed effects models, in addition to BMI, age and sex. When all samples were included both time and UF contributed significantly to UPbrate, but when ON samples were excluded, time was no longer statistically significant. This indicates that in daytime the variation in UPbrate is mainly explained by the variation in UF. In the night UF decreases, due to decreased fluid intake and hormonal factors. But also, the glomerular filtration rate decreases in the night, and in ON samples we cannot disentangle the impact on UPbrate of reduced UF from the impact of other factors. The diurnal variation was also found for UPbcrea, though somewhat attenuated. Creatinine also has a diurnal variation with the lowest excretion rate in ON samples and a positive correlation with UF (Sallsten and Barregard, 2021).

Our results are consistent with those found among the 19 metal workers mentioned above, where a significant relationship between UPbrate and UF was found (Araki et al., 1990). Since these workers were occupationally exposed to metals (in daytime) this may have contributed to the variation of UPbrate.

It has been suggested that evaluations of potential associations between health outcomes and chemical exposures using urinary data should be assessed not only on the basis of biomarkers concentration but also on the basis of mass excretion rate (Hays et al., 2015).

### 4.4. Levels of lead in urine and blood

The urinary lead levels found in the present study are very similar to results in the NHANES study from 2015 to 2016 for subjects older than 20 years for both UPb and UPbcrea (NHANES. Centers for Disease control and Prevention, 2019). Some studies in the general population have shown higher levels but results for non-smokers are not presented separately (Sommar et al., 2014; Hoet et al., 2013; Morton et al., 2014). Significantly higher UPb levels have been found among populations in Belgium and UK (Hoet et al., 2013; Morton et al., 2014) but not for UPbcrea (Morton et al., 2014). In NHANES. Centers for Disease control and Prevention (2019) a clear decreasing trend of both UPb and UPbcrea from 1999 until 2016 can be seen in the age-group above 20 years.

BPb was significantly higher in men than women as shown also in NHANES and in Swedish subjects (Jain, 2016; Bjermo et al., 2013; Wennberg et al., 2017). The geometric mean of 9.5 μg/L found in our study is similar to 9.2 μg/L among non-smokers in the US adult population in the period 2011–2012 (Jain, 2016) and similar to subjects in northern Sweden in 2014 (Wennberg et al., 2017). In a study of Korean elderly (2008–2014) a higher geometric mean level (19 μg/L) was obtained among non-smokers (Lee et al., 2017). In Sweden BPb decreased during 1990–2009 after which no further reduction was found until 2014 (Wennberg et al., 2017). Studies from the early 2000s show in general higher levels in blood (Jain, 2016, Simić et al., 2022).

### 4.5. Limitations and strengths

The number of individuals in this study would have been too small for a study on associations between biomarkers and adverse health effects. The sample is 3–5 times larger than two previous studies of ICC for urinary lead in adults (Sommar et al., 2014; Wang et al., 2016). An important limitation is that the interval between repeated collections was one week and samples collected after longer periods would have been desirable and might have shown lower ICCs. However, the study by Sommar et al. (2014) found ICCs for blood and urinary Pb similar to our results in spite of collection intervals of 2–3 months. A strength in this study is that both urine and blood samples were collected in all individuals.

A small part (1.7%) of the urine had Pb levels below the detection limit, but this should not affect our findings. When urine samples were corrected for dilution some creatinine values fell outside recommended cut-off values (Cocker et al., 2011) and were excluded. For SG we only excluded samples above 1.030, though Ikeda et al. (2003) recommend the following cut-off values for SG; $<1.010$ and $>1.030$. However, the United States Department of Health and Human Services have recommended acceptable values of 1.001-1-030 in their drug testing guidelines (DHHS, 2004; Smolders et al., 2014). Very few samples were below 1.005 but nearly 15% was below 1.010 (Sallsten and Barregard., 2021). Too many samples would have been excluded if a lower limit of 1.010 had been used.

Creatinine correction assumes that creatinine is excreted at a constant rate, but in fact the creatinine excretion rate also varies by urinary flow rate (Araki et al., 1990; Middleton et al., 2019; Sallsten and Barregard, 2021). Equations have been suggested to improve creatine correction (Araki et al., 1990; Middleton et al., 2019). A modified creatinine correction adjustment method might have resulted in somewhat higher ICCs for UPbcrea.

The present study was conducted in non-smoking men and women from a city on the West coast of Sweden. The study by Sommar et al. (2014) included only men from another city in the South of Sweden. Our results might not be valid for people with different dietary or smoking habits.

Subjects were carefully instructed how to collect urine samples at different timepoints and record it. However, we cannot exclude that some errors occurred.

Metal analyses was performed at the National Institute of Occupational Health in Norway using mass spectrometric techniques. Quality control samples were included and showed satisfactory results.

### 4.6. Conclusions

The ICC was excellent for lead in whole blood and erythrocytes but high also for urinary lead adjusted for dilution and the correlation between the two biomarkers were high. Therefore, also UPb adjusted for dilution by creatinine or specific gravity seems to be a biomarker that can be used for exposure assessment in epidemiological studies. There is a diurnal variation of lead in urine with lowest excretion rate in

overnight samples, but ICCs were similar for ON and daytime spot samples. There was a significant positive association between urinary flow rate and lead excretion rate within individuals which explained most of the variation rate in lead excretion in daytimes.

## Author contributions

Conceptualization, G.S. and L.B; Data curation, L.B., G.S., D.G.E, B. B., S.W.; Metal analysis; B.B. Formal analysis and writing original draft; G.S.; writing – review & editing; G.S., L.B., B.B., S.W., D.G.E.; Funding acquisition; L.B., G.S.

## Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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