

Effects of water restriction and water loading on daily urinary excretion of heavy metals and organic substances in metal workers

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ABSTRACT The effects of urinary volume on daily urinary excretion of seven heavy metals and four organic substances were examined in relation to the changes in their plasma and erythrocyte concentrations and urinary creatinine excretion in 19 metal workers. The examination was conducted under the conditions of water restriction and loading for six days. The major findings were as follows: (1) urinary excretion of all heavy metals and organic substances except mercury, together with creatinine excretion, significantly decreased under the water restrictive condition whereas under the water loading condition their excretion significantly increased and (2) daily variations in urinary excretion of lead, cadmium, chromium, copper, hippuric acid, δ -aminolaevulinic acid, and coproporphyrin did not differ significantly from the variation in urinary excretion of creatinine (profile analysis, $p > 0.05$). It is suggested that glomerular filtration is the major factor determining renal excretory mechanisms of the four heavy metals and three organic substances examined.

We have found that the urinary excretion of various heavy metals and organic substances is significantly affected by urinary volume (UV) in metal workers¹ and in healthy controls.² These findings were consistent with previous observations^{3,4} that 24 hour urinary excretion of lead (Pb) and δ -aminolaevulinic acid (ALA) significantly decreased when UV was reduced. The findings,^{1,2} however, contrasted sharply with previous observations^{3,4} that the excretion of coproporphyrin (CP) and creatinine (Cn) was independent of UV.

To examine the effects of UV on 24 hour urinary excretion of substances further, we have measured the excretions of various heavy metals and organic substances in the urine under the conditions of water restriction and water loading. In addition, daily variations in urinary excretion of substances were compared with the variations in UV, urinary Cn excretion, and plasma and erythrocyte concentrations of those substances using profile analysis.⁵

copper (Cu), and tin) were male metal foundry workers aged 34 to 59. Their blood Pb concentrations ranged from 25 to 59 $\mu\text{g}/\text{dl}$ (mean 39 $\mu\text{g}/\text{dl}$ (1.9 $\mu\text{mol}/\text{l}$)). No subject had ever suffered from renal disease; nor were albuminuria or glucosuria found in any subject.

COLLECTION OF BLOOD AND URINE SAMPLES

The procedure was explained to all subjects and this study was conducted with their informed consent. After maintaining their usual intake of food and liquid for two days (free condition), the subjects drank less than 0.25 l of liquid a day for another two days (water restrictive condition) and were then loaded with more than 3 l of water for two successive days (water loading condition). Twenty four hour urine samples (0700-0700) were collected on all workers every day for six days; venous blood samples were collected at 1000 daily for six days. All subjects were admitted to a special room for health examination at the Medical College of Oita during the six day period, and they ate all meals in the hospital dining room. To minimise the intake of organic mercury, they consumed little fish and shellfish.

ANALYTICAL METHODS

Analytical methods, lower limits of detection, and

Subjects and methods

SUBJECTS

The 19 subjects (workers exposed to Pb, zinc (Zn),

reproducibility of analysis for all substances examined in the present study have been reported previously.¹⁶ Blood, erythrocyte, and urinary Pb concentrations were measured by atomic absorption spectrophotometry (AAS) after wet ashing, chelation by sodium diethyl dithiocarbamate (DDTC), and extraction to water saturated methyl isobutyl ketone (MIBK); the plasma Pb concentration was measured by the method of de Silva.⁷ Plasma, erythrocyte, and urinary concentrations of total (inorganic and organic) mercury (Hg) were determined by the method of Magos.^{8,9} Plasma and erythrocyte concentrations of cadmium (Cd) and Cu were measured by the flameless AAS after deproteinisation by trichloroacetic acid (TCA) and urinary Cd by the method of Subramanian *et al.*¹⁰ Plasma and erythrocyte concentrations of manganese (Mn) and chromium (Cr) were measured by the flameless AAS using the standard addition technique after deproteinisation by TCA; urinary Mn, Cr, and Cu concentrations by the AAS after wet ashing, chelation by DDTC, and extraction to MIBK. Plasma and urinary Zn concentrations were measured by the AAS after deproteinisation by TCA; erythrocyte Zn by the AAS after wet ashing. Plasma and urinary β -2-microglobulin (BMG) concentrations were measured by the radioimmunoassay method; urinary hippuric acid (HA), ALA, and CP by the

benzenesulphonyl chloride method, the methods of Tomokuni and Ogata,¹¹ and of Soulsby and Smith,¹² respectively; urinary Cn by Jaffe's reaction; and urinary specific gravity (SG) by refractometry. The urinary concentration of total urinary solutes (TUS) was calculated from urinary SG and Haeser's index.

STATISTICAL ANALYSIS

Differences in daily urinary excretion and plasma and erythrocyte concentrations of substances were tested by the paired sample *t* test. Parallelisms of daily urinary excretion of substances to daily urinary volume, Cn excretion, and plasma and erythrocyte concentrations of substances were analysed by profile analysis using the F test.⁵

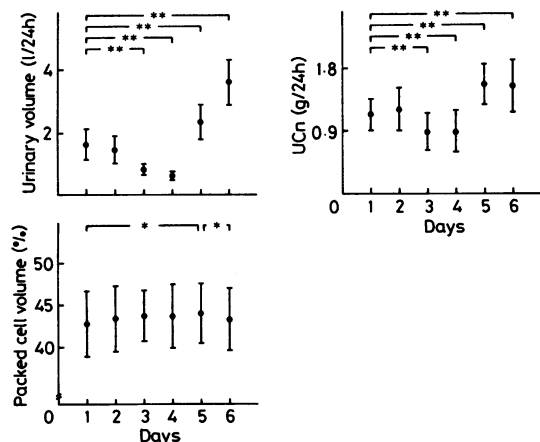


Fig 1 Daily variations in urinary volume, urinary creatinine (UCn), and packed red blood cell volume for six days in 19 metal workers (mean \pm SD). Days 1 and 2 represent free condition, 3 and 4 show water restrictive condition, and 5 and 6 are water loading condition. Packed red blood cell volume was measured at 1000 on all six days. * and ** indicate significant differences between two days at levels of $p < 0.05$ and 0.01 , respectively. Urinary volume and UCn on day 2 also differed significantly from those on days 3 to 6 ($p < 0.01$). (1 g/24 h for UCn corresponds to 8.8 mmol/24 h.)

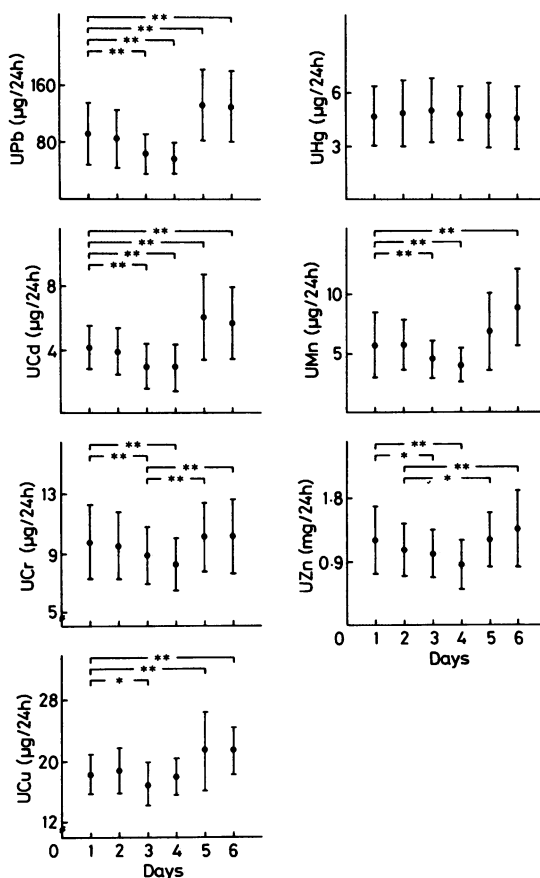


Fig 2 Daily variations in urinary lead (UPb), mercury (UHg), cadmium (UCd), manganese (UMn), chromium (UCr), zinc (UZn), and copper (UCu) for six days in 19 metal workers. Examination days, vertical lines, and signs as in fig 1. (1 μ g/24 h for UPb, UHg, UCd, UMn, UCr, UZn, and UCu correspond to 4.8, 5.0, 8.9, 18, 19, 15, and 16 nmol/24 h, respectively.)

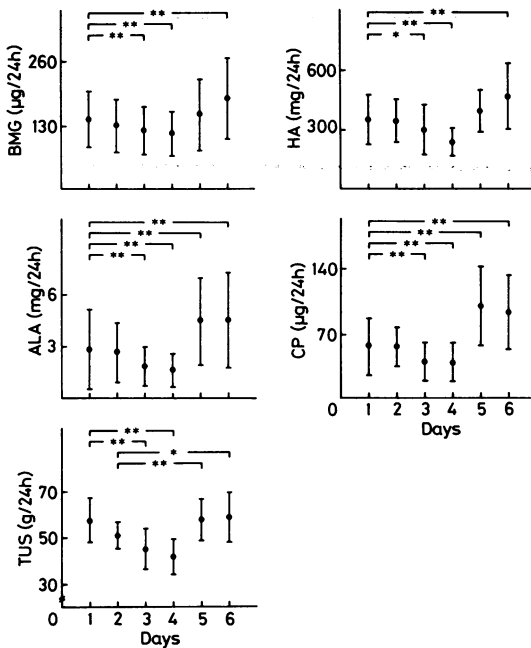


Fig 3 Daily variations in urinary β -2-microglobulin (BMG), hippuric acid (HA), δ -aminolaevulinic acid (ALA), coproporphyrin (CP), and total solutes (TUS) for six days in 19 metal workers. Examination days, vertical lines, and signs as in fig 1. (1 mg/24 h for BMG, HA, ALA, and CP correspond to 0.085, 5.6, 7.6, and 1.5 μ mol/24 h, respectively.)

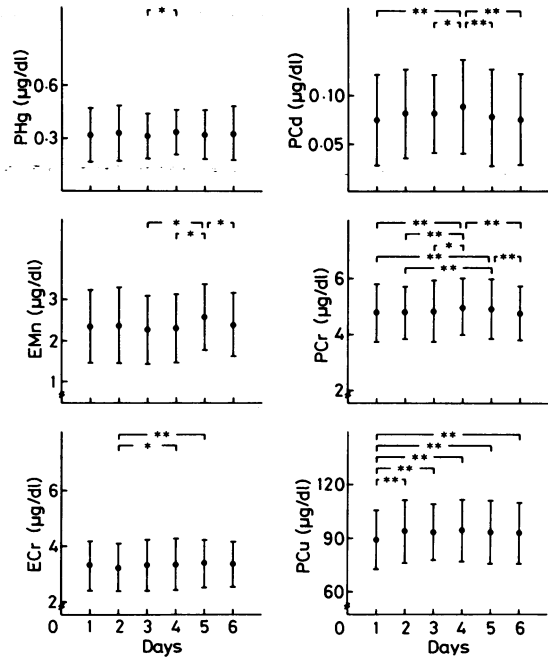


Fig 4 Daily variations in mercury, cadmium, chromium, and copper in plasma (PHg, PCd, PCr, and PCu) and manganese and chromium in erythrocytes (EMn and ECr) for six days in 19 metal workers. Examination days, vertical lines, and signs as in fig 1. 1 μ g/dl for PHg, PCd, EMn, PCr, ECr, and PCu correspond to 0.050, 0.089, 0.18, 0.19, 0.19, and 0.16 μ mol/l, respectively.

Results

The daily urinary excretion of each heavy metal and organic substance except Hg, and UV, Cn, and TUS, significantly decreased under the water restrictive

condition, and significantly increased under the water loading condition (figs 1–3). The concentrations of Hg, Cd, Cr, and Cu in plasma, the concentrations of Mn and Cr in erythrocytes, and packed red blood cell volume were significantly higher in the fourth or fifth

Parallelisms of daily urinary excretion of heavy metals, organic substances, total solutes, and creatinine to daily urinary volume and creatinine excretion, and to plasma and erythrocyte concentrations of each substance for six days in 19 metal workers under conditions of water restriction and loading: profile analysis (F values)

	Urinary volume	Urinary creatinine	Plasma concentration of each substance	Erythrocyte concentration of each metal
Lead	8.266**	1.163	14.471**	10.307**
Mercury	8.844**	5.516**	2.605*	0.488
Cadmium	4.230**	1.028	25.544**	20.852**
Manganese	4.889**	4.692**	18.579**	11.507**
Chromium	7.320**	1.653	14.194**	4.542**
Zinc	5.245**	4.955**	2.915*	1.885
Copper	6.902**	2.008	13.591**	14.970**
β -2-microglobulin	3.526*	3.666**	11.910**	—
Hippuric acid	2.968*	1.376	—	—
δ -Aminolaevulinic acid	6.415**	1.243	—	—
Coproporphyrin	11.613**	1.258	—	—
Total solutes	9.062**	6.903**	—	—
Creatinine	4.964**	—	—	—

*p < 0.05; **p < 0.01.

—Plasma and erythrocyte concentrations were not measured.

examination days, or both (figs 1 and 4).

The results of profile analysis indicated that daily variations in urinary excretion of Pb, Cd, Cr, Cu, HA, ALA, and CP did not significantly differ from the variation in urinary Cn excretion (table). On the other hand, daily variations in all urinary heavy metals and organic substances, together with urinary Cn and TUS, differed significantly from the variation in UV; the variations in all urinary heavy metals and BMG differed significantly from the variations in the plasma concentrations of the metals and BMG; and the variations in all urinary heavy metals except Hg and Zn differed significantly from the variations in the erythrocyte concentrations of the metals (table).

Discussion

Pronounced effects of UV on the daily urinary excretion of all the heavy metals and organic substances except Hg were found; daily excretion of these substances under the water loading condition was nearly twice as large as the excretion under the water restrictive condition (figs 2 and 3). Thus it is essential to take into account the effects of UV in the biological monitoring of these heavy metals and organic substances.

Packed red blood cell volume and the plasma or erythrocyte concentration, or both, of six heavy metals reached the highest levels in the fourth or fifth days of examination—that is, in the later stage of water restriction. This was probably due to dehydration under the water restrictive condition.

The daily variations in urinary excretion of Pb, Cd, Cr, and Cu were closely associated with the variation in urinary Cn excretion but not with the variations in the plasma and erythrocyte concentrations of each metal nor with the variation in UV (table, figs 1, 2). Evidence has been given that in man: (1) glomerular filtration rate (GFR) (inulin or endogenous creatinine clearance) is greatly altered under the conditions of water restriction and loading¹³; (2) substantial amounts of heavy metals are filtered at the glomerulus.¹⁴ These data indicate that a change in GFR is the major factor determining the variations in urinary excretion of those metals under the conditions of water restriction and loading. The same mechanism may also be responsible for the variations in urinary excretion of many organic substances.

Daily variations in urinary excretion of Mn, Zn, and BMG, on the other hand, were significantly associated neither with the variations in urinary Cn excretion nor with the plasma concentrations. Mn, Zn, and BMG have been found to be also filtered at the glomerulus in man.^{14,15} Therefore, the present observation may reflect the complex renal excretory mechanisms of those substances.

Finally, urinary excretion of Hg was independent of

the variations in UV and in urinary excretion of Cn. The latter observation suggests that Hg is not filtered at the glomerulus; most studies by us^{14,16,17} and by others¹⁸ are in line with this finding. It appears that Hg is the only metal that has been found to have such a characteristic renal excretory mechanism.

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