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# Renal Excretion of Trace Elements: Chromium and Copper (38425)

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Chromium (Cr) is recognized as a trace element essential for normal glucose and lipid metabolism in common experimental animals (1, 2). The evidence for a physiological role in man is gradually accumulating (1–6). Chromium deficiency has been suggested as a contributing factor in atherosclerosis (7) and for the impairment of glucose assimilation associated with certain clinical states (1), as well as with normal pregnancy (3–5). In previous studies we demonstrated that both an oral or intravenous glucose load, and also insulin administration, produced an immediate and sustained fall of plasma Cr in normal fasting subjects. Parallel falls of phosphate and potassium occurred also but no change of other plasma trace elements such as Cu or Fe. This observation was interpreted as support for a physiological role for Cr in the insulin-stimulated utilization of glucose in peripheral tissues (3, 4). However, the possibility that glucose or insulin increased the renal excretion of Cr could not be excluded entirely. While it is generally accepted that the kidney is the major route of Cr excretion (1, 2, 6), little other information is available on the renal regulation of Cr excretion . Collins *et al.* (8), using iv loading doses of $Cr^{51}$, found dog plasma clearance values consistent with glomerular filtration and not tubular secretion. In diabetic subjects, Schroeder (9) reported that urinary excretion of Cr increased twofold at 2 hr of an oral glucose tolerance test. Doisy *et al.* (6) also observed increased excretion of loading doses of $Cr^{51}$ in similar patients. Whether the increased Cr excretion of diabetic patients is representative of normal Cr metabolism or is related to changed renal function associated with the disease is not clear.

The purpose of this study was twofold: (a) to determine if a standard glucose load or a water load influences the renal excretion of Cr in normal subjects, and (b) to determine the normal plasma clearance and tubular reabsorption of ultrafilterable Cr. For comparative purposes copper (Cu) excretion was studied.

*Materials and Methods.* Observations were made on healthy volunteer laboratory personnel of both sexes (aged 18–36) on unselected diets. All subjects were fasted from 8:00 PM on the day prior to each experiment to ensure basal rates of renal excretion. On the morning of the experiment, at 7:45 AM and again at 8:00 AM, residual overnight urine was voided. Each subject was given 100 ml water at the start of a 4-hr control period. Urine was collected into polyethylene bottles every hour. At the end of the fourth hour a standard oral glucose load (75 g glucose in approximately 100 ml, Glucola, Ames Co. Inc., Elkhart, IN) was given and urine collections continued for an additional 4 hr. Normal daily activities were not restricted. The procedure was repeated 2–3 weeks later with a water load; 1 liter of distilled water was ingested over a 15-min period at the beginning of the second collection period. Chromium and Cu in urine were determined by atomic absorption with a graphite furnace by methods previously described (10). Creatinine was determined by the alkaline picrate method (11). All determinations were carried out in duplicate.

Dog studies were conducted on episiotomized female mongrels (20–25 kg), trained to lie quietly in a dorsal position with light restraint. The animals were fasted overnight. Urine was collected from the bladder with an indwelling catheter following standard renal clearance technique (12). Glomerular filtration rate (GFR) was measured using creatinine. Diuresis was induced by infusion of hypotonic saline (0.5%) containing creatinine (0.5%) into a brachial vein at constant rates of 3–6 ml/min. A priming dose of creatinine calculated to establish the approp-

riate plasma level was then administered. Following a 1-hr equilibration period, 20-min urine collection periods were started. At the end of each period, the bladder was flushed with 20 ml warm saline, drained, and 15 ml air injected. After diuresis was established (3–6 ml urine/min for 4–6 periods), a prime dose (10 munit/kg body wt) of arginine vasopressin (Sigma Chemical Co., St. Louis, MO) was given iv followed by an iv maintenance infusion of 100 munit/hr/kg body wt. Collection periods were continued during and following cessation of the hormone infusion. Blood samples (5 ml) were drawn from a brachial vein into heparinized plastic syringes. The total number of samples drawn from any one dog was equal to one more than the number of urine collection periods. Urine and plasma Cr and Cu were determined in duplicate by atomic absorption (10). The plasma level of "free" or ultradialyzable Cr and Cu was determined on plasma ultrafiltrates from prewashed Centriflo Ultrafilters CF50A (Amicon Corp., Lexington, MA), at least three times during each experiment. As the percentage of ultrafilterable element did not change appreciably during an experiment, the average value was used to calculate the plasma ultrafilterable concentration for remaining periods. Duplicate creatinine determinations on the diluted urine and tungstate filtrates of plasma were made by an alkaline picrate method (11).

Plasma concentrations for use in the clearance formula or for calculating filtered load (creatinine clearance × plasma concentration) were determined by plotting plasma levels of trace elements and of creatinine versus time of collection and selecting the plasma value interpolated to coincide with the midpoint of the urine collection.

*Results*. The rate of renal excretion of Cr and Cu for male and female adults fasted 16 hr varied considerably between individuals and also for the same individual on different occasions (Tables I and II). Variation between individuals was not due entirely to differences of renal function since it was evident also when cation excretion was expressed in terms of creatinine excretion.

The administration of a standard oral glucose load (75 g) produced no statistically significant effect on the mean renal excretion of Cu for eight

TABLE I. Chromium and Copper Excretion After Oral Glucose Load (75 g).[a]

| | Chromium (Cr) | | | | Copper (Cu) | | | |
|---|---|---|---|---|---|---|---|---|
| | Preload | | Postload | | Preload | | Postload | |
| Subject | A (ng) | B (ng/mg) | A (ng) | B (ng/mg) | A (μg) | B (ng/mg) | A (μg) | B (ng/mg) |
| MB (F, 36 yr) | 247 | 1.38 | 220 | 1.19 | 4.83 | 12.7 | 4.91 | 13.0 |
| BN (F, 25 yr) | 294 | 1.87 | 313 | 1.91 | 3.96 | 22.9 | 4.42 | 26.3 |
| CK (F, 23 yr) | 346 | 1.56 | 102 | 0.65 | 2.94 | 13.2 | 1.38 | 7.6 |
| TW (F, 25 yr) | 504 | 1.83 | 188 | 0.93 | 14.53 | 52.9 | 6.34 | 33.7 |
| BS (M, 26 yr) | 385 | 1.87 | 139 | 0.49 | 4.45 | 21.6 | 2.55 | 9.0 |
| BG (M, 31 yr) | 415 | 1.83 | 325 | 1.85 | 2.22 | 9.8 | 2.35 | 13.4 |
| BO (M, 28 yr) | 459 | 1.43 | 467 | 1.19 | 4.21 | 12.3 | 4.17 | 10.5 |
| CD (M, 18 yr) | 474 | 1.46 | 419 | 0.98 | 2.85 | 8.8 | 4.53 | 10.6 |
| Mean | 391 | 1.65 | 272* | 1.15** | 5.00 | 19.3 | 3.83 | 15.5 |
| ± SEM | 32 | 0.08 | 46 | 0.18 | 1.40 | 5.1 | 0.57 | 3.3 |
| Urine volume (ml) | | | | | | | | |
| Mean | | 198 | | 133 | | 198 | | 133 |
| ± SEM | | 35 | | 28 | | 35 | | 28 |
| Creatinine (mg) | | | | | | | | |
| Mean | | 247 | | 248 | | 247 | | 248 |
| ± SEM | | 25 | | 39 | | 25 | | 39 |

[a] A: Total element excretion during 4-hr control period or 4-hr period after glucose load. B: Ratio element excreted to creatinine excretion. Significance of difference analyzed by paired $t$ test: *$P < 0.05$; ** $P < 0.025$.

TABLE II. Chromium and Copper Excretion after Water Load (1 liter).[a]

| | Chromium (Cr) | | | | Copper (Cu) | | | |
|---|---|---|---|---|---|---|---|---|
| | Preload | | Postload | | Preload | | Postload | |
| Subject | A (ng) | B (ng/mg) | A (ng) | B (ng/mg) | A (μg) | B (ng/mg) | A (μg) | B (ng/mg) |
| BN (F, 25 yr) | 275 | 1.20 | 431 | 1.78 | 4.24 | 18.6 | 6.48 | 26.8 |
| MB (F, 36 yr) | 342 | 1.76 | 697 | 2.78 | 4.12 | 21.2 | 5.41 | 21.6 |
| LK (F, 23 yr) | 278 | 1.73 | 442 | 2.91 | 3.16 | 19.7 | 4.21 | 27.8 |
| TW (F, 25 yr) | 335 | 1.34 | 334 | 1.80 | 7.90 | 31.6 | 22.78 | 68.3 |
| CS (F, 28 yr) | 220 | 0.56 | 603 | 1.72 | 3.64 | 9.3 | 8.52 | 24.4 |
| BS (M, 26 yr) | 196 | 0.67 | 750 | 2.54 | 3.55 | 12.2 | 16.82 | 57.0 |
| CD (M, 18 yr) | 181 | 0.89 | 722 | 2.43 | 4.89 | 14.9 | 9.34 | 31.4 |
| BO (M, 28 yr) | 299 | 0.76 | 536 | 1.79 | 4.50 | 11.5 | 6.44 | 21.5 |
| HM (M, 26 yr) | 205 | 0.70 | 410 | 1.36 | 2.79 | 9.5 | 5.68 | 18.8 |
| DG (M, 25 yr) | 262 | 0.82 | 534 | 1.73 | 3.65 | 11.5 | 5.74 | 18.6 |
| Mean | 259 | 1.04 | 546*** | 2.08*** | 4.24 | 16.0 | 9.14* | 31.6** |
| ± SEM | 18 | 0.14 | 46 | 0.17 | 0.45 | 2.2 | 1.89 | 5.4 |
| Urine volume (ml) | | | | | | | | |
| Mean | | 210 | | 936 | | 210 | | 936 |
| ± SEM | | 32 | | 77 | | 32 | | 77 |
| Creatinine (mg) | | | | | | | | |
| Mean | | 279 | | 283 | | 279 | | 283 |
| ± SEM | | 24 | | 18 | | 24 | | 18 |

[a] A: Total amount element excreted during 4-hr control period or 4-hr period after water load. B: Ratio of element to creatinine excretion. Significance of difference analyzed by paired $t$ test: *$P < 0.01$; **$P < 0.005$; ***$P < 0.001$.

fasting subjects observed over a 4-hr period (Table I), but significant reduction of Cr excretion was effected by the glucose load in six of eight subjects of both sexes ($P < 0.05$). The decrease in Cr excretion was not accounted for by changes in GFR since a decrease occurred also in the individual excretion ratios, Cr:creatinine ($P < 0.025$). For the group the mean creatinine excretion before (4-hr period) and after (4-hr period) the glucose load was unchanged, while the decrease in Cr excretion averaged 30%. Figure 1 shows the mean hourly excretion of Cr for five subjects. Chromium excretion decreased promptly within the first hour after glucose load, with only a gradual return to normal.

Diuresis, induced by an oral water load (1 liter), effected a marked increase in the renal excretion of both trace elements (Table II). For the entire group of subjects, Cr and Cu excretion increased an average of 111 and 116%, respectively. These changes in renal excretion occurred in nearly every subject and were highly significant statistically ($P < 0.005$). Similar increases of Cr and Cu excretion rates were obtained when their excretion was expressed as a ratio of creatinine excretion, and therefore they were independent of any significant change in GFR.

Figure 2 shows the diuresis induced by the water load in these subjects was maximal 2 hr after water ingestion, but urine flow returned to normal by the end of 4 hr, when approximately 90% of the ingested water volume was collected as urine (Table II). Figure 2 shows also that the increase in excretion rate of Cr and Cu correlated with the extent of the diuresis, although concentrations of the elements in the dilute urine decreased. At maximal urine flows Cr decreased from 1.81 ± 0.28 SEM ng/ml during control period to 0.44 ± 0.05 SEM ng/ml, and for Cu, 21.9 ± 3.3 to 5.6 ± 0.43 SEM ng/ml.

In dog studies, the plasma clearance of ultrafilterable Cr and Cu, the percentage of glomerular filtered load excreted, and the effect of urine flow on these parameters were determined. Figure 3 illustrates the results of a typical experiment demonstrating the effect of diuresis produced by infusion of 0.5% saline and an-

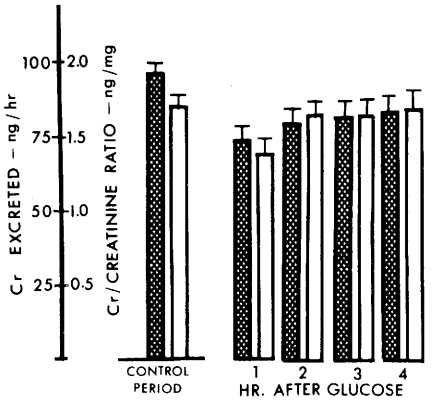


FIG. 1. Effect of an oral glucose load (75 g) on the hourly excretion of urinary Cr (hatched bars) and the ratio of Cr to creatinine excretion (open bars). Bars and vertical lines represent mean ± SEM for five normal subjects.

tidiuresis by vasopressin infusion. For six dogs, before onset of diuresis, the plasma clearance of ultrafilterable Cr and Cu was 0.21 ± 0.26 and 0.43 ± 0.14 SEM ml/min, respectively. With diuresis, Cr and Cu excretion increased in proportion to the rate of urine flow. Vasopressin infusion decreased urine flow and element excretion. At maximal diuresis, excretion was 150% greater than at the minimal urine flow rates produced by vasopressin. Calculated as the percentage of the filtered load, both Cr and Cu excretion rates were highly related to urine flow rate ($r$ = 0.84 and 0.86, respectively—Fig. 3). Vasopressin did not exert an intrinsic effect, distinct from antidiuretic activity, on Cr or Cu excretion. As shown in Fig. 3, the correlation between element excretion and urine flow rate was independent of the mode of varying the rate of flow. A similar high correlation was obtained also for the plasma clearance of ultrafilterable Cr and Cu and urine flow rate.

The plasma Cr and Cu concentrations (2.00 ± 0.02 and 3.00 ± 1 SEM ng/ml) did not change significantly during the experiment despite diuresis and increased element excretions. The "free" or ultrafilterable Cr and Cu also did not change significantly, and averaged 41.3 and 20.6% of the total plasma concentration, respectively.

From Fig. 3 it is apparent that, of the glomerular filtered load, less than 3.5% of Cr was excreted, while Cu excretion approximated 1% at maximum diuresis. For the dog, reabsorption of the elements was therefore greater than 96% with diuresis and 98% with normal urine flow.

*Discussion.* The finding that the renal excretion of Cr of normal fasting subjects is decreased after an oral glucose load is consistent with previous work (3, 4) which showed that an oral or iv glucose load, or insulin administration, produced a prompt and sustained fall in plasma Cr concentration. In contrast, plasma Cu levels were not changed (3, 4), and in the present study Cu excretion was also unchanged. Normally, total plasma concentrations of Cr and Cu of fasting subjects are maintained within narrow ranges: 4–7 ng/ml and 0.9–1.3 μg/ml, respectively (3, 4, 10, 14, 15). Plasma ultrafilterable or "free" Cr and Cu are in equilibrium with protein-bound elements, and for Cr are 35% of the total plasma concentration (unpublished observations) and for Cu, 7–10%. There is evidence that the "free" elements are complexed with amino acids, small peptides, nicotinic acid, and possibly other small-molecular-weight ligands (2, 16, 21). These ligands may represent a readily diffusible form, which exchanges with tissues and are filtered by the kidney. In view of the postulated role of Cr in enhancing insulin-stimulated assimilation of glucose (2, 3), the fall in plasma Cr and decreased Cr excretion observed

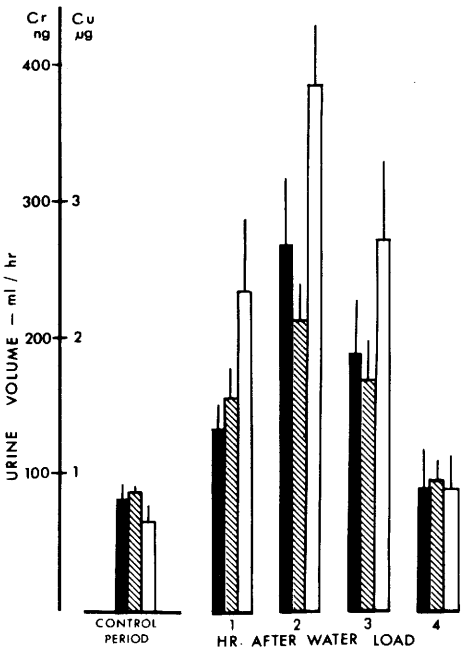


FIG 2. Effect of diuresis (open bars) produced by an oral water load (1 liter) on the hourly excretion of urinary Cr (solid bars) and Cu (shaded bars). Bars and vertical lines represent mean ± SEM for five normal subjects.

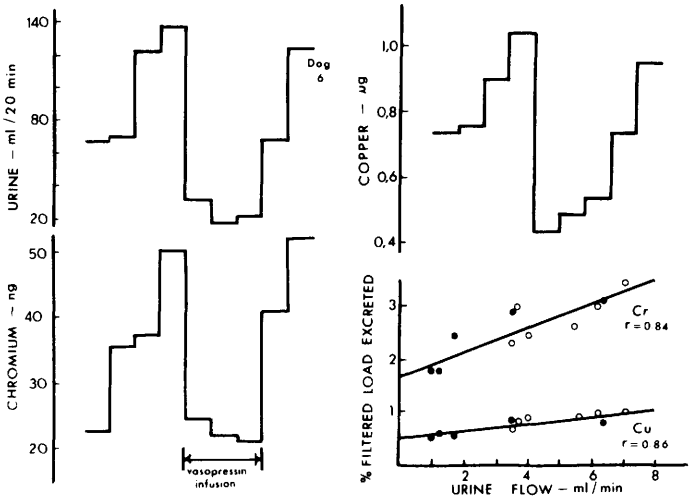


FIG. 3. Results of a typical dog experiment demonstrating the effect of urine flow rate on the rate of excretion of Cr and Cu. Diuresis was produced by intravenous hypotonic saline infusion and antidiuresis by vasopressin infusion. Lower right: Correlation between rate of urine flow and Cr and Cu excreted in urine, expressed as the fraction of plasma ultrafilterable element filtered at the glomerulus. Each point represents a urine collection period; solid points indicate periods during and immediately after vasopressin infusion.

after a glucose load can be interpreted as a movement of diffusible Cr from plasma to sites of action of insulin in peripheral tissues. The decreased renal excretion of Cr may reflect a decreased glomerular filtered load as a result of lowered plasma Cr concentration.

The results of this study show that during the fasting state the Cr and Cu filtered by the kidney are almost completely reabsorbed. From the experiments in man, an approximation of the fraction of glomerular filtered Cr and Cu that is excreted in the urine can be obtained from a comparison of the renal clearances of the elements to creatinine clearance. When calculated from the rates of excretion of the elements and of creatinine (Tables I and II) and the normal fasting plasma ultrafilterable levels noted above, plasma clearance values for Cr and Cu averaged 0.73 and 0.21 ml/min, respectively. The Cu clearance accords with previously reported values (17, 18). Comparison of these values with creatinine clearance provides average values for the excreted fraction of Cr and Cu of 0.66 and 0.17%, respectively, indicating that the normal reabsorption for both metals is greater than 99%. This conclusion is supported by direct observation in dogs in which the fraction of filtered Cr and Cu excreted in the urine at normal flow rates averaged 1.4 and 0.5%, respectively (Fig. 3). In both man and dog experiments water diuresis markedly increased the excretion of Cr and Cu (Table II, Figs. 2 and 3), and also increased the percent of filtered load excreted, but the percent of the filtered load reabsorbed did not decrease appreciably and at high urine flow rates was greater than 97% (Fig. 3). The correlation noted between excretion and urine flow, as influenced by water diuresis or vasopressin, may be accounted for by tubular permeability changes or increased tubular flow rates with urine dilution.

The high reabsorption by the kidney, both in the fasting state and during water diuresis, suggests that active reabsorption mechanisms may exist in the renal tubule for these trace elements. During marked diuresis in both man and dog the concentrations of Cr and Cu are lower in urine than normally observed in plasma ultrafiltrates (Fig. 2), which also suggests an active transport. These observations contrast with the kidney handling of a freely diffusible solute such as urea, which has a reabsorption ranging from 70% of the filtered load at low urine flows to 15% with water diuresis (22). Both Cr and Cu are ultrafilterable in urine (8, 15); however, it is not known if they are reabsorbed as free ions or ligand-bound complexes as they may exist in plasma. The very stable chelates, EDTA–Cr and penicillamine–Cu, are excreted without significant reabsorption (17, 19), but physiological plasma complexes, *e.g.*, with amino acids, could possibly be transported by shared transport systems.

The supposition of active transport mechanisms for Cr and Cu reabsorption also implies maximal reabsorption rates (Tm). In Wilson's disease urine Cu is often greatly elevated (20), and urine Cr is markedly increased in monkeys maintained on high dietary intake of the metal, resulting in a twofold increase of plasma Cr (unpublished data). The excretion rates of Cr and Cu of normal fasting subjects show both individual and daily variations (Tables I and II), possibly in response to dietary intake and requirement (1, 2, 10, 15). If the normal filtered load for these trace elements is close to the reabsorptive Tm, then a small increment in plasma concentration, and hence filtered load, would result in a significant increase in their renal excretion. Our findings suggest the kidney may play a role in the conservation of these essential trace elements. While intestinal absorption, biliary excretion, storage, and other mechanisms may be principal factors maintaining homeostatic balance of Cr and Cu (1, 2, 13, 14), kidney mechanisms for reabsorption of these metals may be important during periods of fasting, increased requirements, *e.g.*, pregnancy or dietary deficiency states. Furthermore, a low Tm for these metals may provide a protective mechanism for excessive absorption.

*Summary*. The ingestion by normal adults of a standard oral glucose load (75 g) resulted in a significant decrease of the fasting rate of urinary excretion of the trace metal chromium. This effect was not accompanied by any significant parallel change in GFR. Copper excretion rate was not significantly changed by the glucose load. In contrast with the effect of a glucose load, diuresis, induced by a water load, increased the excretion rate of both chromium and copper by more than 100%. The increases in metal excretion with diuresis were not attributable to increased GFR.

Observations on the renal excretion of chromium and copper in the dog showed that tubular reabsorption of chromium and copper was greater than 98% in normal fasting conditions. In dogs receiving intravenous infusions of hypotonic saline and of vasopressin, a marked correlation was obtained between urine flow rate, the chromium and copper excretion rates, and also the fraction of the filtered loads of the metals excreted. However, tubular reabsorption of chromium and copper was greater than 97% even at maximal urine flow rates. These observations suggest that the kidney of both man and dog may possess an active tubular reabsorptive capacity for chromium and copper.

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