Hyperzincuria in IDDM Women

Relationship to Measures of Glycemic Control, Renal Function, and Tissue Catabolism

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Eighteen women with insulin-dependent diabetes mellitus (IDDM) and 15 nondiabetic women participated in a study of the relationship of zincuria to measures of glycemic control, renal function, and tissue catabolism. In the IDDM women, mean ± SE glycosylated hemoglobin was 9.8 ± 0.5%, and fasting plasma glucose was 189 ± 19 mg/dl; duration of diabetes averaged 15 yr. In comparison with control women, the IDDM women excreted four times as much zinc in the urine. However, the total plasma zinc concentration was significantly higher in the IDDM than in the control women (14.7 vs. 13.4 μM). The increased urinary zinc loss in the IDDM women was not related to urine volume, urinary glucose excretion, fasting plasma glucose concentration, percent glycosylated hemoglobin, or an increased glomerular filtration rate. Total urinary protein losses were four times higher in the IDDM women than in the control women, and these urinary protein losses correlated with the urinary zinc losses ($P < .007$). There was no relationship between urinary zinc and the excretion of any of the amino acids, urea, or ammonia. The results of this study show that hyperzincuria in diabetes is not associated with lower plasma zinc levels. An increased zinc absorption, decreased intestinal zinc excretion, or increased tissue catabolism may support higher plasma zinc levels.
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Hyperzincuria is consistently found in people with diabetes mellitus (1–5) and in animal models of the disease (6). Factors that govern the renal zinc losses in diabetic patients have not been elucidated, nor is it clear that this excessive zinc excretion affects their overall zinc status. Serum or plasma zinc levels reportedly have been lower than (3,7), higher than (4,8), or the same as (1,5,9) in nondiabetic patients. Insulin treatment has reduced zinc excretion in diabetic humans (1) and animals (6,10), but the total zinc losses were still above normal levels.

Elevated urinary protein is a frequent observation in diabetes. The cause of diabetic proteinuria is not known, but it may be associated with alterations in the glomerular basement membrane (11,12) and an increased glomerular filtration rate (GFR; 13–15). The protein losses often include significant quantities of albumin (16–18), which may influence zinc losses. Approximately 60–70% of the total plasma zinc is associated with albumin; the remaining zinc is tightly bound to α_2-macroglobulin (19). Zinc loosely bound to albumin comprises ~98% of the exchangeable serum zinc pool. The remaining 2% is amino acid bound, with cysteine and histidine as the major amino acid–zinc ligands (20). Infusion of an amino acid mixture or of either cysteine or histidine in dogs caused a significant increase in urinary zinc excretion, presumably due to an increase in the plasma amino acid–bound zinc, which is thought to be the ultrafilterable zinc fraction (21,22).

Tissue catabolism is a potential contributor to circulating zinc. The cortisol secretion rate is elevated during metabolic stress in diabetic individuals (23,24). Because ~60% of total body zinc is in muscle (25), cortisol-induced muscle catabolism may release zinc that is subsequently excreted in the urine. A direct relationship between plasma cortisol and urinary zinc has been reported (26). Demineralization of bone could also re-

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lease zinc into the circulation, because bone contains ~30% of total tissue zinc (25). Reduced bone mass is a frequent observation in insulin-dependent diabetes mellitus (IDDM; 27–30).

Based on these findings, the overall goal of our study was to identify factors related to urinary zinc losses in women with IDDM. Our specific objectives were to *1*) assess the effect of hyperzincuria in IDDM women on zinc status, *2*) determine if hyperzincuria in these women was related to glycemic control, and *3*) correlate urinary zinc losses with measures of renal function and tissue catabolism.

MATERIALS AND METHODS

Eighteen IDDM and 15 nondiabetic women agreed to participate in this cross-sectional study. The protocol was approved by the Committee for Protection of Human Subjects at the University of California at Berkeley and Children's Hospital of San Francisco, California. Each subject consented to keep a written food intake record for 3 consecutive days while consuming her normal diet, provide a 24-h urine collection on the 3rd day, and to come in to have fasting blood drawn on the morning of the 4th day. The women were instructed to abstain from taking vitamin or mineral supplements for at least 2 wk before their evaluation. Study periods were scheduled so that none of the women were menstruating at the time of urine collection.

Specimen collection and preparation. The women were each given a tared plastic container and a plastic funnel for urine collection. Both items had been washed in Radiacwash (Atomic Products, Center Moriches, NY), a cleansing solution and metal chelator, and rinsed with low-mineral content, triply deionized water (Aqua Media, Menlo Park, CA) to remove all exogenous zinc. After determination of total urine volume, aliquots were frozen in polyethylene bottles until analyzed. Baker reagent-grade concentrated nitric acid was added (1% vol/vol) to the urine aliquot for zinc analysis to keep the mineral solubilized. A fasting blood sample was collected by venipuncture into zinc-free Vacutainer tubes (Becton Dickinson, Rutherford, NJ) fitted with 21-gauge stainless-steel needles and plastic adapters. Within 1 h after collection, plasma and serum were separated from whole blood by centrifugation for ~20 min at 2500 rpm. Aliquots were then frozen (−20 or −70°C) until time of analysis.

Analytical procedures. Plasma samples were analyzed for total, albumin-bound, and α_2-macroglobulin–bound zinc with the method of Giroux (31). The albumin- and α_2-macroglobulin–bound zinc were separated with a 20% polyethylene glycol reagent. After dilution with triply deionized water, the albumin-bound zinc concentration (supernatant) and total zinc (from 2nd plasma sample) were determined by flame atomic absorption spectrophotometry (AAS; Perkin-Elmer model 560, Mountain View, CA). The α_2-macroglobulin–bound zinc was calculated as the difference between total zinc and albumin-bound zinc. Urinary zinc was also determined by flame AAS after a fivefold dilution with triply deionized water. Aqueous zinc standards were prepared from a stock standard solution (zinc reference standard, 1000 ppm, American Scientific, McGaw Park, IL). A National Bureau of Standards wheat flour standard was analyzed as a quality control. The obtained value was 10.0 μg/g in all samples compared to a certified value of 10.6 μg/g.

Diabetic control was assessed by measurement of glycosylated hemoglobin, fasting plasma glucose, and urinary glucose. Glycosylated hemoglobin was measured with a colorimetric procedure (courtesy of S. Deering, Clinical Laboratory, Children's Hospital of San Francisco, CA). Plasma glucose was determined by a glucose oxidase/peroxidase colorimetric assay (kit no. 510, Sigma, St. Louis, MO). Urinary glucose was assayed by an ultraviolet procedure utilizing hexokinase and glucose-6-phosphate dehydrogenase (kit no. 16-UV, Sigma).

GFR was approximated from creatinine clearance with the calculation

$$(UF \cdot UC/SC) \cdot 1.73/SA$$

where UF is urine flow in ml/s, UC is urinary creatinine in μM, SC is serum creatinine in μM, and SA is surface area in square meters. The factor 1.73, the external surface area of an average person, corrects for differing kidney masses. Urinary creatinine was measured by an automated alkaline picrate method (Technicon Autoanalyzer method N-11a, Chauncey, NY). Serum creatinine was analyzed colorimetrically (procedure no. 555, Sigma).

Total urinary protein was determined with a dye-binding procedure (Bio-Rad protein assay, Richmond, CA). Total serum protein was analyzed by the Lowry method (32). Serum albumin was analyzed utilizing a bromcresol green dye-binding technique (procedure no. 630, Sigma). Urinary albumin and serum α_2-macroglobulin were determined by radial immunodiffusion (Behring Diagnostic, La Jolla, CA).

Plasma and urinary amino acids, urea, and ammonia were analyzed at the Bruce Lyon Memorial Research Center at Children's Hospital (Oakland, CA) with a Beckman 6300 high-performance liquid-chromatography system with ninhydrin detection. Urinary cortisol was determined by radioimmunoassay (no-spin cortisol radioimmunoassay kit, Cambridge Medical Diagnostics, Billerica, MA).

Nutrient intakes were calculated and analyzed by a computer program with the Minilist data bank (33) and a cross-reference index (34) for foods not included in the Minilist.

Statistics. Statistical analyses were done with a Statistical Package for Social Sciences program. The data appeared to have a nonnormal distribution, so differences in measured parameters between the diabetic and nondiabetic groups were analyzed nonparametrically with

the Mann-Whitney test. Pairwise correlations reported are Spearmann's nonparametric correlation coefficients. On tests of significance, $P < .05$ was taken to be the critical value.

RESULTS

Subjects. IDDM women without nephropathy were recruited from a diabetes clinic at Children's Hospital in San Francisco and from the Greater Bay Area. Their mean age was 30 ± 6 yr with mean ± SE weight and height of 62 ± 7 kg and 164 ± 7 cm, respectively. Healthy women without a history of diabetes were recruited from the University of California at Berkeley community for a control group. Their characteristics were similar to those of the IDDM women; average age was 27 ± 5 yr, mean weight was 57 ± 4 kg, and mean height was 165 ± 7 cm. The duration of diabetes ranged from 4 to 35 yr, with a mean of 15 yr. Diabetes was managed by conventional therapy (insulin injections) in all subjects except one who used a continuous subcutaneous insulin-infusion pump. Insulin doses averaged 40 ± 17 U/day with varying combinations of regular, intermediate-acting, and long-acting insulin preparations. The maximum amount of zinc injected with insulin was 57 μg/day.

Calculated dietary intakes were similar for both groups for all nutrients except total carbohydrates, dietary fiber, sucrose, and copper. Intakes of these nutrients were significantly lower in the IDDM women. The energy intake of both groups was less than the recommended 2000 kcal/day for this age group (35). The IDDM group reported an intake of 1565 ± 249 kcal/day; the control group reported an intake of 1716 ± 291 kcal/day. The zinc intake of both groups averaged 8.5 mg/day or 57% of the 15 mg recommended allowance (35). The diets selected provided ~5 mg zinc/1000 kcal. Copper intake was lower in the IDDM group compared with control women (1.1 vs. 1.5 mg/day). Both values are below the recommended intake of 2–3 mg/day (35). The zinc-to-copper intake ratios for both groups fell within the suggested range of 5–7.5.

Glycemic control. Assessment of glucose homeostasis was measured by glycosylated hemoglobin, fasting plasma glucose, urinary glucose output, and 24-h urine volume (Table 1). The glycosylated hemoglobin for 2 IDDM women was within the normal range, i.e., 4.5–7.6% of total hemoglobin. The values for the remaining IDDM women ranged from 8.0 to 14.5%. The fasting plasma glucose levels were more than twice as high as the levels for the control women (189 vs. 86 mg/dl; conversion factor for glucose from mg/dl to mM is 0.05551). Insulin administration was necessary in 10 of the 18 IDDM women before blood collections. There was no difference in mean plasma glucose values in women who had or had not taken insulin (185 vs. 194 mg/dl, respectively). Urinary glucose losses were >300 times those of the control women (58 vs. 0.2 g/24 h or 321.9 vs. 1.1 mmol/24 h). The 2 IDDM women with normal glycosylated hemoglobin values had the lowest urinary glucose losses (0.18 and 0.29 g/24 h or 1.0 and 1.6 mmol/24 h) and normal zinc losses (5.1 and 9.1 μmol/24 h). Their protein excretion was also normal (18 and 37 mg/24 h). Total urine volume in a 24-h period did not differ between the IDDM and control women.

TABLE 1
Parameters of diabetic control

	IDDM women (n = 18)	Control women (n = 15)	P
Glycosylated hemoglobin (% of total)	9.8 ± 0.5	6.2 ± 0.1	<.0001
Fasting plasma glucose (mg/dl)	189 ± 19	86 ± 1	<.0001
Urinary glucose (g/24 h)	57.66 ± 14.73	0.16 ± 0.01	<.0001
Urine volume (ml/24 h)	1842 ± 118	1594 ± 243	NS

Values are means ± SE. IDDM, insulin-dependent diabetic.

Urinary zinc. The IDDM women excreted significantly more zinc over a 24-h period than did the control women ($P < .00001$; Table 2). Total zinc output was four times greater in the IDDM women than in the control women (14.4 vs. 3.7 μmol/24 h). Because total urine volume did not differ significantly between the two groups, the increase in zinc excretion was due primarily to an increase in the concentration of zinc in the urine. The urinary zinc concentration of the IDDM women was 2.7 times that of the controls (8.3 vs. 3.1 μM). Urinary zinc losses were not correlated with urinary glucose excretion, fasting plasma glucose concentration, or percent glycosylated hemoglobin.

Plasma total zinc and zinc-protein fractions. Mean total plasma zinc was significantly higher in the IDDM women than in the control women by ~9% (14.7 vs. 13.4 μM; Table 2). Normal values in our laboratory range from 12.2 to 16.8 μM. The higher total plasma zinc levels in the IDDM women were associated with significantly higher α_2-macroglobulin–bound zinc levels; the albumin-bound zinc levels were essentially the same in both groups.

These changes in zinc-protein fractions seem to be unrelated to the concentrations of the serum proteins per se. Total serum α_2-macroglobulin levels did not differ between the two groups (Table 2). Serum albumin concentrations were significantly lower in the IDDM women than in the control women, although all values in both groups were within the normal range of 35–50 g/L. Because the absolute amount of zinc associated with albumin did not differ between the two groups, this reduction in total circulating albumin did not influence the amount of albumin-bound zinc. If albumin can bind

TABLE 2
Urinary zinc, plasma zinc fractions, and zinc-binding proteins

	IDDM women (n = 18)	Control women (n = 15)	P
Total urinary zinc (μmol/24 h)	14.4 ± 1.2	3.7 ± 0.3	<.00001
Urinary zinc concentration (μM)	8.3 ± 0.9	3.1 ± 0.5	<.00001
Total plasma zinc (μM)	14.7 ± 0.4	13.4 ± 0.6	<.05
α_2-Macroglobulin–bound zinc (μM)	6.4 ± 0.3	5.2 ± 0.3	<.05
Percent of total	43 ± 1	39 ± 1	<.05
Albumin-bound zinc (μM)	8.4 ± 0.2	8.2 ± 0.3	NS
Percent of total zinc	57 ± 1	61 ± 1	NS
Serum albumin (g/L)	44.2 ± 0.7	46.1 ± 0.4	<.02
Serum α_2-macroglobulin (g/L)	3.6 ± 0.2	3.2 ± 0.2	NS

Values are means ± SE. IDDM, insulin-dependent diabetic.

10–11 μmol zinc · g^{-1} albumin · L^{-1} serum (36), only 3% of the albumin-zinc binding sites will normally be occupied by zinc. This small reduction in serum albumin will, therefore, have an insignificant effect on the capacity of zinc binding.

Results of the plasma amino acid concentrations demonstrated that the IDDM women had significantly higher cysteine concentrations (60 vs. 38 μM; $P < .005$) and significantly lower glutamine concentrations (434 vs. 480 μM; $P < .05$) than the control women. Although not significantly different, the concentrations of the branched-chain amino acids—leucine, isoleucine, and valine—tended to be higher in the IDDM women than in the control women (460 vs. 406 μM). Higher circulating levels of the branched-chain amino acids in the fasting IDDM women may reflect increased breakdown and mobilization of muscle protein.

GFR, urinary protein, and amino acids. GFR, estimated by creatinine clearance, was significantly higher in the IDDM women than in the control women (2.08 vs. 1.37 ml/s; $P < .01$). This increased GFR was associated with a significantly lower serum creatinine concentration in the IDDM women (70 vs. 90 μM; $P < .005$) and a tendency toward a higher urinary creatinine excretion (11.5 vs. 9.7 mmol/24 h; $P = .078$). Although both the GFR and urinary zinc excretion were significantly higher in the IDDM women, the correlation of the two measurements did not reach significance.

Total urinary protein losses were four times higher in the IDDM women than in the control women (0.16 vs. 0.04 g/24 h; $P < .002$). Urinary albumin levels, measured by radialimmunodiffusion, were undetectable in all of the control women and in 14 of the 18 IDDM women. The remaining 4 IDDM women excreted 4–25 mg albumin/24 h. The IDDM women also excreted significantly more urea and ammonia than the control women (Table 3).

Total urinary amino acid losses in a 24-h period were 1.6 times higher in the IDDM women than in the control women (11.05 vs. 6.9 mmol/24 h). The excretion levels of the majority of the individual amino acids measured (including amino acid–zinc ligands cysteine and histidine) were significantly higher in the IDDM women than in the control women (Table 3). Urinary zinc losses correlated significantly with urinary protein losses in the IDDM women ($r = .61$, $P < .01$) but were unrelated to the levels of urea, ammonia, histidine, cysteine, or any of the other amino acids in the urine.

Muscle and bone catabolism. Muscle catabolism was assessed indirectly from measurements of urinary 3-methylhistidine excretion (37) and 24-h urinary cortisol excretion. Urinary cortisol excretion reflects the free or unbound circulating cortisol levels that are involved in the regulation of cortisol secretion by the adrenal cortex (38). Urinary cortisol levels were significantly higher in the IDDM women than in the control women (410 ± 160 vs. 310 ± 100 nmol/day; $P < .01$), but total urinary 3-methylhistidine excretion did not differ between the two groups (Table 3). There was a weak relationship between urinary zinc and urinary cortisol ($r = .422$, $P < .08$). There was no relationship between urinary zinc levels and 3-methylhistidine. Plasma zinc levels were not associated with either parameter of protein catabolism. Urinary hydroxyproline per millimeter creatinine excretion, a measure of bone breakdown (39), was greater in the IDDM women than in the control women (69 vs. 55 μmol hydroxyproline/mmol creatinine; $P = .015$). Neither plasma nor urinary zinc concentrations were related to hydroxyproline excretion.

TABLE 3
Urinary amino acids, ammonia, and urea (μmol/24 h)

	IDDM women (n = 18)	Control women (n = 15)	P
Phosphoserine	270.2 ± 26.3	193.0 ± 12.0	<.01
Taurine	946.1 ± 136.5	402.5 ± 86.5	<.002
Hydroxyproline	781.1 ± 63.0	522.0 ± 40.0	<.005
Threonine	278.9 ± 55.3	108.7 ± 17.5	<.002
Serine	625.4 ± 104.5	261.2 ± 32.1	<.0005
Asparagine	178.4 ± 47.9	42.2 ± 17.6	<.01
Glutamine	486.5 ± 84.2	283.3 ± 36.5	<.01
Glycine	2437.6 ± 527.8	1220.3 ± 160.1	NS
Alanine	498.8 ± 82.0	195.3 ± 23.4	.0005
Cysteine	173.0 ± 30.7	57.3 ± 14.4	.0005
Leucine	119.5 ± 11.3	59.3 ± 9.5	<.005
Tyrosine	140.7 ± 19.4	68.7 ± 12.1	<.005
Phenylalanine	112.6 ± 32.9	40.7 ± 23.9	<.05
Lysine	278.4 ± 48.4	158.8 ± 35.5	NS
L-Methylhistidine	1383.5 ± 282.7	1484.0 ± 390.9	NS
Histidine	1044.2 ± 174.8	559.9 ± 72.0	<.05
3-Methylhistidine	342.1 ± 25.6	278.1 ± 21.0	NS
Anserine	46.1 ± 33.7	147.0 ± 66.3	NS
Urinary urea	397 ± 37	258 ± 81	<.01
Urinary ammonia	61 ± 12	29 ± 4	<.01

Values are means ± SE. IDDM, insulin-dependent diabetic.

DISCUSSION

Our results of elevated urinary zinc excretion are consistent with previous reports of diabetic hyperzincuria (1–5). The increased losses of urinary zinc were not associated with a decline in plasma zinc or any known clinical signs of altered zinc status, e.g., dermatitis. Plasma zinc concentrations were in fact significantly higher in the diabetic group. These differences cannot be explained by dietary zinc intake because both experimental groups reported very similar zinc intakes.

There are several possible explanations for increased plasma zinc in the presence of hyperzincuria. Absorption of zinc may be enhanced in diabetes. Animal studies showed intestinal hyperplasia and increased absorption of hexoses, amino acids, zinc, and copper in streptozocin-induced diabetic rats (40–43). In contrast, a study of IDDM patients suggested that zinc was malabsorbed by these subjects, based on a reduced plasma zinc response to a large oral dose (44). An animal study found that net absorption of zinc was similar in diabetic and control rats, but the reverse mucosa-to-lumen flux was decreased in all intestinal segments of the diabetic rats (45). Another study showed no difference in zinc absorption between diabetic and control rats consuming equivalent amounts of zinc, but intestinal zinc excretion was reduced in the diabetic rats (46). These studies suggest that the decrease in net excretion of zinc from the intestinal tract may be a response by the diabetic rat to conserve zinc in compensation for hyperzincuria.

Increased tissue catabolism associated with overnight fasting may account for the higher plasma and urinary zinc levels in the IDDM women. In healthy women, prebreakfast plasma zinc concentrations were ~20% higher than the concentrations before a meal at 1800 the previous evening (47). The overnight cortisol response to hypoglycemia may have been exaggerated by diabetes. This increased tissue catabolism overnight and subsequent zinc release may be a factor in the increased fasting (or prebreakfast) plasma zinc concentrations observed in diabetes and in the increased urinary zinc losses.

Bone mineral content (BMC) declines ~10% within the first 5 yr of clinical diabetes and is associated with increased urinary excretion of calcium and phosphorus (30). Parameters of zinc metabolism have not been measured in relation to BMC; however, demineralization would release zinc as well as calcium and phosphorus. Urinary excretion of hydroxyproline is positively correlated to bone resorption (48); however, a portion of the urinary hydroxyproline is also derived from nonbone collagen (39). Urinary hydroxyproline excretion was significantly increased in our IDDM women and may reflect increased bone resorption. In another study, hydroxyproline excretion in diabetic subjects did not differ from that in normal subjects (49).

In addition to higher total circulating plasma zinc, the distribution of zinc between the two major zinc-binding proteins in the plasma was altered in diabetes. Zinc bound to α_2-macroglobulin was increased in the IDDM women, whereas albumin-bound zinc levels were similar to those of the control group. Variations in total serum zinc concentrations in healthy subjects usually are accounted for by changes in the zinc associated with albumin, whereas α_2-macroglobulin–bound zinc remains constant (36). Elevated levels of α_2-macroglobulin have been observed in diabetic patients (7,50), but levels of α_2-macroglobulin–bound zinc were the same as in control subjects (7). In contrast, serum α_2-macroglobulin concentrations were not increased in our diabetic population. Zinc is tightly bound to α_2-macroglobulin and, therefore, is not readily exchangeable, as opposed to albumin, which carries it loosely. An increase in the binding of zinc to α_2-macroglobulin may reduce the amount of ultrafilterable zinc and may thus be a zinc-conservation mechanism.

Hyperzincuria was not related to any measurements of glycemic control in our population. Previous studies have found a positive correlation between glucosuria and zinc excretion (4,5). One reported glycosylated hemoglobin values ranging from 7.2 to 25% (4); the range was lower and narrower in our study (from 5 to 14.5%). This suggests poorer glucose homeostasis may be more strongly associated with alterations in zinc excretion; however, the relationship between glucose metabolism and urinary zinc excretion remains unclear.

Measurements of renal function were significantly altered in the IDDM women. GFR was 34% higher in IDDM women than in control women, but it was not related to urinary zinc output. Elevated GFR and filtration fraction have been previously observed in diabetes (13,14). We did not measure the ultrafilterable fraction of plasma zinc and were therefore unable to measure zinc reabsorption or fractional excretion. Significantly elevated levels of urinary protein, amino acids, urea, and ammonia were also observed in the IDDM women. Diabetic proteinuria is a common clinical observation, although the precise mechanism is not well understood. Increased transglomerular loss due to structural alterations of the basement membrane and reduced tubular reabsorption are possible explanations (14,17,18). Zinc excretion in non-insulin-dependent diabetic patients was increased when proteinuria was present, but contrary to our findings, no correlation was observed (44). Hyperzincuria has also been observed in patients with albuminuria, but no correlation was found (51). Our method for urinary albumin analysis was not sensitive enough to indicate whether a relationship existed between albumin and zinc excretion. Hyperzincuria and amino aciduria have been observed during intravenous alimentation (52,53). It was found that the excretion of free amino acids was not elevated; instead, sugar-amine compounds were the source of the elevated urinary amino acids. Zinc appeared to have a high affinity for chelation with the sugar-amine complexes and was excreted in this form. Increased concentrations of glycosylated serum

albumin (54,55) and glycosylated amino acids and peptides have been found in the urine of diabetic subjects (56). Based on these findings, it is possible that glycosylated amino acids or peptides chelated with zinc contribute to the elevated urinary zinc in diabetes.

In summary, this study showed that diabetic hyperzincuria was not associated with a decline in plasma zinc nor with glycemic control and that IDDM women had higher plasma zinc concentrations than nondiabetic control women. Possible factors that may account for the higher plasma zinc levels include increased zinc absorption, decreased intestinal zinc excretion, or increased tissue catabolism. Although elevated GFR was observed in IDDM women, it was not correlated with increased urinary zinc losses. A significant correlation was found between urinary zinc and urinary protein concentration.

REFERENCES

1. Tarui S: Studies on zinc metabolism. III. Effect of the diabetic state on zinc metabolism: a clinical aspect. *Endocrinol Jpn* 10:9–15, 1963
2. Pidduck HG, Wren PJJ, Evans, DAP: Hyperzincuria of diabetes mellitus and possible genetical implications of this observation. *Diabetes* 19:240–47, 1970
3. Kumar S, Rao KSJ: Blood and urinary zinc levels in diabetes mellitus. *Nutr Metab* 17:231–35, 1974
4. Canfield WK, Hambidge KM, Johnson LK: Zinc nutriture in type I diabetes mellitus: relationship to growth measures and metabolic control. *J Pediatr Gastroenterol Nutr* 3:577–84, 1984
5. McNair P, Kiilerich S, Christiansen C, Christiansen MS, Madsbad S, Transbol I: Hyperzincuria in insulin treated diabetes mellitus—its relation to glucose homeostasis and insulin administration. *Clin Chim Acta* 112:343–48, 1981
6. Lau AL, Failla ML: Urinary excretion of zinc, copper and iron in the streptozotocin-diabetic rat. *J Nutr* 114:224–33, 1984
7. Kiilerich S, Christiansen C: Distribution of serum zinc between albumin and α_2-macroglobulin in patients with different zinc metabolic disorders. *Clin Chim Acta* 154:1–6, 1986
8. Mateo MCM, Bustamante JB, Cantalapiedra MA: Serum zinc, copper and insulin in diabetes mellitus. *Biomedicine* 29:56–58, 1978
9. Chooi MK, Todd JK, Boyd ND: Influence of age and sex on plasma zinc levels in normal and diabetic individuals. *Nutr Metab* 20:135–42, 1976
10. Vander AJ, Victery W, Germain C, Holloway D: Insulin is a physiological inhibitor of urinary zinc excretion in anesthetized dogs. *Am J Physiol* 244:E535–40, 1983
11. Kimmelstiel P, Kim OJ, Beres J: Studies on renal biopsy specimens with the aid of the electron microscope. I. Glomeruli in diabetes. *Am J Clin Pathol* 38:270–79, 1962
12. Spiro RG: Search for a biochemical basis of diabetic microangiopathy. *Diabetologia* 12:1–14, 1976
13. Ditzel J, Junker K: Abnormal glomerular filtration rate, renal plasma flow, and renal protein excretion in recent and short-term diabetics. *Br Med J* 2:13–19, 1972
14. Mogensen CE: Kidney function and glomerular permeability to macromolecules in early juvenile diabetics. *Scand J Clin Lab Invest* 28:79–90, 1971
15. Mogensen CE: Glomerular filtration rate and renal plasma flow in long-term juvenile diabetes without proteinuria. *Br Med J* 4:257–59, 1972
16. Steno Study Group: Effect of 6 months of strict metabolic control on eye and kidney function in insulin dependent diabetics with background retinopathy. *Lancet* 1:121–24, 1982
17. Viberti GC, Pickup JC, Jarrett RJ, Keen H: Effect of control of blood glucose on urinary excretion of albumin and beta-2 microglobulin in insulin dependent diabetes. *N Engl J Med* 300:638–41, 1979
18. Parving HH, Noer I, Deckert T, Ervin PE, Nielsen SL, Lungsoe J, Mogensen CE, Rorth M, Svendsen H, Trap-Jensen J, Lassen NA: The effect of metabolic regulation on microvascular permeability to small and large molecules in short-term juvenile diabetics. *Diabetologia* 12:161–66, 1976
19. Parisi AF, Vallee BL: Isolation of a zinc alpha-2 macroglobulin from human serum. *Biochemistry* 9:2421–26, 1970
20. Giroux EL, Henkin RI: Competition for zinc among serum albumin and amino acids. *Biochim Biophys Acta* 273:64–72, 1972
21. Levine AS, McClain CJ, Silvis SE: Urinary zinc excretion rises with infusion of amino acids and/or glucose (Abstract). *Clin Res* 28:765A, 1980
22. Yunice AA, King RW, Kraikitpanitch S, Haygood CC, Lindeman RD: Urinary zinc excretion following infusions of zinc sulfate, cysteine, histidine, or glycine. *Am J Physiol* 235:F40–45, 1978
23. Garces LY, Kenny FM, Drash A, Preeyasombat C: Cortisol secretion in acidotic and nonacidotic juvenile diabetes mellitus. *J Pediatr* 74:517–22, 1969
24. Sperling MA, Bacon G, Kenny FM, Drash AL: Cortisol secretion in acidotic and nonacidotic diabetes mellitus. *Am J Dis Child* 124:690–92, 1972
25. Henkin R: Zinc in humans. In *Zinc*. Baltimore, MD, University Park, 1979, p. 123–72 (Natl. Res. Counc., Comm. on Med. and Biol. Effects of Environmental Pollutants, Subcomm. on Zinc)
26. Henkin R: On the role of adrenocorticosteroids in the control of zinc and copper metabolism. In *Trace Element Metabolism in Animals—2*. Baltimore, MD, University Park, 1974, p. 647–51
27. Levin ME, Vincenza CB, Avioli LV: Effects of diabetes mellitus on bone mass in juvenile and adult-onset diabetes. *N Engl J Med* 294:241–45, 1976
28. McNair P, Madsbad S, Christiansen C, Faber OK, Transbol I, Binder C: Osteopenia in insulin-treated diabetes mellitus: its relation to age at onset, sex, and duration of disease. *Diabetologia* 15:87–90, 1978
29. Hui SL, Epstein S, Johnson CC Jr.: A prospective study of bone mass in patients with type I diabetes. *J Clin Endocrinol Metab* 60:74–80, 1985
30. McNair P, Madsbad S, Christiansen MS, Christiansen C, Faber OK, Binder C, Transbol I: Bone mineral loss in insulin-treated diabetes mellitus: studies on pathogenesis. *Acta Endocrinol* 90:463–72, 1979
31. Giroux EL: Determination of zinc distribution between albumin and alpha-2 macroglobulin in human serum. *Biochem Med* 12:258–66, 1975
32. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ: Protein

measurement with the Folin phenol reagent. *J Biol Chem* 193:265–75, 1951
33. Pennington JA: *Dietary Nutrient Guide*. Westport, CT, AVI, 1976
34. Murphy S: *Dietary Correlates of Trace Element Status for Young Women in Nhanes II*. PhD thesis. Berkeley, Univ. of California, 1984
35. National Academy of Sciences, National Research Council, Food and Nutrition Board: *Recommended Dietary Allowances*. 9th ed. Washington, DC, Natl. Acad. Sci., 1979, p. 144–47.
36. Foote JW, Delves HT: Albumin bound and alpha-2 macroglobulin bound zinc concentrations in the sera of healthy adults. *J Clin Pathol* 37:1050–54, 1984
37. Young VR, Munro HN: 3-Methylhistidine and muscle protein turnover: an overview (Abstract). *Fed Proc* 37:2291, 1978
38. Streeten DHP, Anderson GH, Dalakos TG, Seeley D, Mallov JS, Eusebio R, Sunderlin FS, Badawy SZ, King RB: Normal and abnormal function of the hypothalamic-pituitary-adrenocortical system in man. *Endocr Rev* 5:371–94, 1984
39. Laitinin O: Clinical applications of urinary hydroxyproline determination. *Acta Med Scand Suppl* 577:1–57, 1974
40. Scheal HP, Wilson HD: Effects of diabetes on intestinal growth and hexose transport in the rat. *Am J Physiol* 220:1739–45, 1971
41. Lal D, Schedl HP: Intestinal adaptation in diabetes: amino acid absorption. *Am J Physiol* 227:827–31, 1974
42. Gourley GR, Korsmo HA, Olsen WA: Intestinal mucosa in diabetic rats: studies of microvillus membrane composition and microviscosity. *Metabolism* 30:1053–58, 1983
43. Craft NE, Failla ML: Zinc, iron, and copper absorption in the streptozotocin-diabetic rat. *Am J Physiol* 244:E122–28, 1983
44. Kinlaw WB, Levine AS, Morley JE, Silvis SG, McClain CJ: Abnormal zinc metabolism in type II diabetes mellitus. *Am J Med* 75:273–77, 1983
45. Ghishan FK, Greene HL: Intestinal transport of zinc in the diabetic rat. *Life Sci* 32:1735–41, 1983
46. Johnson WT, Canfield WK: Intestinal absorption and excretion of zinc in streptozotocin-diabetic rats as affected by dietary zinc and protein. *J Nutr* 115:1217–27, 1985
47. English JL, Hambidge KM, King JC, Kern KL, Pritts JL, Stall CD: The effect of evening meals on pre-breakfast plasma zinc concentration (Abstract). *Fed Am Soc Exp Biol J* 2:A635, 1988
48. Charles P, Poser JW, Mosekilde L, Jensen FT: Estimation of bone turnover evaluated by ^{47}Ca-kinetics: efficiency of serum bone gamma-carboxy-glutamic acid-containing protein, serum alkaline phosphatase and urinary hydroxyproline excretion. *J Clin Invest* 76:2254–58, 1985
49. Benoit FL, Theil GB, Watten RH: Hydroxyproline excretion in endocrine disease. *Metabolism* 12:1072–82, 1963
50. Pozzilli P, Mario UD, Javicoli M, Chinca M, Orsini M: Alpha-2 macroglobulin: its variability in diabetes. *Horm Metab Res* 12:409–10, 1980
51. McCance RA, Widdowson EM: The absorption and excretion of zinc. *Biochem J* 36:692–96, 1942
52. Van Rij AM, McKenzie JM, Dunckley JV: Excessive urinary zinc losses and amino aciduria during intravenous alimentation. *Proc Univ Otago Med School* 53:77–78, 1975
53. Freeman JB, Stegink LD, Meyer PD, Fry LK, Denbesten L: Excessive urinary zinc losses during parenteral alimentation. *J Surg Res* 18:463–69, 1975
54. Dolhofer R, Wieland OH: Increased glycosylation of serum albumin in diabetes mellitus. *Diabetes* 29:417–22, 1980
55. Day JF, Thorpe SR, Baynes JW: Nonenzymatically glucosylated albumin. *J Biol Chem* 254:595–97, 1979
56. Brownlee M, Vlassara H, Cerami A: Measurement of glycosylated amino acids and peptides from urine of diabetic patients using affinity chromatography. *Diabetes* 29:1044–47, 1980