

Copper, Zinc, Manganese, and Magnesium Status and Complications of Diabetes Mellitus

Robert M. Walter, Jr., MD
Janet Y. Uriu-Hare, PhD
Katherine Lewis Olin, BS
Michelle H. Oster, MS
Bradley D. Anawalt, MD
James W. Critchfield, PhD
Carl L. Keen, PhD

Objective: To evaluate copper, zinc, manganese, magnesium, and other indices of peroxidative status in diabetic and nondiabetic human subjects. **Research Design and Methods:** Convenience sample of 57 insulin-dependent or non-insulin-dependent diabetic subjects recruited from the diabetes clinic of the University of California, Davis, Medical Center and 28 nondiabetic subjects recruited from the staffs of the Departments of Internal Medicine and Nutrition. Individuals conducting laboratory analyses were blind to subject group. A fasting blood sample was collected from all subjects and appropriately processed for future analyses. A 24-h urine collection was obtained in a subset of subjects. **Results:** Hyperzincuria and hypermagnesuria were evident in diabetic subjects compared with control subjects. There were no differences in plasma magnesium or whole-blood manganese between groups. Plasma copper was higher and plasma zinc was lower in diabetic than in control subjects. When data were viewed with respect to specific diabetes-associated complications, diabetic subjects with retinopathy, hypertension, or microvascular disease had higher plasma copper concentrations compared with both diabetic subjects without complications and with control subjects. There were no significant differences between control and diabetic subjects in erythrocyte copper-zinc superoxide dismutase activity or whole-blood glutathione peroxidase or glutathione reductase activities. Plasma peroxide concentrations were higher in diabetic than control subjects. **Conclusions:** Diabetes can alter copper, zinc, magnesium, and lipid peroxidation

status. Perturbations in mineral metabolism are more pronounced in diabetic populations with specific complications. It is not known whether differences in trace element status are a consequence of diabetes, or alternatively, whether they contribute to the expression of the disease. *Diabetes Care* 14:1050-56, 1991

Diabetes mellitus is a heterogeneous disease characterized by an absolute or relative deficiency of insulin and insulin resistance. Clinical manifestations include hyperglycemia, glycosuria, altered protein, fat, and carbohydrate metabolism, and chronic complications resulting from macro- and microvascular pathology. Accepted etiologic factors include genetics, viral infections, autoimmunity, and obesity, the latter of which clearly contributes to insulin resistance.

Alterations in the metabolism of several trace elements, including copper (1,2), zinc (3-7), manganese (8-11), and the macroelement magnesium (12), have been associated with impaired insulin release, insulin resistance, and glucose intolerance in experimental animals and humans. Although there have been numerous studies evaluating the mineral status of diabetic subjects, these studies yielded inconsistent results. For example, normal (13) and increased (14) serum and plasma levels of copper have been found in diabetic individuals. Hyperzincuria has been found in insulin-dependent (type I; 15) and non-insulin-dependent (type II; 16) diabetic subjects, but low (14) and high (17) concentrations of plasma zinc have been reported in diabetic compared with control subjects. Blood manganese levels in diabetic subjects have been reported to be high (18), low (19), or unchanged (20). Hypomagnesemia and hyper-

From the Departments of Internal Medicine and Nutrition, University of California, Davis, Davis, California.

Address correspondence and reprint requests to Robert M. Walter, Jr., MD, Department of Internal Medicine, University of California, Davis, 4301 X Street, Sacramento, CA 95817.

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magnesuria have been noted, particularly when the diabetes was poorly controlled in some (21,22) but not all (23,24) studies. Differences in the above results may reflect study populations that varied greatly with respect to diabetic control and/or the presence of micro- and macrovascular disease. It has been suggested that perturbations in mineral metabolism are more pronounced when metabolic control is poor or if vascular complications are present. Changes in peroxidative status may also be anticipated when trace element status is altered. Elevated levels of lipoperoxides have been reported in diabetic patients, and peroxidative damage has been proposed as a contributory factor in diabetic retinopathy (25) and vascular disease (26).

In this study, we evaluated the copper, zinc, manganese, and magnesium status in diabetic subjects, most of whom were selected because of poor glycemic control and/or significant retinopathy. Plasma lipid peroxide concentrations, whole-blood glutathione peroxidase and glutathione reductase activities, and erythrocyte copper-zinc superoxide dismutase (CuZnSOD) activities were also indices of peroxidative status.

RESEARCH DESIGN AND METHODS

Fifty-seven diabetic subjects were recruited from the diabetes clinic of the University of California, Davis, Medical Center. Fifty-four percent were women with a mean \pm SE age of 48 ± 2 yr. Twenty-eight nondiabetic subjects were recruited from the staffs of the Departments of Internal Medicine and Nutrition. This group was normotensive, nonmedicated, nonobese, free of apparent illness, and 54% female with a mean \pm SE age of 35 ± 2 yr. No subjects had taken vitamin or mineral supplements for at least 1 mo before sampling. All subjects were ambulatory and gave informed consent as approved by the Human Subjects Research Committee of the University of California, Davis. The duration of diabetes ranged from 1 mo to 51 yr. Fifty-four percent had type I diabetes. Of the type II diabetic subjects, 77% were treated with insulin and the remainder with sulfonylureas. Insulin dosage ranged from 18 to 110 U/day. Fifty percent of the diabetic subjects had hypertension (diastolic pressure >100 mmHg). Forty-six percent had macrovascular disease, including peripheral, coronary, and/or cerebrovascular disease. Seventy-four percent had retinopathy confirmed by ophthalmologic consultation. Fifty-one percent had nephropathy with persistent macroalbuminuria, which we defined as >250 mg/24 h. All serum creatinine levels were <0.22 mM, and hypoalbuminemia was not observed. Thirty-seven percent of diabetic subjects were obese when obesity was defined as weight $>120\%$ of ideal body weight. The diabetic subjects were not acutely ill, lacked major chronic unrelated disease, and had normal serum hepatic enzymes.

An overnight fasting blood sample was collected by venipuncture into heparinized tubes (Sarstedt, Prince-

ton, NJ) and centrifuged for 20 min (3000 rpm at 4°C). Whole-blood and plasma aliquots were refrigerated or frozen for later analyses. Urine was collected over a 24-h period in wide-mouthed plastic bottles and frozen. For mineral analysis, all materials used in the collection and storage of samples were cleaned with nitric acid, repeatedly washed with distilled deionized water, and stored under dust-free conditions.

Plasma copper and zinc were measured for all participants. Plasma magnesium, ceruloplasmin activity, lipid peroxide concentrations, erythrocyte SOD activity, whole-blood manganese concentration, glutathione reductase and glutathione peroxidase activities, 24-h urinary zinc, and magnesium and creatinine concentrations were measured in subsets of subjects. HbA_{1c} concentrations were determined by high-performance liquid chromatography as an index of diabetes control (27). Plasma and urinary glucose concentrations were determined by the glucose oxidase method (Beckman Glucose Analyzer II, Fullerton, CA). Albumin in urine was determined by the sulfosalicylic acid method.

Zinc, copper, and magnesium concentrations in plasma were measured by flame-atomic absorption spectrophotometry (AAS; IL551, Instrumentation Laboratories, Wilmington, MA). Urine was ashed with concentrated HNO₃, and zinc and magnesium were determined with AAS (28). Manganese in whole blood was measured by graphite-furnace atomic spectrophotometry (29). Plasma ceruloplasmin activity was measured by following the oxidation of *o*-dianisidine dihydrochloride as described by Schosinsky et al. (30). Erythrocyte SOD activity was assayed by the method of Marklund and Marklund (31) after extraction of hemoglobin (32) and expressed as units of activity per milligram hemoglobin. Hemoglobin concentration was determined by the cyanomethemoglobin colorimetric procedure (kit 525, Sigma, St. Louis, MO). Plasma lipoperoxides were measured by the method of Yagi (33), and the results were expressed as nanomoles malondialdehyde per milliliter blood. Blood glutathione reductase activity was determined by the method of Goldberg and Spooner (34). Blood glutathione peroxidase activity was measured after preparation of hemolysates with Drabkin's reagent (35). Results for the latter two enzymes were expressed as micromoles NADPH oxidized/minute/milligrams hemoglobin.

Students' unpaired *t* test, one-way analysis of variance, and regression analyses were used for statistical analysis of data. The significance of observed differences among the groups was evaluated with Scheffe's post hoc test (36). *P* < 0.05 was significant.

RESULTS

Based on stepwise and multiple regression analyses, obesity, age, and duration of diabetes were not significant predictors for most parameters measured in this study. When significant differences were noted for obe-

TABLE 1
Indices of glucose, trace element, and antioxidant status in nondiabetic and diabetic subjects

	Control	Diabetic
Plasma		
Glucose (mM)	4.4 ± 0.1 (28)*	13.8 ± 0.8 (56)†
Ceruloplasmin activity (U/L)	155.8 ± 17.7 (8)	214.2 ± 20.2 (19)
Copper (μM)	13.2 ± 0.6 (27)*	16.6 ± 0.8 (57)†
Zinc (μM)	11.7 ± 0.6 (28)*	9.9 ± 0.4 (57)†
Zinc-copper (μM)	0.96 ± 0.06 (27)*	0.65 ± 0.03 (57)†
Magnesium (mM)	0.77 ± 0.02 (22)	0.73 ± 0.02 (55)
Whole blood		
Manganese (μM)	0.19 ± 0.02 (12)	0.16 ± 0.02 (15)
Urine		
Glucose (mol/mol creatinine)	33.9 ± 11.3 (10)*	2865.7 ± 467.8 (17)†
Zinc (nmol/mol creatinine)	397.8 ± 41.8 (12)*	2319.9 ± 362.7 (19)†
24-h urinary Zinc (nmol/day)	4.43 ± 0.76 (12)*	21.26 ± 3.21 (19)†
Magnesium (mmol/mol creatinine)	232.8 ± 30.5 (12)*	418.1 ± 46.3 (18)†
24-h urinary Mg (nmol/day)	2.28 ± 0.26 (12)*	3.91 ± 0.40 (18)†
Antioxidant status		
Erythrocyte copper-zinc superoxide dismutase (U/mg Hb)	0.98 ± 0.02 (17)	1.05 ± 0.02 (36)
Plasma peroxide (nmol MDA/ml)	4.77 ± 0.23 (20)*	6.46 ± 0.28 (38)†
Whole-blood glutathione (nmole NADPH · min ⁻¹ · mg Hb ⁻¹)		
Peroxidase	8.09 ± 0.47 (7)	10.04 ± 0.62 (18)
Reductase	4.60 ± 0.30 (7)	5.10 ± 0.20 (18)

Values are means ± SE.

n given in parentheses.

**P* < 0.05 vs. diabetic subjects.

†*P* < 0.05 vs. control subjects.

sity, age, or duration of diabetes, these effects were stated. When compared with control subjects, diabetic subjects (HbA_{1c} 9.1 ± 0.3%) exhibited significant alterations in trace element and antioxidant status (Table 1). With the exception of plasma magnesium, which was lower in type II (0.69 ± 0.02 mM) compared with type I (0.79 ± 0.04 mM) diabetic subjects, there were no differences between types observed for any other variable. Data for type I and type II diabetic subjects were therefore pooled. Neither HbA_{1c}, plasma glucose concentration, nor insulin dosage correlated with any measured parameter. Overall, diabetic subjects had higher plasma copper concentrations compared with control subjects. Copper levels were higher in diabetic women (18.4 ± 1.3 μM) compared with diabetic men (14.7 ± 0.7 μM) and was the only sex difference noted in the study. Plasma copper concentrations did not correlate with the duration of diabetes (*P* = 0.11). Although plasma ceruloplasmin activity tended to be higher in diabetic compared with control subjects, it did not reach statistical significance (*P* = 0.092). Plasma copper and ceruloplasmin were highly correlated (*R*² = 0.91) in both control and diabetic populations.

In contrast to plasma copper, plasma zinc concentrations and the zinc-copper ratio were lower in diabetic subjects. Neither plasma magnesium nor whole-blood manganese concentrations differed between groups, although obese diabetic subjects had lower plasma magnesium than normal-weight diabetic subjects. Plasma

magnesium correlated positively with duration of diabetes. Although there was no correlation between age and plasma magnesium concentrations when diabetic and nondiabetic subjects were pooled, there was a positive correlation between age and plasma magnesium in the nondiabetic subgroup (*r* = 0.181, *P* = 0.048). This correlation was not noted in the diabetic subgroup.

Hyperzincuria and hypermagnesuria were evident in diabetic compared with control subjects and did not correlate with albuminuria. Diuretic use (hydrochlorothiazide, triamterene, furosemide) among diabetic subjects did not affect any measured parameters. Plasma peroxide concentrations were higher in diabetic subjects in whom positive correlations with age and obesity were noted. However, erythrocyte CuZnSOD, glutathione peroxidase, and glutathione reductase activities were similar between groups.

The data were also viewed with respect to the presence of specific diabetes-associated complications. Plasma copper levels were higher in diabetic subjects with retinopathy, hypertension, or macrovascular disease compared with control subjects (Fig. 1). Diabetic subjects without retinopathy, hypertension, or macrovascular disease had plasma copper concentrations intermediate between the control subjects and diabetic subjects with disease and did not differ from either group. Subjects with all three complications had a >50% increase in plasma copper compared with subjects without these complications.

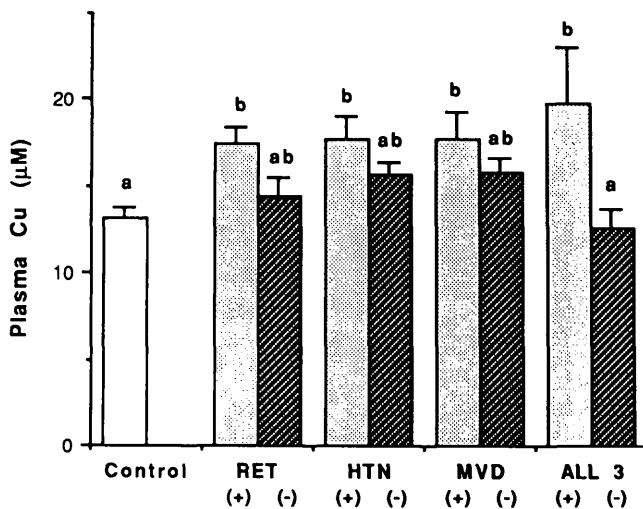


FIG. 1. Values are means \pm SE for control vs. diabetic subjects with and without select complications. Plasma copper concentrations of nondiabetic control (\square) and diabetic subjects with (\square) and without (\blacksquare) the presence of retinopathy (RET), hypertension (HTN), macrovascular disease (MVD), or all 3 complications. $P < 0.05$ for a and b.

No consistent association of plasma zinc and the above complications was observed. Plasma magnesium concentrations were lower in diabetic subjects without retinopathy (0.64 ± 0.03 mM) than in those with retinopathy (0.76 ± 0.02 mM) or in control subjects (0.76 ± 0.02 mM). Similarly, this pattern for magnesium was seen when hypertension was examined. Diabetic subjects lacking all three complications had lower magnesium levels (0.58 ± 0.04 mM) than those with the corresponding complications (0.79 ± 0.05 mM) or control subjects (0.76 ± 0.02 mM). No other measured parameter exhibited consistent associations with complications.

CONCLUSIONS

This study shows that diabetes alters mineral metabolism; however, the magnitude of the changes can be influenced by other clinical characteristics. Of interest are our data with respect to copper status. Consistent with other studies, we noted that diabetic subjects are characterized by increased plasma copper compared with control subjects. Note that the higher diabetic mean value for copper is due to the higher concentrations in diabetic subjects with retinopathy, hypertension, or macrovascular disease. The values for copper in subjects without the above complications were similar to those for control subjects. Noto et al. (37) also reported higher serum copper concentrations in diabetic subjects with macro- and microangiopathy and/or altered lipid metabolism compared with diabetic subjects without any complications or to nondiabetic control subjects. Interestingly, high plasma copper or cerulo-

plasmin has been found in other disease states, such as lymphocytic leukemia (38), inflammation (39), atherosclerosis (37,40), and hypertension in the absence of diabetes in humans and animals (41). Sjögren et al. (42) found that despite elevated plasma copper concentrations, type I diabetic subjects had lower concentrations of copper in muscle biopsies compared with control subjects, indicating possible copper depletion in muscle. Whether abnormal copper status in the face of elevated plasma copper occurs in other tissues such as the eye and blood vessels remains to be investigated.

Consistent with the findings of some investigators (14), we observed that plasma zinc concentrations are lower in diabetic than control subjects. Plasma zinc is not the only pool of zinc that is affected by diabetes. Type I and type II diabetic subjects have lower concentrations of zinc in lymphocytes, granulocytes, and platelets compared with control subjects (43). Thymulin, a biologically active zinc-dependent thymic hormone involved in the maturation and differentiation of the thymus-derived T-lymphocyte line, is reduced in diabetes (44). That this altered zinc status might be deleterious is suggested in the report by Niewoehner et al. (45) who showed improvement in T-lymphocyte response to phytohemagglutinin with zinc supplementation in type II diabetic subjects.

We noted marked hyperzincuria in diabetic subjects. Note that in our study, urinary zinc did not correlate significantly with plasma glucose concentrations, urinary glucose concentrations, HbA_{1c}, proteinuria, or diuretic use. The nearly sixfold increase in urinary zinc excretion in the diabetic subjects may be a contributing factor to the hypozincemia. For example, similar to the argument of Canfield et al. (46), if one assumes a typical dietary intake of 11 mg zinc/day (168 nmol) with an absorption of $\sim 30\%$, daily urinary zinc excretion would be $\sim 9\%$ of absorbed zinc for control subjects and 42% for diabetic subjects. Whether the high urine zinc losses are offset by increased absorption and/or decreased endogenous fecal loss remains unknown.

Urinary magnesium excretion was higher in diabetic than control subjects, but among diabetic subjects, neither plasma nor urinary magnesium correlated with plasma or urine glucose or HbA_{1c}. However, we did note that plasma magnesium levels in nonretinopathic (0.64 ± 0.03 mM) and normotensive (0.67 ± 0.03 mM) diabetic subjects were lower than those in control subjects (0.76 ± 0.02 mM) and hypertensive (0.79 ± 0.03 mM) and retinopathic (0.76 ± 0.02 mM) diabetic subjects. Consistent with the above, plasma magnesium was lowest in diabetic subjects with all three complications compared with control and diabetic subjects with hypertension, retinopathy, and macrovascular disease. These findings contrast with the previously observed association of hypomagnesemia with retinopathy (47) and ischemic heart disease (48).

Whole-blood manganese is thought to be a better index of manganese status than plasma manganese (29). Our finding of no difference between diabetic and con-

tol subjects may provide the most complete assessment of diabetic manganese status. Although altered manganese metabolism has been associated with diabetes in one subject (11), and manganese deficiency results in a diabeteslike condition in experimental animals (8,9), in this study, we found no evidence of altered manganese status in diabetic compared with control subjects. Consistent with this finding, in a recent clinical trial on the efficacy of manganese supplementation in the treatment of type II diabetic patients, we were unable to demonstrate a glucose-lowering effect of the element (49), in contrast to Rubenstein et al. (11). These observations suggest that although altered manganese metabolism may be a factor in the etiology of diabetes in selected cases, manganese deficiency does not appear to be a common occurrence in diabetic subjects.

Based on 24-h recall food records, there were no marked differences between diabetic and nondiabetic subjects with regard to mineral intakes. In our opinion, the altered mineral status of diabetes may be due in part to the well-recognized cytokine response to vascular damage. During the acute phase response, liver metallothionein is induced with a resulting decrease in plasma zinc; an increase in plasma copper and ceruloplasmin concentrations also results. We question whether the alteration may reflect a response to retinal or vascular injury via an acute phase response, or whether hypercupremia could contribute to the pathogenesis of retinopathy, hypertension, or macrovascular disease. HbA_{1c} or blood glucose concentrations were not different between subjects with and without vascular disease. Merely reporting diabetic control may overlook altered mineral metabolism in diabetic subpopulations.

We assessed another index of copper status, erythrocyte CuZnSOD activity. Although the nonenzymatic glycosylation of erythrocyte CuZnSOD in diabetes results in a reduction in its activity (50), erythrocyte CuZnSOD activity was similar between diabetic and control subjects in our study and that of Arai et al. (50). There were no differences in glutathione peroxidase and reductase activities in diabetic compared with control subjects. However, plasma peroxide concentrations were higher in diabetic than control subjects. Elevated levels of serum lipoperoxides have been reported in diabetic subjects (25,51), and excessive lipid peroxidation has been suggested to be a potential biochemical lesion associated with the development of diabetic angiopathy (25) including retinopathy, nephropathy, or atherosclerosis. Godin et al. (52) found that the lipids in erythrocytes from human diabetic subjects have an increased in vitro susceptibility to peroxidation. The high concentration of glucose may contribute to the higher plasma peroxide levels seen with diabetes. Glucose and other monosaccharides can enolize and reduce molecular O₂, generating hydrogen peroxide and free radical intermediates (53). Because glycosylation reactions are more notable in diabetes, particularly when poorly controlled, the increased oxidative activity, free radical pro-

duction, and glycosylation of proteins may play a role in the initiation of atherosclerosis, retinopathy, hypertension, or the pathogenesis of other diabetic complications. Further studies investigating whether other components of the antioxidant defense system such as vitamin C, vitamin A, and glutathione are also altered in diabetic subjects will be of value in better defining the role of oxidative damage in diabetes.

In summary, in this study, diabetes was associated with altered copper, zinc, and magnesium metabolism. The perturbations in mineral status were particularly pronounced in diabetic subjects with specific clinical complications including retinopathy, hypertension, and macrovascular disease. Note that the diabetic subjects in our study were primarily nonreferral, ambulatory, on conventional therapy, and free of acute illness, unrelated chronic disease, and renal failure. Further investigation of other diabetic subjects is clearly warranted. It remains to be determined whether the differences in trace element status in this study are a simple consequence of diabetes or if they in turn contribute to the clinical expression of the disease.

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