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Urinary Zinc and Its Relationships with Microalbuminuria in Type I Diabetics

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ABSTRACT

We investigated whether zincuria is associated with microalbuminuria in type I (insulin-dependent) diabetics (IDDM). In 169 IDDM, 215 overnight urine samples were collected for simultaneous assay of zinc and albumin. In 76 samples with excessive microalbuminuria (>15 mg/L), zincuria was higher than in the 139 other samples (0.83 ± 0.06 vs 0.58 ± 0.03 mg/L $p < 0.001$), though zincuria and microalbuminuria were not significantly correlated. An exercise provocation test was performed in 78 IDDM. Although microalbuminuria increased, zincuria did not change during the test. Another group of 83 IDDM underwent urinary zinc determination over a period of 1 h of recumbency. The 48 patients who had a zincuria higher than the mean + 2 SD of control values had higher microalbuminuria at rest (48 ± 16 μg/min vs 12 ± 2 $p < 0.01$) and after exercise (111 ± 33 vs 42 ± 14 $p < 0.02$) than the remaining 35 subjects. Both subgroups did not differ for zinc intake and zincemia. Thus, incipient nephropathy as detected by the measurement of microalbuminuria is associated with a highly significant increase in zinc excretion, which is not proportional to albumin leakage, nor is it amplified during exercise. Hyperzincuria is not explained by an increase in zinc intake and does not result in hypozincemia.

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INTRODUCTION

Zinc has been implicated in insulin metabolism and also in wound healing, both of relevance to diabetics (*1*). Abnormalities of zinc status have been reported in humans and in animal models of diabetes, and were suggested to play some role in the pathogenesis of diabetic complications (*2,3*). Some patients with diabetes mellitus presented with increased urinary losses of zinc (*4*). Presumably (*1*), some of them may become zinc deficient, though in general the plasma zinc is not affected. Another urinary parameter that is frequently abnormal in diabetics is albumin excretion. Microalbuminuria (i.e., albumin excretion under the range usually detected by reagent strips, but higher than usual values) is believed to be a marker of incipient diabetic nephropathy, announcing further decline in kidney function (*5*). Since zinc binds to proteins and is massively excreted in urine together with albumin in proteinuric patients (*1*), we investigated the relationships between zincuria and microalbuminuria in IDDM, with three different protocols: the overnight urine sampling, the exercise-provocation test, and 1-h morning sampling in recumbent position under water oral load.

MATERIALS AND METHODS

In the first protocol, overnight urine sampling was performed in 169 type I diabetics (61 females, 108 males, age: 17–74 yr, duration of disease: 1–33 yr) during a standard checkup in a hospital for their diabetes mellitus. Inclusion criteria were insulin dependency, with no macroalbuminuria (defined as urinary albumin higher than 200 mg/L) previously detected, and a systolic blood pressure lower than 140 mm Hg. In the second protocol, an exercise-provocation test was performed as previously reported (*6*). It started at 9 AM. Patients (22 females, 56 males, age: 16–46 yr) remained for 1 h recumbent and drank during this period 600 mL of water (Mont Roucous, France, which is not reported to contain Zn^{2+}). The following hour started with 20 min of strenuous inframaximal exercise on an ergometric bicycle. Heart rate was monitored on electrocardioscope in order to increase the work load every 5 min, reaching a final step at 200/min minus the age, which was maintained for 5 min. After exercise, patients remained recumbent until the end of the second hour. They urinated at the beginning of the first hour and at the end of both the first and the second hour, in order to allow a determination of resting and exercise-induced microalbuminuria. Inclusion criteria for the patients entering this study were similar to the first protocol. In the third protocol, another group of 83 IDDM selected according to the criteria of the second protocol was studied. First, the patients underwent urinary zinc determination over a period of 1 h of recumbency, while they had to drink 600 mL of water (2 glasses every 20 min). They were then divided

into two subgroups according to their zincuria (expressed in μg/min, as in all the excretion rates during these kinds of short-duration tests). Subgroup A (48 patients) had a zincuria higher than 0.8 μg/min (mean ± 2 SD of control values), whereas the remaining 35 subjects had values within the control range (subgroup B). Nutritional assessment was performed in order to evaluate daily zinc intake (including zinc given by daily insulin injections). An exercise test, as described in the second protocol, was then performed.

Microalbuminuria was measured by immunonephelometry (Behring laser nephelometer) according to Marre and coworkers (*7*), with the kit "N Microalbuminurie" from Behring. The lower limit of sensitivity of this method is 1.3 mg/L. Its intraassay coefficients of variation range between 3.2–6.14% (n = 10). Urinary zinc was assayed by flame atomic absorption spectrophotometry. The lower limit of sensitivity of this method is 0.0125 mg/L. Its coefficient of variation is 7.2% (n = 9). Total glycosylated hemoglobin (HbA1) was measured with the kit "fast Hemoglobins" from Eurobio and expressed as a percentage of total hemoglobin. Antiinsulin antibodies were measured with the method of Christiansen (*8*). Results are expressed as a percentage of radiolabeled insulin bound by the serum, so that a value of 1% indicates a binding capacity of 200 μU/mL of insulin by the sample. Statistical comparisons were performed with Mann-Whitney's nonparametric test for unpaired data.

RESULTS

In the first protocol, the 169 patients can be divided into two groups according to their urinary albumin excretion rate, since this parameter was abnormally high (>15 μg/mL) in 59 subjects (group A) and normal in the 110 others. Seventy-six overnight samples of urine were studied in the first group, and 139 in the latter. Comparison between the two groups indicates that subjects who have excessive microalbuminuria also exhibit significantly higher systolic blood pressure (13.3 ± 0.2 vs 12.3 ± 0.2 $p < 0.01$) and glycosylated hemoglobin values (11.9 ± 0.2 vs 10.3 ± 0.2 $p < 0.01$) than the subjects of group B. In contrast, they are matched for sex (male/female ratio 40/19 vs 68/42), age (45.7 ± 2.2 vs 41.9 ± 1.4 yr), duration of diabetes (12.9 ± 1.3 vs 12.8 ± 0.9 yr), diastolic blood pressure (7.5 ± 0.1 vs 7.5 ± 0.1), and antiinsulin antibodies (3.6 ± 0.8 vs 3.8 ± 0.6). In the samples drawn for overnight measurements of microalbuminuria, zinc urinary concentration, as well as zinc urinary excretion rate, are significantly higher in group A than in group B (Fig. 1). On the 215 overnight samples, zinc and albumin excretion rates could not be correlated (r = 0.006, n.s.). Similarly, no statistical relationship was found among urinary zinc excretion, blood pressure, and glycosylated hemoglobin.

In the second protocol (Fig. 2), no significant increase in zinc urinary

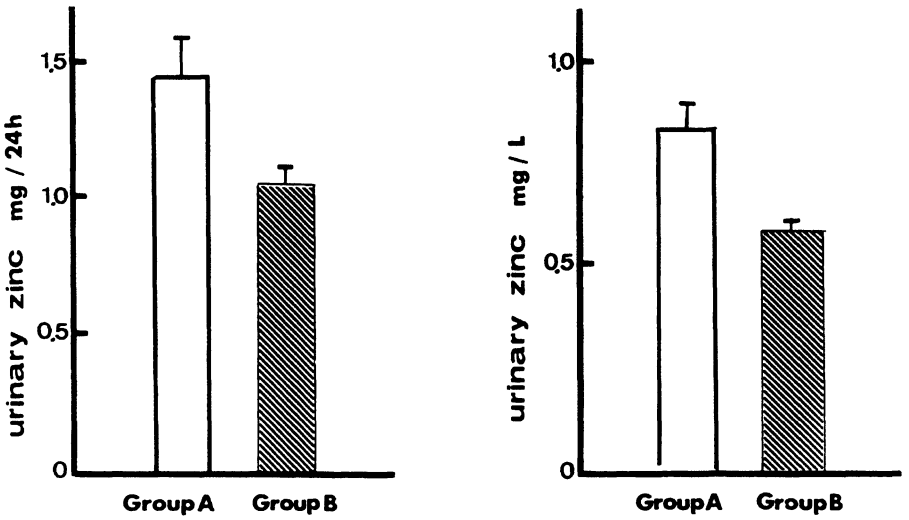

Fig. 1. Comparison of urinary zinc excretion between the 59 diabetics (group A) with excessive microalbuminuria (>15 μg/mL) and the 110 others (group B) with normal albumin excretion. Zincuria is expressed as a flow rate (left) or a concentration (right). In both cases, differences are highly significant ($p < 0.001$ and $p < 0.00001$ respectively).

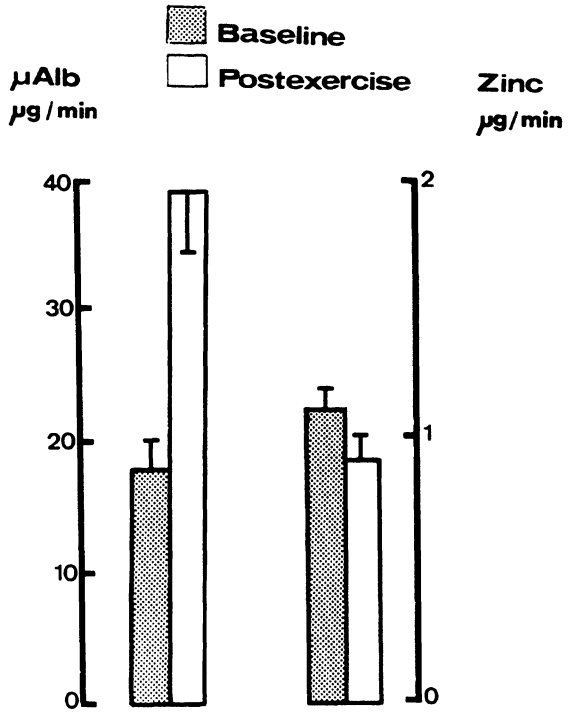

Fig. 2. Influence of a standardized submaximal exercise on urinary albumin excretion and zinc excretion. No significant increase in zinc urinary excretion was found, whereas albumin excretion rate was increased ($p < 0.01$).

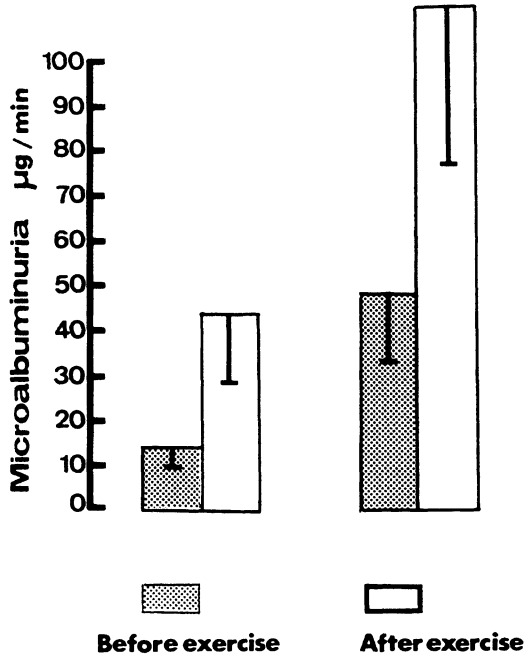

Fig. 3. Patients (83 IDDM) were divided into two subgroups according to their zincuria. Subgroup A (48 patients, right panel) had a zincuria higher than 0.8 µg/min (mean ± 2 SD of control values), whereas the remaining 35 subjects had normal urinary zinc (subgroup B, left). Differences between A and B: at rest $p < 0.02$; after exercise $p < 0.01$.

excretion was found during the excercise test (zincuria before exercise: 1.12 ± 0.09 µg/min; zincuria after exercise: 0.92 ± 0.07), whereas albumin excretion rate, as expected, was increased (at rest: 17.4 ± 2.2 µg/min; after exercise: 39.5 ± 5.8 µg/min; $p < 0.01$). In the third protocol (Fig. 3), another group of 83 IDDM underwent urinary zinc determination over a period of 1 h of recumbency. Patients were divided into two subgroups according to their zincuria. Subgroup A (48 patients) had a zincuria higher than 0.8 µg/min (mean ± 2 SD of control values), whereas the remaining 35 subjects had urinary zinc within the control range (subgroup B). Nutritional assessment indicated that both subgroups had similar zinc intake. Serum zinc values were also measured: they did not differ between the two subgroups (A: 0.92 ± 0.04; B: 0.84 ± 0.04 µg/mL). By contrast, microalbuminuria was higher in subgroup A at rest (48 ± 16 vs 12 ± 2 $p < 0.01$) and after exercise (111 ± 33 vs 42 ± 14 $p < 0.02$).

DISCUSSION

In this article, we present data suggesting that diabetics with abnormally high albumin excretion rate during the night also have an increased urinary zinc loss. Unfortunately, in this preliminary study, nei-

ther serum nor tissue zinc were measured. Thus, whether such an increased urinary loss could result in zinc deficiency remains speculative. Indirect evidence for such a deficiency in some diabetics has been reported (*1*). In the genetically diabetic (Db/Db) mice, tissue zinc deficiency has been also described (*9*). We postulate, as a working hypothesis, that an increased urinary loss of zinc could play some role in such abnormalities. However, the third protocol of the study, which was undertaken in order to answer this question, fails to detect any reduction in serum zinc in hypozincemic patients, probably because zinc intake is sufficient in these patients. We hypothesize that type II diabetics, who do not receive daily insulin preparations containing zinc, may be at risk for such a deficiency.

By which mechanism do patients with incipient nephropathy excrete increased quantities of zinc in urine? Prasad (*1*) suggests two potential causes of zinc loss in renal diseases: failure in tubular reabsorption and excretion of protein-zinc complexes across the glomerulus. Nevertheless, no correlation between zincuria and microalbuminuria was detected in our study. An acute rise in albumin excretion rate during the exercise test is not associated with an increase in zincuria. This seems to indicate that zinc excretion does not directly result from glomerular albumin leakage. Our results suggest rather that microalbuminuria is a marker of a complex glomerular dysfunction in which zinc loss is also increased, independently of albumin excretion. Recent reports (*10*) have shown that diabetics with excessive microalbuminuria also have abnormalities in lipoproteins and fibrinogen blood levels. Therefore, multiple biologic abnormalities seem to be associated with the early stages of renal diabetic disease, and they may include disorders in trace element metabolism.

Our subgroup with increased microalbuminuria also had, as expected, higher values of blood pressure and HbA1. Glycemic equilibrium and blood pressure are well known to influence albumin excretion strongly in diabetes (*5*). However, no direct influence of these parameters on zincuria can be suggested from our statistical data, nor has it been reported, to our knowledge, in current literature. A possible influence of higher dietary protein intake on both glomerular albumin leakage and zinc excretion can also be hypothesized, since protein catabolism has been reported to increase zincuria (*1*).

For this reason, we investigated the relationships between Zn intake and zincuria in the third protocol. Results presented above suggest that hyperzincuria is not explained by an increase in zinc intake, since the values of zinc provided daily by alimentation and insulin treatment are similar in normozincuric and hyperzincuric subjects. In addition, hyperzincuria does not result in hypozincemia, probably because in these diabetics, zinc intake is sufficient. However, in type II diabetics receiving no insulin and therefore no parenteral exogenous zinc, incipient diabetic nephropathy may be a factor for zinc loss and zinc deficiency: This last point remains to be determined.

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