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Effect of Hypothyroidism on Intestinal Zinc Absorption and Renal Zinc Disposal in Five-Sixths Nephrectomized Rats

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Abstract: Both hypothyroid (Hypo) and hypozincemia are commonly observed in patients and animals with chronic renal failure (CRF). In CRF whether the hypothyroid plays a role in the pathogenesis of hypozincemia is unclear. This study is designed to investigate the effects of hypothyroid on intestinal zinc absorption and urinary zinc excretion in 5/6 nephrectomized (Nx) rats, because plasma zinc balance is attained through a controlled rate of intestinal uptake as well as renal reabsorption. Intestinal zinc absorption was carried out in jejunum and ileum segments by an in vivo perfusion technique and the renal zinc disposal was evaluated by a conventional method using a standard formula to calculate the zinc tubular reabsorption and the excretion of urinary zinc in 5/6 Nx rats with hypothyroidism. The Hypo-NxT rats showed a significant decrease in the rate of intestinal zinc absorption and in the response of plasma zinc levels during intestinal zinc perfusion compared with Eu-NxT rats. They also had significantly lower levels of mucosal zinc and MT as well as lower content of liver zinc than Eu-NxT rats after intestinal zinc perfusion for 80 min. Hypo-NxT rats showed low plasma zinc levels, but had a similar output of pancreaticobiliary zinc and excretion of 24-h urine zinc compared with the Eu-NxT rats. When 2% alcohol intestinal perfusion was used to produce water diuresis, the Hypo-NxT rats presented a higher excretion of urinary zinc than the Eu-NxT rats did, especially during 2% alcohol intestinal zinc perfusion. In the Hypo-NxT rats, the lower plasma zinc levels may thus result from the hypothyroid because it reduces intestinal zinc absorption. Increasing the urine flow rate may aggravate the reduction of plasma zinc level in Hypo-NxT rats because of the increased excretion of urinary zinc. [The Japanese Journal of Physiology 55: 211–219, 2005]

Key words: zinc, chronic renal failure, hypothyroidism, intestinal zinc absorption.

Zinc is an essential nutrient in humans and animals because it is a critical component of numerous metalloenzymes and zinc-dependent transcription factors [1]. Thus it is involved in many biological activities, such as fuel metabolism [2], protein synthesis [3], hormone metabolism [4, 5], and cell division and differentiation [6–8]. Zinc is also implicated as a neuromodulator of certain postsynaptic neurons in the brain [9]. Because of these important functions, nutritional zinc deficiency can have devastating consequences to human and animal health, including growth retardation, immune system dysfunction, and mental disorder. Some diseases have been suggested to induce zinc deficiency, such as chronic uremia [10–12], sickle cell disease [13], chronic alcoholism [14], Crohn's disease [15], and the genetic disorder acrodermatitis enteropathica [16]. Hypozincemia is a main feature of zinc deficiency and is commonly found in patients with renal insufficiency [17, 18] or in uremic patients on hemodialysis [19, 20]. Our recent studies suggested that both the decreased intestinal zinc absorption [21, 22] and the decreased renal tubular reabsorption of zinc

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Table 1. Metabolic parameters in 5/6 nephrectomized rats with hypothyroidism and euthyroidism.

Status	*n*	Body weight (g)		Food intake (g/day)	Plasma levels			Urine output (ml/day)	Urine protein (mg/day)
		Initial	Final		Cr (mg/dl)	T_3 (ng/ml)	T_4 (ng/ml)		
Normal	10	193.5 ± 5.5	315.2 ± 16.5	22.3 ± 1.6	0.52 ± 0.03	1.98 ± 0.18	96.4 ± 3.1	24.7 ± 0.9	3.7 ± 0.5
Hypo-NxT	12	193.8 ± 5.9	295.5 ± 14.3	18.6 ± 1.5	1.21 ± 0.05*	0.85 ± 0.18*†	67.2 ± 2.5*†	23.5 ± 1.1	8.9 ± 0.8*
Eu-NxT	14	194.4 ± 5.4	308.1 ± 12.8	21.2 ± 1.8	1.12 ± 0.05*	1.95 ± 0.22	98.5 ± 3.5	26.4 ± 1.2	9.8 ± 0.8*

Values are expressed as mean ± SE. *n*, no. of rats; Cr, creatinine; T_3, triiodothyronine; T_4, thyroxine; Hypo-NxT, hypothyroid-NxT; Eu-NxT, euthyroid-NxT. *Significantly different from normal control value ($p < 0.05$); †Significantly different from Eu-NxT value ($p < 0.05$).

[23, 24] might be the main causes of hypozincemia, because the kidney and intestine play important roles in the maintenance of plasma zinc homeostasis [25].

Hypothyroid is often found in patients with chronic renal failure [26–28] because the kidney plays an important role in the metabolism, degradation, and excretion of several thyroid hormones [29]. Zinc deficiency has been found to decrease serum T_4 and T_3 concentrations and to blunt TSH response to TRH [30]. Zinc supplementation has corrected the T_3 and T_4 values toward normal in patients receiving intermittent peritoneal dialysis therapy [30]. In uremia, whether the hypothyroid plays a role in the pathogenesis of hypozincemia is unclear because in vitro study suggests that hypothyroid reduces Zn^{2+} uptakes in brush-border membranes (BBM) of rat intestinal and renal tubules [31]. The effects of hypothyroid on Zn^{2+} uptakes in the in vivo studies of intestine and renal tubules are not delineated. Therefore we attempted to use an in vivo intestinal zinc perfusion model to evaluate the effect of hypothyroid on intestinal zinc absorption and to study the effect of hypothyroid on the handling of renal tubular zinc in rats with CRF.

MATERIALS AND METHODS

Reagents and animals. T_4 and T_3 enzyme immunoassay test kits were obtained from Maxim Biotech, Inc (South San Francisco, CA). All other chemicals were purchased from Sigma Chemical (St. Louis, MO).

Adult male Sprague-Dawley strain rats, weighing 180–200 g at the start of each experiment, were obtained from the animal center of National Yang-Ming University and were kept at 25°C in a 12-h light-dark cycle. All animals were fed with a standard rat chow, which contained 23% protein and an average of 50-ppm total zinc, and had free access to food and water. The rats were given a two-stage five-sixths nephrectomy (5/6 Nx) to induce CRF for 2 weeks later. The 5/6 Nx rats were randomly divided into two groups as follows: hypothyroid-NxT (Hypo-NxT) and euthyroid-NxT (Eu-NxT). Hypo-NxT was induced as described by Choudhury et al. [32], but it is modified 1.5 mg/ml 6-n-propyl-2-thiouracil (PTU) was dissolved in distilled water and administered orally via a gastric tube at a bid dose of 20 ml/kg for 3 weeks. Euthyroid rats were administered orally via gastric tube with an equivalent amount of solvent. The procedure for 5/6 Nx was described in detail in our previous report [33]. Sham-operated normal control rats were simultaneously performed in present study.

Absorption studies were performed at the end of the hypothyroid treatment. Prior to the absorption study, 24-h urine samples were collected from all animals in a Nalgene plastic metabolic cage, and the animals were fasted for 16 hours. The samples were used to assay the concentrations of urine protein, urinary zinc and urinary creatinine, and the standard formula Cx = UxV/Px was used to calculate the renal clearances of creatinine and zinc, where Ux and Px represent the concentrations of creatinine and zinc in urine and plasma respectively, and V represent the urine flow rate in milliliters per minute [34].

On the day of absorption study, the preparation and surgery of the animals were described in detail in our previous report [22]. A cannula was inserted in the common duct proximal to the ampulla of Vater to collect pancreaticobiliary juice. Before urine collection, the bladder was emptied with a 27 G needle, using 1 ml 0.3% phenol red zinc-free saline solution to perfuse the bladder and collect the urine. The total volume of urine was calculated for 1 ml perfusate shifts as reflected by changes of the concentration of phenol red, which was used as a non-absorbed marker. The perfusate was then used to determine the urinary excretion of zinc. The blood samples were obtained through the femoral artery, and base line blood sam-

Table 2. Effects of hypothyroid on renal handling of zinc in 5/6 nephrectomized rats.

Status	*n*	Plasma Zn (μg/ml)	UZn.V (μg/day)	Ccr (ml/min)	CZn^{2+} (μl/min)	CZn^{2+}/Ccr Ratio (%)	$TRZn^{2+}$ (%)
Normal	10	1.31 ± 0.04	4.88 ± 0.21	1.58 ± 0.11	2.62 ± 0.14	0.17 ± 0.11	99.8 ± 0.11
Hypo-NxT	12	1.05 ± 0.04*†	5.65 ± 0.22*	0.56 ± 0.07*	3.71 ± 0.21*	0.66 ± 0.19*	99.3 ± 0.19*
Eu-NxT	14	1.16 ± 0.04*	6.38 ± 0.35*	0.69 ± 0.09*	3.91 ± 0.32*	0.54 ± 0.14*	99.4 ± 0.14*

Values are expressed as mean ± SE. *n*, no. of rats; Zn, zinc; UZnV, urinary zinc excretion; Ccr, renal creatinine clearance; CZn^{2+}, renal zinc clearance; $TRZn^{2+}$, zinc tubular reabsorption; Hypo-NxT. Hypothyroid-NxT; Eu-NxT, euthyroid-NxT. *Significantly different from normal control value ($p < 0.05$), †Significantly different from Eu-NxT value ($p < 0.05$).

ples were used to determine plasma zinc, creatinine, and urea nitrogen concentrations as well as the T_4 and T_3 levels.

Absorption studies. The absorption studies were performed in a peristaltic pump (Perista minipump; Atta) to drive the perfusate at a rate of 2 ml/min through the inflow catheter into the intestinal segment as in our previous description [22]. To increase the urine flow rate, we prepared the 450 mg/*l* $ZnSO_4$-$7H_2O$ perfusion solution in a 2% ethanol solution containing 0.072% NaCl, 0.2% D-glucose, and 3 mg/ml phenol red, buffered with 20 mM TRIS (tris[hydroxy methyl]aminomethane, Sigma 7-9 biochemical buffer, USA) to induce water diuresis. This was because the 2% ethanol solution has been shown to inhibit the secretion of antidiuretic hormone by the posterior pituitary [35].

Prior to zinc sulfate perfusion, intestinal zinc secretion was measured in all investigated rats with a 40 ml, 2% zinc-free ethanol solution containing 0.072% NaCl, 0.2% D-glucose, and 3 mg/ml phenol red buffered with 20 mM TRIS, pH 6.2 and maintained at 37°C. The small intestine was perfused with this solution for 30 min to assess appreciable amounts of zinc secretion through the intestinal wall, which may alter the results of the absorption studies.

Before zinc sulfate perfusion, the basal pancreaticobilary juice and urine secretions were collected for 30 min to determine their secretion volume and amount of zinc output. Next, the zinc sulfate was perfused to study the effect of intestinal zinc absorption on the secretion of bile and urinary zinc. The volume of pancreaticobilary secretion was determined by weight and the result was used to determine the amount of output in pancreaticobiliary zinc.

At the end of the experiment, blood was withdrawn from the abdominal aorta. The liver was flushed with 20 ml heparinized 0.9% NaCl solution via the abdominal aorta, and partial liver tissue samples were then harvested to assay the content of liver zinc. The perfused intestines were immediately excised and opened along the horizontal axis to remove the contents, then washed twice in 30 ml ice-cold 0.9% NaCl solution. The mucosal lining of the intestine was scraped from the serosa with a glass slide and stored at –25°C before zinc, MT and total protein were measured.

Biochemical analysis. Routine methods were used to measure the concentrations of plasma urea nitrogen, creatinine and albumin as well as the concentration of urine creatinine on an automatic biochemistry analyzer (Express Plus, Ciba-Corning Diagnostics Corp., Mass., USA). A urine protein assay kit (Bio-Systems. SA, Costa Brava, Barcelona, Spain) was used to measure the concentration of urine protein, as described by Orsonneau *et al.* [36]. An atomic absorption spectrophotometer (AAS) was used to determine the concentrations of zinc and MT in all samples, including plasma, urine, and tissue samples in a Perkin Elmer 3110 AAS. Plasma urea nitrogen and creatinine levels and the clearance of renal creatinine were used to monitor the development of chronic uremia.

Determination of tissue Zn, MT and total protein levels. Fresh or frozen tissue samples weighing about 0.4 g each, including liver and intestinal mucosa were homogenized in 1 ml cold 0.25 M saccharose solution with the use of a homogenizer (PT 1200; Polytron). The tissue samples were homogenized at full speed for 1–2 min. Homogenates were diluted with 1 ml cold 0.25 M saccharose solution and then mixed and centrifuged at 650 × g for 10 min at 4°C to remove the particles.

The resultant supernatants were then used to measure the contents of zinc, MT and total protein. The analysis of tissue total protein was performed on the same automatic biochemistry analyzer (Express Plus, Ciba-Corning Diagnostics Corp., Mass., USA) following Lowry's method. A total of 0.2 ml of the resultant homogenates was digested in a 95°C dry heat bath with 0.2 ml 12 N nitric acid. The residue was then dissolved in 2 ml deionized distilled water, and subjected to AAS to determine zinc level. Zinc concentrations in the intestine mucosa were expressed as μg/mg

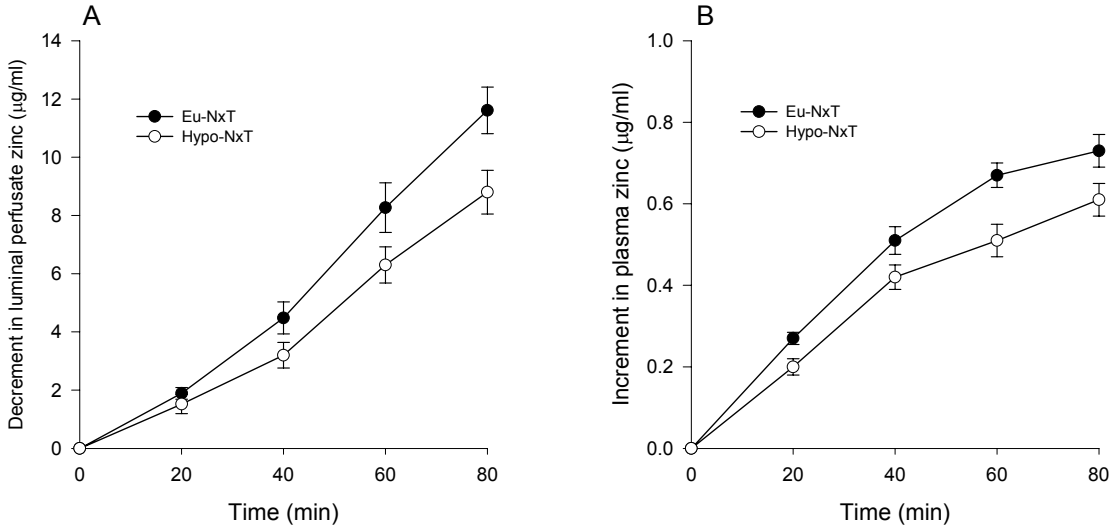

Fig. 1. A: Intestinal zinc absorption. Decrement in luminal perfusate zinc resulting from the absorption of intestinal zinc in Eu-NxT and Hypo-NxT rats. **B: Response of plasma zinc level.** Increment in plasma zinc resulting from the intestinal luminal perfusion of 450 mg// $ZnSO_4$-$7H_2O$ at a rate of 2 ml/min in Eu-NxT and Hypo-NxT rats. All values represent the mean ± SE.

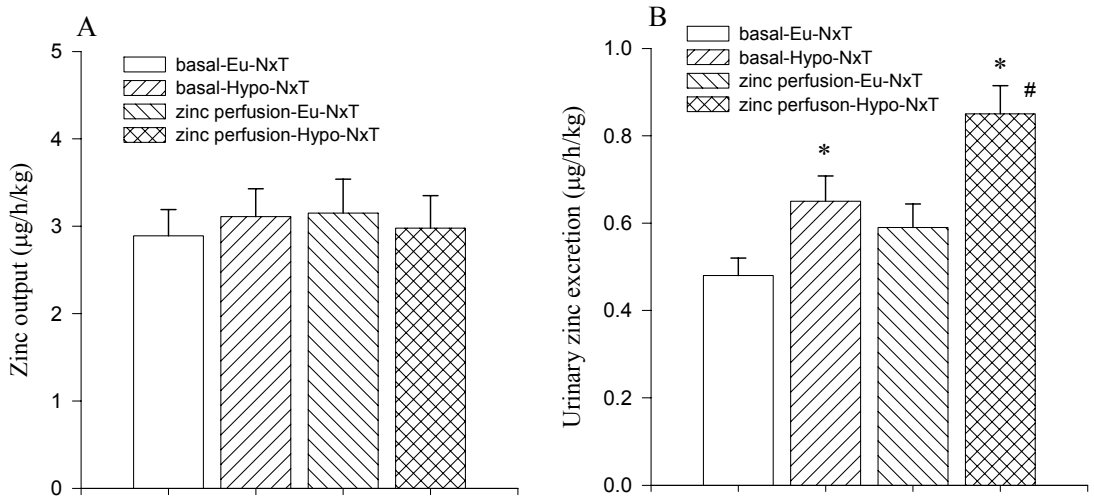

Fig. 2. A: Pancreaticobiliary zinc secretion. Pancreaticobiliary zinc secretion during basal and 450 mg// $ZnSO_4$-$7H_2O$ intestinal perfusion period in Eu-NxT and Hypo-NxT rats. **B: Urinary zinc excretion.** Urinary zinc excretion during basal and 450 mg// $ZnSO_4$-$7H_2O$ intestinal perfusion period in Eu-NxT and Hypo-NxT rats. All values represent the mean ± SE. Urinary zinc excretion significantly different from values obtained from the Eu-NxT rats ($^*p < 0.05$) and obtained from the basal-Hypo-NxT rats ($^\#p < 0.05$) in B.

protein, and in the liver tissue they were expressed as μg/g wet weight. MT concentrations were measured by the modified silver saturation hemolysate method [37]. The procedure and calculation were described in detail in our previous report [22].

Statistical analysis. The results are expressed as mean ± SE. Statistical software (SPSS, Chicago, I11., USA) was used to perform the statistical analysis. A Student's unpaired *t*-test and a one-way analysis of variance (ANOVA) were used to evaluate the variance between the different groups. The area under the curve (AUC) of the plasma zinc response and the alteration of luminal perfusate zinc were calculated by using the trapezoidal rule. These areas were examined by means of ANOVA and compared with each other by using Duncan multiple range tests. The differences were considered significant at a p value less than 0.05.

RESULTS

The levels of plasma creatinine and urea nitrogen in the 5/6 Nx rats were approximately twice those of the sham-operated normal rats (Table 1), and the renal creatinine clearance (Ccr) was significantly lower than in the sham-operated normal rats (Table 2). Thus, the 5/6 Nx rats appeared to suffer CRF. In the 5/6 Nx rats including Hypo-NxT and Eu-NxT rats, the excretions of urinary protein and zinc as well as renal zinc clearance (CZn^{2+}) and the ratio of CZn^{2+}/Ccr were found to increase compared with that in sham-operated normal rats (Tables 1 and 2). Because the intake of dietary zinc was not restricted in these 5/6 Nx rats so that the features of zinc deficiency, such as anorexia and growth retardation, were not observed in them including Hypo-NxT and Eu-NxT rats (Table 1).

The metabolic effects in relation to hypothyroid in 5/6 Nx rats are shown in Table 1. Serum T_3 and T_4 levels were reduced significantly in Hypo-NxT rats compared with Eu-NxT rats. There was no significant difference in body weights and food intake between the Hypo-NxT and Eu-NxT rats. Hypothyroid was not found to affect the urinary protein excretion or the urine flow rate in the 5/6 Nx rats.

The effect of hypothyroid on the renal handling of zinc was presented in Table 2. The plasma zinc level in the Hypo-NxT rats was significantly lower than in the Eu-NxT rats, but the values of CZn^{2+} and CZn^{2+}/Ccr ratio were not significantly different between the Hypo-NxT and the Eu-NxT rats. Because the CZn^{2+}/Ccr ratio presented the same profile of fractional excretion of zinc, the values of fractional reabsorption of zinc ($TRZn^{2+}$) were calculated by 1 – CZn^{2+}/Ccr ratio. Thus the values of $TRZn^{2+}$ were not significantly reduced in Hypo-NxT rats. We expressed the fractional reabsorption of zinc in terms of percentage.

During the 80-min perfusion period, the percentage of reduced zinc concentrations in the luminal perfusate in CRF rats with hypothyroid was significantly less than that of CRF rats with euthyroid (8.7 ± 1.8% vs. 11.8 ± 2.1, $p < 0.05$). The response of the plasma zinc level over time was performed in Eu-NxT and Hypo-NxT and is shown in Fig. 1B. The variance of area under the curve (AUC) for the change in the luminal perfusate zinc levels (306.5 ± 28.9 vs. 408.9 ± 32.5 μg/ml for 80 min, Fig. 1A) and the variance of AUC for the response in the plasma zinc levels (36.3 ± 1.5 vs. 31.2 ± 1.2 μg/ml for 80 min, Fig. 1B) demonstrated a statistical significance between the Eu-NxT and the Hypo-NxT rats ($p < 0.05$).

A 2% alcohol intestinal perfusion was performed to induce water diuresis and the increase of urine flow rate from mean 16.3 μl/min to mean 31.7 μl/min (Fig. 4B). The Hypo-NxT rats presented a high urinary zinc excretion compared with Eu-NxT rats (Fig. 2B). Hypothyroid was not found to increase basal zinc output in pancreaticobiliary juice (Fig. 2A). The 80-min intestinal zinc perfusion did not increase the output of pancreaticobiliary zinc in Eu-NxT and Hypo-NxT rats, but the excretion of urinary zinc significantly ($p < 0.05$) increased in Hypo-NxT rats (Fig. 2B). Following this perfusion, the Hypo-NxT rats showed lower contents of mucosal zinc and MT (Fig. 3, A and B) as

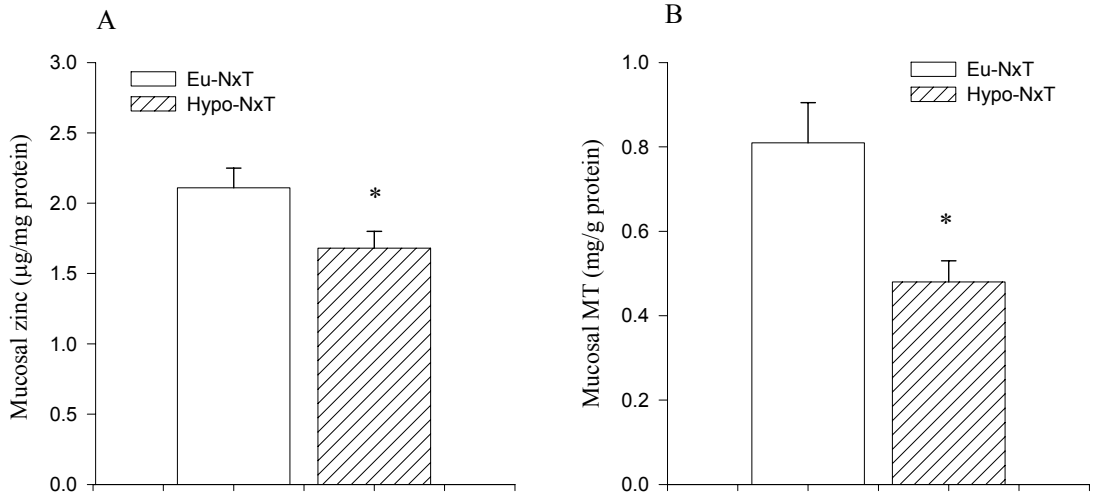

Fig. 3. A: Content of mucosal zinc. The content of mucosal zinc after 450 mg/l $ZnSO_4$-$7H_2O$ intestinal perfusion in Eu-NxT and Hypo-NxT rats. **B: Content of mucosal metallothionein.** The content of mucosal metallothionein (MT) after 450 mg/l $ZnSO_4$-$7H_2O$ intestinal perfusion. All values represent the mean ± SE. The contents of mucosal zinc and MT significantly differ from values obtained from the Eu-NxT rats (*$p < 0.05$).

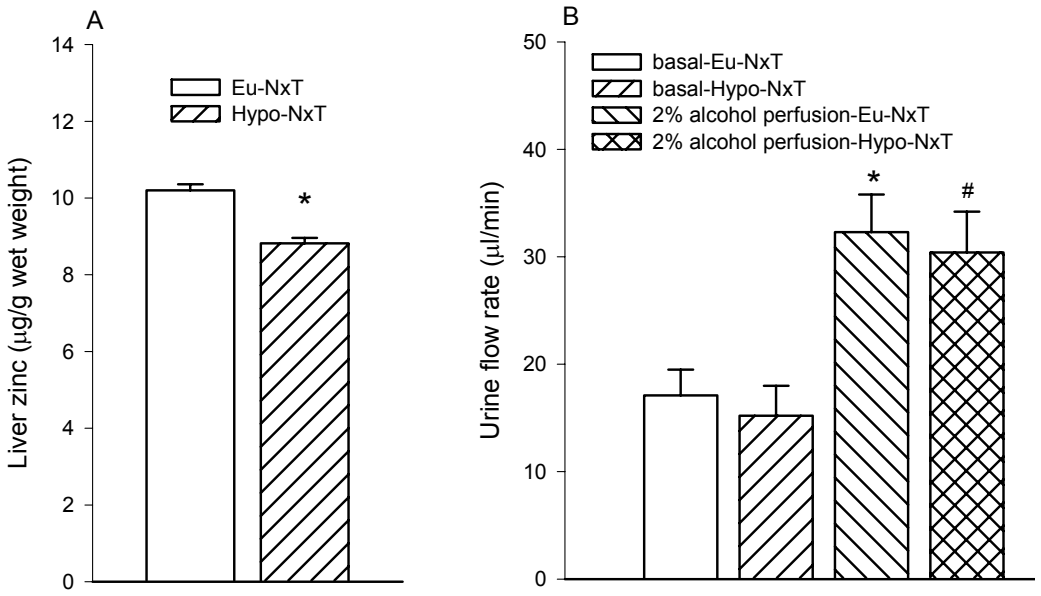

Fig. 4. A: Content of liver zinc. After 450 mg// $ZnSO_4$-$7H_2O$ intestinal perfusion, the content of liver zinc in Eu-NxT and Hypo-NxT rats. **B: Comparison of urine flow rate.** The comparison of urine flow rate between 2% alcohol diuresis and 24-h basal urine collection in these 5/6 Nx with euthyroid and hypothyroid. The content of liver zinc significantly different from values obtained from the Eu-NxT (*$p < 0.05$) in A. The urine flow rate significantly different from values obtained from the basal-Eu-NxT rats (*$p < 0.05$) and obtained from the basal-Hypo-NxT rats ($^{\#}p < 0.05$) in B. All values represent the mean ± SE.

well as a lower level of zinc in the liver compared with that in the Eu-NxT rats (Fig. 4A).

DISCUSSION

In mammals, zinc is absorbed through the BBM of small intestine from diet and transported through blood to the tissues and cells where zinc is needed [38].The present study demonstrated that reduced serum T_3 and T_4 levels in rats with CRF decrease the absorption of intestinal zinc and the activity of renal tubular zinc uptake, which confirms the report of Prasad *et al.* [31] that hypothyroid had reduced the Zn^{2+} uptake activity in both intestinal and renal BBM. The thyroid hormone status has been suggested to modulate zinc transport activity in intestinal and renal BBM through an alteration in the number of Zn^{2+} transporters [31]. Thus in regulating the intestinal zinc absorption or urinary zinc excretion, the numbers of Zn^{2+} transporters may play an important role because zinc transportation into and out of cells is completed by a series of transporters [39]. Two families of zinc transporters are identified in mammals [40–44]. The ZnT proteins appear to function either by transporting zinc out of the cells or by sequestering zinc into intracellular compartments [40–42]. In contrast, the Zip proteins appear to function in the uptake of zinc into cytoplasm [43, 44].

In the present studies, we measured the intestinal zinc absorption directly to evidence the effect of hypothyroid on intestinal zinc absorption. The results of the present study demonstrate that Hypo-NxT rats had decreased intestinal zinc absorption because a low absorption rate was found during the intestinal zinc perfusion. The decreased intestinal zinc absorption may result from the reduction of luminal zinc absorption from the lumen into intracellular compartment, because zinc absorption in rat intestine has been considered to consist of two sequential stages (i) movement from the lumen into the mucosal tissue, and (ii) movement from mucosa into plasma compartments [45]. The decreased movement of zinc from lumen into the mucosal tissue may reduce the content of mucosa zinc and the transport of zinc across the basolateral membrane to the plasma. This is possible because the absorbed zinc from lumen is either integrated into intracellular zinc pool(s) or is rapidly (within minutes) transported across the basolateral membrane to the plasma compartment [46]. A low content of mucosal zinc and a low response of plasma zinc concentrations were found in Hypo-NxT rats. Therefore the uptake activity of zinc in intestinal BBM might be reduced in Hypo-NxT rats.

MT is an intracellular metalloprotein and is present in many organs of the body, especially the intestine, liver and kidney. In the intestine, it may play a role

in detoxification to prevent the potentially deleterious actions of excessive zinc absorption thereby in the intestine the MT is rapidly synthesized after zinc is absorbed [47], thus preventing zinc toxicity because MT can sequester the excess zinc [48]. The present study demonstrated that after the 80-min intestinal zinc perfusion, the Hypo-NxT rats had lower mucosal zinc and MT levels than the Eu-NxT rats did because Hypo-NxT reduces the absorption of intestinal zinc. However, the direct effect of hypothyroid on intestinal MT synthesis is not yet known.

After zinc has been absorbed into portal circulation, it is bound to albumin and is then rapidly taken up by the liver [47]. Therefore the liver zinc levels have been shown to fluctuate as a result of transient changes in dietary zinc status [49]. After the 80-min intestinal zinc perfusion, the lower liver zinc level in Hypo-NxT rats than in the Eu-NxT rat may thus be a result of decreased intestinal zinc absorption.

The GI tract is the major pathway of zinc excretion [50]. Fecal zinc consists of unabsorbed dietary zinc and the excretion of endogenous zinc. Thus adjustment in fecal endogenous zinc excretion has been suggested as the primary means of maintaining zinc homeostasis, since a short-term dietary zinc restriction can increase the absorption of intestinal zinc and decrease fecal endogenous zinc loss [51, 52]. The sources of intestinal endogenous zinc are mainly derived from pancreas and biliary zinc secretion. These results show that hypothyroid does not increase the secretion of pancreaticobiliary zinc; however, decreased intestinal zinc absorption may impair the balance of plasma zinc because it may increase the excretion of endogenous zinc from feces and impair the GI system's role in maintaining zinc homeostasis. It has been suggested that the GI tract is a primary site to maintain zinc homeostasis in mammals [53], since it controls the absorption of dietary zinc and the excretion of endogenous zinc. In zinc deficiency, the GI tract compensates by increasing the velocity of zinc absorption by brush border membrane vesicles. Inversely, in zinc excess it decreases zinc absorption and increase endogenous zinc excretion [47]. Therefore in Hypo-NxT, the low plasma zinc level may partially attribute to the reduction of intestinal zinc absorption. However, further studies are required to elucidate the effect of hypothyroid on the excretion of fecal zinc.

Hypothyroidism has been suggested to decrease the Zn^{2+} uptake activity in renal BBM [31], which may reduce zinc reabsorption from the renal tubules and then increase urinary zinc excretion, because most of the filtered zinc is reabsorbed along kidney proximal tubules and/or distal tubules, averaging approximately 98.5–99.5% [54, 55]. The present study, however, in using the 24-hours urine collection to evaluate the handing of renal zinc, finds no increase in urinary zinc excretion or decrease in the fractional reabsorption of zinc in Hypo-NxT rats. On the contrary urinary zinc excretion is found to slight decrease (Table 2). The result may be caused by the reduction of plasma zinc level, which decreases the filtered load of zinc in glomerular cells and minimizes the possible increase of urinary zinc excretion as a result of the reduction of tubular zinc reabsorption in Hypo-NxT rats. Because the filtered load of zinc in glomerular cells is the major source of urinary zinc excretion, in a nonpathological condition or nondrug treatment the urinary zinc excretion is suggested to be related to the plasma zinc level [56]. Thus a low plasma zinc level and a low urinary excretion of zinc have been found in patients with hypothyroidism [57]. In the present study, we also find that during intestinal zinc perfusion, urinary zinc excretion was found to increase, especially in Hypo-NxT rats, which may result from the elevation of the plasma zinc level after intestinal zinc perfusion.

In our previous study [24], we found that urinary zinc excretion is positively related to urine flow rate. Thus tubular flow rate can affect the reabsorption of renal tubule zinc and alter urinary zinc excretion [58]. Therefore without alteration in plasma ultrafilterable zinc status, some drugs have been found to enhance the excretion of urine zinc because they inhibit the tubular zinc reabsorption, such as citrate and mannitol diuresis [58]. In the present study, by using 2% alcohol to induce water diuresis we find that the Hypo-NxT rats present a higher urinary zinc excretion than the Eu-NxT rats do, especially during intestinal zinc perfusion. The result may be because increasing the urine flow rate by alcohol diuresis worsens the abnormality of renal tubule zinc uptake activity in these Hypo-NxT rats. This has resulted because hypothyroidism has been suggested to decrease Zn^{2+} uptake activity in renal BBM [31]. Thus the results of the present study have clearly demonstrated that hypothyroidism has increased urinary zinc excretion when the tubular flow rate is increased.

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REFERENCES

1. Berg JM and Shi Y: The galvanization of biology: A growing appreciation for the roles of zinc. Science 271: 1081–1085, 1996
2. Lowe NM, Woodhous LR, and King JC: A comparison of the short-term kinetics of zinc metabolism in women during fasting and following a breakfast meal. Br J Nutr 80: 363–370, 1998
3. Clegg MS, Keen CL, and Hurley LS: Biochemical pathologies of zinc deficiency. In Zinc in Human Biology, ed. Mills CF, Springer-Verlag, London, pp 136–140, 1989
4. Mills CF, ed.: Zinc in human Biology, Springer-Verlag, London, pp 95–259, 1989
5. Morley JE, Gordon J, and Hershman JM: Zinc deficiency, chronic starvation and hypothalamic-pituitary-thyroid function. Am J Clin Nutr 33: 1767–1770, 1980
6. Coleman JE: Zinc proteins: enzymes, storage proteins, transcription factors and replication proteins. Annu Rev Biochem 61: 897–946, 1992
7. Vallee BL and Falchuk KH: The biochemical basis of zinc physiology. Physiol Rev 73: 79–118, 1993
8. Wu FXH and Wu CW: Zinc in DNA replication and transcription. Annu Rev Nutr 7: 251–271, 1987
9. Cuajungco MP and Lees GJ: Zinc metabolism in the brain: relevance to human neurodegenerative disorder. Neurobiol Dis 4: 137–169, 1997
10. Atkin-Thor E, Goddard BW, O'Nion J, Stephen RL, and Kolff Wj: Hypogeusia and zinc depletion in chronic dialysis patients. Am J Clin Nutr 31: 1948–1951, 1978
11. Mahajan SK, Gardiner W, and Abbasi AA: Hypogeusia in patients on hemodialysis. Proc Clin Dial Transplant Forum 8: 20–24, 1978
12. Mahajan SK, Prasad AS, Rabbani P, Briggs WA, and McDonald FD: Zinc deficiency: a reversible complication of uremia. Am J Clin Nutr 36: 1177–1183, 1982
13. Prasad AS: Zinc deficiency in patients with sickle cell disease. Am J Clin Nutr 75: 181–182, 2002
14. Valberg LS, Flanagan, FPR, Ghent CN, and Chamberlain MJ: Zinc absorption and leukocyte zinc in alcoholic and nonalcoholic cirrhosis. Dig Dis Sci 30: 329–333, 1985
15. Matsui T: Zinc deficiency in Crohn's disease. J Gastroenterol 33: 924–925, 1998
16. Dillaha CJ, Lorincz AL, and Aavik OR: Acrodermatitis enteropathica: review of the literature and report of a case successfully treated with diodoquin. J Am Med Assoc. 152: 509–512, 1953
17. Condon CH and Freeman RM: Zinc metabolism in renal failure. Ann Intern Med 73: 531–536, 1970
18. Zumkley H, Bertram HP, Lison A, Knoll O, and Losse H: Aluminum, zinc and copper concentrations in plasma in chronic renal insufficiency. Clin Nephrol 12: 18–21, 1979
19. Mansouri K, Halsted JA, and Gombos EA: Zinc, copper, magnesium and calcium in dialyzed and non-dialyzed uremic patients. Arch Intern Med 125: 88–93, 1970
20. Mahler DJ, Walsh JR, and Haynie GD: Magnesium, zinc and copper in dialysis patients. Am J Clin Path 56: 17–23, 1971
21. Wang SC, Chen YS, Chen SM, and Young TK: Possible site of decreased intestinal zinc absorption in Chronic uremic rats. Nephron 89: 208–214, 2001
22. Chen SM, Liao JF, Kuo CD, and Ho LT: Intestinal absorption and biliary secretion of zinc in rats with chronic renal failure. Nephron Physiol 96: 113–120, 2004
23. Chen SM, Chen TW, and Young TK: Renal excretion of zinc in patients with chronic uremia. J Formosan Med Assoc 89: 220–224, 1990
24. Young TK, Wang SC, Chen SM, and Shi CC: Decreased tubular reabsorption of zinc in rats with chronic uremia. Chinese J Physiol 39: 43–48, 1996
25. Ranaldi G, Perozzi G, Truong-Tran A, Zalewski P, and Murgia C: Intracellular distribution of labile Zn(II) and zinc transporter expression in kidney and MDCK cells. Am. J. Physiol. 283: F1365–F1375, 2002
26. Semple CG, Beastall GH, Henderson IS, Thomson JA, and Kennedy AC: Thyroid function and continuous ambulatory peritoneal dialysis. Nephron 32: 249–252, 1982
27. Monzani F, Panichi V, and De Negri F: Iodine and thyroid dysfunction in uremia. Clin Nephrol 34: 44–47, 1990
28. Lim VS: Thyroid function in patients with chronic renal failure: Am J Kidney Dis 38: S80–S84, 2001
29. Lebkowska U, Malyszko J, and Mysliwiec M: Thyroid function and morphology in kidney transplant recipients, hemodialyzed and peritoneally dialyzed patients. Transplant Proc 35: 2945–2948, 2003
30. Arreola F, Paniagua R, Perez A, Diaz-Bensussen S, Junco E, Villalpando S, and Exaire E: Effect of zinc treatment on serum thyroid hormones in uremic patients under peritoneal dialysis. Horm Metab Res 25: 539–542, 1993
31. Prasad R, Kumar V, Kumar R, and Singh KP: Thyroid hormones modulate zinc transport activity of rat intestinal and renal brush-border membrane. Am J Physiol 276: E774–E782, 1999
32. Choudhury S, Chainy GBN, and Mishro MM: Experimentally induced hypo- and hyper-thyroidism influence on the antioxidant defence system in adult rat testis. Andrologia 35: 131–140, 2003
33. Chen SM and Young TK: Effect of parathyroid hormone on antioxidant enzyme activity and lipid peroxidation of erythrocytes in five-sixths nephrectomized rats. Nephron 73: 235–242, 1996
34. Wetzels JFM, Huysmans FTM, and Koene RAP: Creatinine as a marker of glomerular filtration rate. Neth J Med 33: 144–153, 1988
35. Young TK, Tsai SM, Chen SM, and Lin CW: Diminution of antidiuretic and natriuretic response to arginine vasopressin in rats with five sixths nephrectomy. Chinese J Physiol 36: 205–209, 1993
36. Orsonneau JL, Douet P, Massoubre C, Lustenberger P, and Bernard S: An improved pyrogallol red-molybdate method for determining total urinary protein. Clin Chem 35: 2233–2236, 1989
37. Scheuhammer ByAM and Cherian MG: Quantification of metallothionein by siliver saturation. Methods Enzymol 205: 78–83, 1991
38. Reyes JG: Zinc transport in mammalian cells. Am J Physiol 270: C401–C410, 1996

39. Harris ED: Cellular transporters for zinc. Nutr Rev 60: 121–124, 2002
40. Palmiter RD and Findley SD: Cloning and functional characterization of a mammalian zinc transporter that confers resistance. EMBO J 14: 639–649, 1995
41. Huang L, Kirschke CP, and Gitschier J: Functional characterization of a novel mammalian zinc transporter, Zn T6. J Biol Chem 277: 26389–26395, 2002
42. Palmiter RD, Cole TB, and Findley SD: Zn T-2, a mammalian protein that confers resistance to zinc by facilitating vesicular sequestration. EMBO J 15: 1784–1791, 1996
43. Gaither LA, and Eide DJ: Functional expression of the human hZip2 Zinc transporter. J Biol Chem 275: 5560–5564, 2000
44. Gaither LA and Eide D: The human Zip1 transporter mediates zinc uptake in human K562 erythroleulemia cells. J Biol Chem 276: 22258–22264, 2001
45. Hoadley JE, Leinart AS, and Cousins RJ: Kinetic analysis of zinc uptake and serosal transfer by vascularly perfused rat intestine. Am J Physiol 252: G825–G31, 1987
46. Cousins RJ: Regulation of zinc absorption: Role of intracellular ligands. Am J Clin Nutr 32: 339–345, 1979
47. Cousin RJ: Absorption, transport and hepatic metabolism of copper and zinc: special reference to metallothionein and ceruloplasmin. Physiol Rev 65: 238–309, 1985
48. Palmiter RD: Molecular biology of metallothionein gen expression. Experientia 52(suppl): 63–80, 1987
49. McCormick CC, Menard MP, and Cousin RJ: Induction of hepatic metallothionein by feeding zinc to rats of depleted zinc status. Am J Physiol 240: E414–E421, 1981
50. Krebs NF: Overview of zinc absorption and excretion in the human gastrointestinal tract. J Nutr 130: 1374S–1377S, 2000
51. Sian L, Krebe NF, Westcott JE, Fengliang L, Tong L, Miller LV, Sonko B, and Hambidge M: Zinc homeostasis during lactation in a population with a low zinc intake. Am J Clin Nutr 75: 99–103, 2002
52. Lee DY, Prasad AS, Hydrick-Adair C, Brewer G, and Johnson PE: Homeostasis of zinc in marginal human zinc deficiency: role of absorption and endogenous excretion of zinc. J Lab Clin Med 122: 549–556, 1993
53. King JC, Shames DM, and Woodhouse LR: Zinc homeostasis in humans. J Nutr 130: 1360S–1366S, 2000
54. Brandao-Neto J, Silva CAB, Shuhama T, Silva JA, and Oba L: Renal handling of zinc in insulin-dependent diabetes mellitus patients. BioMetals 14: 75–80, 2001
55. Chen SM, Ho JM, and Young TK: Renal excretion of zinc in normal Chinese subjects. Chin Med J 39: 99–104, 1987
56. Baer M and King J: Tissue zinc levels and zinc excretion during experimental zinc depletion in young men. Am J Clin Nutr 39: 556–570, 1984
57. Dolev E, Deuster PA, Solomon B, Trostmann UH, Wartofsky L, and Burman KD: Alterations in Magnesium and zinc metabolism in thyroid disease. Metabolism 37: 61–67, 1988
58. Victery W, Smith JM, and Vander AJ: Renal tubular handling of zinc in the dog. Am J Physiol 241: F532–F539, 1981