Role of Metallothionein and Cysteine-Rich Intestinal Protein in the Regulation of Zinc Absorption by Diabetic Rats

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ABSTRACT

Hyperzincuria and low Zn absorption in diabetic animals and humans have prompted speculation that diabetics are more susceptible to Zn deficiency. There is little information, however, describing the effects of diabetes on the biochemical mechanisms of intestinal Zn transport. We evaluated Zn absorption in streptozotocin-induced diabetic rats based on a model of Zn transport in which cysteine-rich intestinal protein serves as an intracellular carrier that is inhibited by metallothionein (MT). Apparent absorption and retention of Zn and Cu in rats fed a purified diet were measured in a balance study 15–17 d after induction of diabetes. The rate of ^{65}Zn absorption from isolated intestinal segments, molecular distribution of ^{65}Zn in mucosal cytosol, and tissue MT levels were measured on d 20–22. Food consumption, and thus Zn and Cu intake, by diabetic rats was twice that of controls. Although fractional absorption (percent) of Zn and Cu was lower in the diabetic rats, net absorption ($\mu\text{g}/100\text{ g body weight/d}$) was higher. The higher net absorption in the diabetic group was offset, however, by higher urinary excretion, so that Zn and Cu retention was similar in both groups of animals. Low fractional absorption is attributable to the down-regulation of intestinal Zn transport, as indicated by the lower rate of ^{65}Zn

absorption from isolated intestinal segments in the diabetic rats. Down-regulation of intestinal transport is in turn attributable to higher concentrations of intestinal MT, which resulted in more ^{65}Zn in the mucosal cytosol bound to MT, an inhibitor of Zn transport, and less to cysteine-rich intestinal protein. These observations are similar to those previously described in rats consuming normal amounts of a diet containing excess Zn and are consistent with an antagonistic relationship between cysteine-rich intestinal protein and MT in Zn transport. The results suggest that decreased Zn absorption in diabetic rats is not a disease-related defect in Zn transport but instead is a homeostatic response to high Zn intake caused by overconsumption of a diet with a moderate Zn content. (*Pediatr Res* 37: 321–327, 1995)

Abbreviations

BW, body weight
CRIP, cysteine-rich intestinal protein
HEPES, *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid
MT, metallothionein
IDDM, insulin-dependent diabetes mellitus
STZ, streptozotocin

Diabetes is uniformly characterized by hyperzincuria in studies with humans (1–5) or animal models with either spontaneous or chemically induced diabetes (6–8). Controversy persists, however, regarding the effects of diabetes on Zn absorption and other aspects of Zn metabolism and homeostasis. Using a variety of methods, experimental models, and measures of Zn absorption, different investigators have interpreted their results to indicate decreased (9–11), increased (4, 12), or no difference (13, 14) in Zn absorption by diabetics. Similar inconsistencies regarding an effect on serum Zn concentration are also evident (1–8, 10–16). Conclusive evidence of high urinary Zn loss combined with limited evidence of depressed Zn absorption has prompted speculation that diabetics may be at greater risk of Zn deficiency (3, 10, 15, 17), and

that dietary Zn supplementation should be recommended (15, 17). However, because excessive Zn intake may inhibit normal Cu metabolism (18, 19), more conclusive evidence of decreased Zn absorption and risk of Zn deficiency is needed before dietary Zn supplements can be confidently recommended to diabetic patients.

Until recently, this evidence has been unavailable because of limited information about the biochemical mechanisms of intestinal Zn transport. It has been recognized for some time that Zn absorption is inversely correlated with intestinal concentrations of MT, a low-molecular weight intracellular Zn-binding protein (20, 21). Because intestinal MT gene expression is positively correlated with dietary Zn intake, it was suggested that MT has an essential role as a “mucosal block” in the homeostatic regulation of Zn transport during periods of high Zn intake. Although several studies have reported elevated levels of MT in livers and kidneys of diabetic rats (12, 16, 22),

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an effect of diabetes on intestinal MT has not been demonstrated. High liver and kidney MT concentration in diabetics is not considered indicative of high Zn status, because stress, glucocorticoid hormones, IL, and other factors also may mediate MT synthesis in these tissues (21, 23–26). The intestine, in contrast, is refractory to most mediators of liver MT synthesis other than heavy metals (23, 25–28).

Further insight into a possible mechanism of intestinal Zn transport followed the identification of CRIP in the soluble fraction of rat intestinal mucosa (29, 30). It was shown that in rats fed a moderate amount of Zn, CRIP binds a large proportion of ^{65}Zn entering the cell from the lumen, whereas very little was bound to MT. In contrast, ^{65}Zn bound to CRIP, and MT was markedly reduced and increased, respectively, in rats fed a high-Zn diet (180 mg/kg) that induces intestinal MT. It was concluded that high Zn intake stimulated the synthesis of intestinal MT, which inhibited binding and transport of Zn by CRIP. A model of Zn absorption was proposed in which CRIP serves as a diffusible intracellular carrier in transcellular mucosal Zn transport, whose function is competitively inhibited by MT to limit absorption and maintain Zn homeostasis during periods of high Zn intake.

The CRIP gene is developmentally regulated and predominantly expressed in the intestine (31, 32). It is also stimulated by glucocorticoid hormones in neonatal intestine (32, 33), suggesting a possible role in intestinal development. Unlike MT, CRIP gene expression is not regulated by short-term changes in dietary Zn (34), suggesting that homeostatic regulation of Zn absorption by acute high Zn intake is mediated primarily through the up-regulation of intestinal MT gene expression. Because the low Zn absorption reported in diabetics may be related to changes in intestinal CRIP or MT synthesis and their interaction, the present study examined Zn and Cu absorption and metabolism in a rat model of IDDM in the context of the model of intestinal Zn transport described above.

METHODS

Animals and diet. Male Sprague-Dawley rats (125–150 g) were obtained from Harlan Sprague-Dawley (Indianapolis, IN) and individually housed in stainless-steel cages in an environmentally controlled room (25°C, 12-h/12-h light/dark cycle). Food and deionized, distilled water were supplied *ad libitum* 5 d before the experiment and throughout the study. The composition of the egg white-based diet (Research Diets, Inc., New Brunswick, NJ) was based on the AIN-76 formulation (35, 36) and supplemented with ZnCO_3 and CuSO_4 . The diet was analyzed by atomic absorption spectrophotometry to contain 31 mg of Zn and 6 mg of Cu/kg of diet. Pair feeding to remove the confounding effects of food intake was not used in this study, because diabetic rats pair fed to the intake level of control rats exhibited high mortality in pilot studies. Care and treatment of experimental animals received prior institutional approval and followed recommended guidelines (37).

Materials. Unless otherwise stated, all chemicals were at least reagent grade and were obtained from Sigma Chemical Co. (St. Louis, MO) or U.S. Biochemical (Cleveland, OH).

Diabetes induction. After dietary acclimation, eight rats were randomly selected and injected s.c. with STZ to induce diabetes. Each dose (65 mg of STZ/kg BW) was prepared by dissolving 0.02 g of STZ in 0.2 mL of buffer (sodium citrate, 10 mol/L, pH 4.5) immediately before injection. Controls received a sham injection of buffer without STZ. Blood glucose levels were regularly monitored (One Touch II, Lifescan, Inc., Milpitas, CA) using one drop of whole blood obtained by clipping the tip of the tail between two glass microscope slides. Severe hyperglycemia, *i.e.* blood glucose greater than 11.1 mmol/L (200 mg/dL), was confirmed in all but one of the STZ-injected rats 48 h after injection. The nonresponding rat was removed from the study.

Zn and Cu balance study. All animals were individually housed in suspended stainless-steel metabolic cages from 14 to 21 d after the induction of diabetes. Food and water intake were recorded and urine and feces quantitatively collected daily on d 15–17, inclusive. The Zn contents of the diet, urine, and feces were determined by atomic absorption spectrophotometry as described below. Apparent absorption and retention of Zn and Cu were calculated as follows.

Apparent absorption data are presented as either fractional or net absorption. Fractional absorption is the amount of Zn or Cu absorbed expressed as a proportion of intake; these data have been converted to percentages for ease of interpretation. Net absorption represents the amount of Zn or Cu absorbed and is expressed as both μg per d and μg per d/100 g BW to normalize for differences in BW.

Zn and Cu measurements. The Zn and Cu contents of diet, urine, feces, plasma, and HPLC fractions were measured by air-acetylene flame atomic absorption spectrophotometry (model 3030B, Perkin Elmer, Norwalk, CT). Plasma, collected by cardiac puncture in heparinized syringes immediately after measurement of the rate of Zn absorption, was diluted 1:4 (vol/vol) in deionized, distilled water. Diet and homogenized feces were dried in a convection oven to constant weight, then ashed by microwave digestion (model MDS 2000, CEM, Matthews, NC). A measured amount (~ 0.2 g) of dried diet or feces was transferred to a Teflon digestion vessel, mixed with 5 mL of HNO_3 and 2 mL of HCl , then digested for 5 and 30 min at 50 and 100% power, respectively. After cooling, each sample was diluted to 100 mL with deionized, distilled water and analyzed.

Rate of Zn absorption. The rate of Zn absorption from isolated intestinal segments was measured on d 20–22 after the induction of diabetes. Each rat was anesthetized (60 mg of sodium pentobarbital/kg BW) and placed on a warm heating pad to maintain surface temperature at approximately 37°C. After median laparotomy, a 15-cm segment of the proximal small intestine immediately distal to the pancreatic and bile duct was rinsed with 10 mL of buffered saline (HEPES, 10 mmol/L, pH 7.5; NaCl, 154 mmol/L), ligated, then injected intraluminally with 1 mL of buffered saline containing 25 $\mu\text{mol/L}$ $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ and 200 kBq of ^{65}Zn (Du Pont New England Nuclear, Wilmington, DE). The isolated segment was returned to the body cavity for 30 min, then excised and flushed with cold homogenization buffer (HEPES, 10 mmol/L, pH 8.0; NaCl, 154 mmol/L; phenylmethylsulfonyl fluoride, 0.2

mmol/L; leupeptin, 0.6 mg/L; pepstatin A, 0.9 mg/L) into a test tube to quantitatively collect the ^{65}Zn remaining in the lumen. The ^{65}Zn content of the luminal fluid and the excised intestinal segment was determined by gamma spectroscopy (model 1272 Clinigamma, Pharmacia LKB, Gaithersburg, MD). Uptake was calculated as disappearance of ^{65}Zn from the lumen, intestinal accumulation of luminal Zn as the amount of ^{65}Zn remaining in the tissue of the intestinal segment after flushing, and absorption by subtracting intestinal accumulation from uptake. Isotope data were converted to molar concentrations based on the Zn content of the injected radioisotope solution and converted to rates based on the length of the absorption period.

Molecular distribution of intracellular ^{65}Zn . After rinsing, the excised intestinal segment was placed on a cold glass plate and slit longitudinally. The mucosal layer was then removed from the underlying submucosa by gently scraping the luminal surface with a glass microscope slide. Homogenates of intestinal mucosa and liver were prepared 1:3 (wt/vol) in homogenization buffer using a Potter-Elvehjem glass-Teflon tissue grinder. After centrifugation at $40\,000 \times g$ for 30 min at 4°C (model RC2-B, Sorval, Newtown, CT), the supernatant fraction was removed and filtered (Millex-GS, $0.22\ \mu\text{m}$, Millipore, Bedford, MA). Intracellular Zn-binding constituents were separated by size-exclusion HPLC using a $1 \times 30\text{-cm}$ Superdex 75 column (Pharmacia, Piscataway, NJ) and were eluted at $0.5\ \text{mL/min}$ (~ 300 pounds per square inch) with buffered saline (HEPES, $10\ \text{mmol/L}$, pH 7.4; NaCl, $154\ \text{mmol/L}$; NaN_3 , $0.2\ \text{g/L}$). The ^{65}Zn and Zn contents of the collected fractions ($0.5\ \text{mL}$) were determined by gamma spectroscopy and atomic absorption spectrophotometry, respectively. Mean recovery of ^{65}Zn from the Superdex 75 column was 96% and ranged from 83 to 112%.

MT concentration. The MT concentration of liver and intestinal mucosa was determined by [^{109}Cd]Hb affinity assay as previously described (23).

Statistical analysis. All data are expressed as the mean $\pm$ SEM and were analyzed by unpaired *t* test. Unless otherwise noted, significant differences between treatments were determined at $p < 0.05$. Variance homogeneity was tested by variance ratio, and heterogeneous data were transformed (log or square root) before statistical analysis.

RESULTS

General metabolism. During the balance study, the diabetic rats exhibited marked hyperphagia, consuming more than twice as much food as controls (Table 1). Polydipsia and polyuria, typically observed in uncontrolled diabetes as a mechanism to increase glucose excretion, were also clearly evident. Despite higher food intake, diabetic rats lost weight

during the balance study in marked contrast to the weight gained by the control rats. Mean weight gain for the entire 21-d study was significantly lower in diabetic ($18.5\ \text{g}$) than in control ($116.2\ \text{g}$) rats.

Zn and Cu balance. The approximately 2-fold higher food intake by the diabetic rats resulted in a similar increase in Zn (Table 2) and Cu (Table 3) intake. Both urine and fecal Zn and Cu excretion were also significantly higher in the diabetic rats than in controls. For a more complete picture of the effect of diabetes on Zn and Cu absorption, it is important to evaluate apparent absorption in terms of both fractional and net absorption. Fractional absorption (percent of intake) of Zn and Cu was significantly lower in the diabetic rats, indicating down-regulation of absorption in response to high Zn and Cu intake. However, because intake was higher in the diabetic group, lower fractional absorption resulted in net absorption ($\mu\text{g/d}$) equal to that observed in control rats. When net absorption was normalized to account for the lower BW of the diabetic rats, net absorption expressed as micrograms per $100\ \text{g BW/d}$ was significantly higher in the diabetic group. The greater amount of Zn and Cu absorbed by the diabetic rats was exactly offset, however, by higher urinary Zn and Cu excretion, resulting in Zn and Cu retention (absorption minus urinary excretion) that was similar in both groups. These data indicate that, despite markedly higher Zn and Cu intakes, the diabetic rats achieved homeostasis by a combination of decreased fractional absorption and increased urinary excretion.

Rate of Zn absorption. The depressing effect of diabetes on fractional absorption can be explained by the down-regulation of Zn transport from the lumen determined by measuring ^{65}Zn absorption from *in situ* isolated intestinal segments (Table 4). Because hyperplasia of the intestinal mucosa is a consistent feature of diabetes (12, 38), these data are presented as both net transport (unadjusted) and weight-normalized transport (per gram of intestine) to account for the significant difference in mucosal mass between diabetic and control rats (1.20 versus $1.04\ \text{g wet weight/15 cm intestine}$, respectively). Net Zn uptake from the lumen during the 30-min incubation period was similar in both groups. Although not significant ($p = 0.09$), net intestinal accumulation of luminal Zn was nearly 25% greater (similar to the increase in mucosal mass) in the diabetic rats than in the controls. In contrast, control rats absorbed nearly twice as much Zn as the diabetic rats. When normalized values were compared, the rate of Zn absorption by controls was more than two times greater than that observed in the diabetic rats. These data indicate that lower fractional absorption of the Zn ingested by the diabetic rats is attributable to a down-regulation of intestinal Zn transport mechanisms.

Table 1. Effect of diabetes on general metabolism in the rat

Treatment (n)	Food intake (g/d)	Water intake (mL/d)	Urine volume (mL/d)	Feces wt (g/d)	BW change (g/d)
Control (8)	15.6 ± 0.4	26.3 ± 1.0	8.8 ± 0.7	0.81 ± 0.07	2.76 ± 0.63
Diabetes (7)	32.9 ± 1.1	235.5 ± 10.3	232.2 ± 12.3	2.29 ± 0.08	-1.00 ± 0.45
<i>p</i>	<0.0001	<0.0001	<0.0001	<0.0001	0.0004

Values are means $\pm$ SEM. Balance data were collected on d 15–17 after induction of diabetes with STZ.

Table 2. Zinc balance in rats

Treatment (n)	Zn intake ($\mu\text{g/d}$)	Urine Zn excretion ($\mu\text{g/d}$)	Fecal Zn excretion ($\mu\text{g/d}$)	Apparent Zn absorption				
				Fractional (%)	Net		Zn retention	
					$\mu\text{g/d}$	$\mu\text{g}/100\text{ g BW/d}$	$\mu\text{g/d}$	$\mu\text{g}/100\text{ g BW/d}$
Control (8)	493 $\pm$ 12	6 $\pm$ 1	265 $\pm$ 24	46 $\pm$ 5	229 $\pm$ 25	94 $\pm$ 9	223 $\pm$ 25	92 $\pm$ 9
Diabetes (7)	1037 $\pm$ 35	66 $\pm$ 5	797 $\pm$ 34	23 $\pm$ 2	240 $\pm$ 19	144 $\pm$ 5	173 $\pm$ 16	104 $\pm$ 5
<i>p</i>	<0.0001	<0.0001	<0.0001	0.0003	NS	0.0004	NS	NS

Values are means $\pm$ SEM. Balance data were collected 15–17 d after induction of diabetes with STZ.

Table 3. Copper balance in rats

Treatment (n)	Cu intake ($\mu\text{g/d}$)	Urine Cu excretion ($\mu\text{g/d}$)	Fecal Cu excretion ($\mu\text{g/d}$)	Apparent Cu absorption				
				Fractional (%)	Net		Cu retention	
					$\mu\text{g/d}$	$\mu\text{g}/100\text{ g BW/d}$	$\mu\text{g/d}$	$\mu\text{g}/100\text{ g BW/d}$
Control (8)	99 $\pm$ 2	8 $\pm$ 1	61 $\pm$ 5	38 $\pm$ 5	38 $\pm$ 5	16 $\pm$ 2	31 $\pm$ 4	13 $\pm$ 2
Diabetes (7)	209 $\pm$ 7	24 $\pm$ 2	159 $\pm$ 6	24 $\pm$ 2	50 $\pm$ 5	30 $\pm$ 2	26 $\pm$ 4	16 $\pm$ 2
<i>p</i>	<0.0001	<0.0001	<0.0001	0.0205	NS	0.0002	NS	NS

Values are means $\pm$ SEM. Balance data were collected 15–17 d after induction of diabetes with STZ.

Table 4. Zinc absorption from isolated intestinal segments

Treatment (n)	Net transport			Weight-normalized transport		
	Uptake (nmol)	Intestinal accumulation of luminal Zn (nmol)	Rate of Zn absorption (pmol/min)	Uptake (nmol/g)	Intestinal accumulation of luminal Zn (nmol/g)	Rate of Zn absorption (pmol/min/g)
Control (5)	16.6 $\pm$ 1.0	10.7 $\pm$ 1.0	196 $\pm$ 12	16.0 $\pm$ 1.1	10.3 $\pm$ 0.8	191.0 $\pm$ 17.5
Diabetes (4)	16.3 $\pm$ 0.6	13.2 $\pm$ 0.8	102 $\pm$ 7	13.7 $\pm$ 1.0	11.1 $\pm$ 1.0	86.4 $\pm$ 9.3
<i>p</i>	NS	NS	0.0005	NS	NS	0.0018

Values are means $\pm$ SEM. Data were collected 20–22 d after induction of diabetes with STZ. Uptake was determined by disappearance of ^{65}Zn from the lumen of a 15-cm isolated segment of proximal small intestine 30 min after injection with 1 mL of buffered saline containing 25 $\mu\text{mol/L}$ $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ and 200 kBq of ^{65}Zn . Intestinal accumulation was based on the amount of ^{65}Zn remaining in the tissue of the intestinal segment. The rate of absorption was calculated by subtracting intestinal accumulation from uptake and dividing by time. Isotope data were converted to molar concentrations based on the Zn content of the injected radioisotope solution.

Molecular distribution of intracellular Zn and intestinal MT concentration. The effect of diabetes on the proposed carrier-mediated Zn transport system involving CRIP and MT was evaluated by measuring MT protein and determining the distribution of total Zn and ^{65}Zn in mucosal cytosol analyzed by size-exclusion HPLC (Fig. 1). Overall, total Zn distribution (which includes both luminal Zn and all other Zn previously accumulated in the mucosal cells) was similar in both groups of rats, although cytosol from diabetic rats showed a small increase in the amount of Zn eluting with MT. In contrast, markedly more ^{65}Zn (*i.e.* Zn that recently entered the cell from the lumen of the intestine) eluted in fractions containing MT in the diabetic group. Higher ^{65}Zn binding by MT in the diabetic rats was associated with proportionally lower ^{65}Zn -binding by both CRIP and higher-molecular weight constituents (see Fig. 1 legend). This evidence of higher intestinal MT in the diabetic rats was supported by data obtained by Cd-Hb affinity assay for MT protein (Fig. 2), which showed elevated MT in both liver and intestine of the diabetic rats. Because MT is recognized as an inhibitor of Zn absorption, these data suggest that the down-regulation of intestinal Zn transport, and by extension fractional absorption, is attributable to the up-regulation of intestinal MT. Because high intestinal MT resulted in less luminal Zn bound to CRIP, the inhibitory effect of MT on Zn

transport may be attributable to competitive binding of luminal Zn between MT and CRIP, the proposed intracellular Zn carrier.

Blood glucose and plasma Zn and Cu. Blood glucose analyzed at the time the animals were killed was 25.9 ± 1.5 and 4.5 ± 0.3 mmol/L in diabetic and control rats, respectively. Plasma Zn (2.54 ± 0.23 versus 1.77 ± 0.06 $\mu\text{g/mL}$) and Cu (0.89 ± 0.03 versus 0.76 ± 0.04 $\mu\text{g/mL}$) were both significantly higher in the diabetic rats. The amount of Zn absorbed during the 30-min ^{65}Zn absorption period represented less than 4% of the Zn in the plasma and did not contribute significantly to differences in plasma Zn concentration. These data correlate with the results of the balance data (higher net absorption and higher urinary excretion of Zn and Cu) and suggest that the Zn and Cu status of the diabetic rats was elevated compared with controls.

DISCUSSION

In these experiments, disease-related hyperphagia resulted in Zn and Cu consumption by diabetic rats that was twice that of controls fed the same diet containing a moderate amount of Zn and Cu (31 and 6 mg/kg diet, respectively). Because the growth rate of the diabetic rats was markedly lower, it seems likely

Figure 1. Molecular distribution of ^{65}Zn and total Zn in cytosol from rat intestinal mucosa determined by size-exclusion HPLC ($1 \times 30\text{-cm}$ Pharmacia Superdex-75; exclusion limit, 100 000 molecular weight). *In vivo*-isolated segments of proximal small intestine were injected intraluminally with ^{65}Zn to measure Zn absorption and label intracellular Zn-binding constituents. Significantly more ^{65}Zn ($p < 0.05$) was bound to MT (32 vs 9%) and less to CRIP (14 vs 22%) in cytosol from diabetic rats ($n = 4$) compared with controls ($n = 5$). These data show that Zn entering the cell from the lumen preferentially binds to MT rather than CRIP and other intracellular constituents when MT levels are elevated.

Figure 2. MT protein levels (means $\pm$ SEM) measured by Cd-Hb affinity assay 21 d after induction of diabetes with STZ. The MT content of proximal small intestine and liver of the diabetic rats was significantly higher than that of controls. *, $p < 0.01$.

that their requirement for these trace elements was also lower. But despite higher intake and lower requirement, the balance data showed that Zn and Cu retention by the diabetic rats was similar to that of the controls. These data further indicate that homeostasis was established in diabetic rats by a combination of low fractional absorption and high urinary excretion.

The biochemical basis underlying low fractional absorption by diabetic rats was further evaluated by measuring the rate of ^{65}Zn absorption from *in situ* isolated proximal intestinal segments. The results showed a lower rate of Zn absorption in the diabetic rats, indicating that intestinal Zn transport mechanisms were down-regulated. Measurement of Zn transport using ^{65}Zn assumes that a similar ^{65}Zn -to-Zn ratio is maintained in both treatment groups. Thus the lower rate of absorption observed in the diabetic rats could be a result of isotope dilution. However, prior studies have shown that intestinal Zn concentration is not

affected by diabetes (16), an observation that is supported in our experiments by the lack of treatment difference in the total Zn concentration of mucosal cytosol evaluated by HPLC. Similarly, other investigators also have reported that mucosa-to-lumen Zn transport is similar (12) or lower (11) in diabetic rats than in controls, and that endogenous Zn secretion is similar in both groups (12). Furthermore, our protocol minimizes opportunities for isotope dilution by preflushing the intestine, excluding pancreatic and biliary secretions from the isolated intestinal segment, and using a short interval during which absorption is measured. It is therefore unlikely that isotope dilution represents a source of error in the measurement of the rate of Zn transport in the present experiments.

The observed induction of intestinal MT in the diabetic rats was similar to that previously observed in nondiabetic rats fed a diet containing excess Zn (180 mg/kg diet) for 4 d (30). Both groups also exhibited altered molecular distribution of intracellular ^{65}Zn in the intestinal mucosa, with proportionally more ^{65}Zn bound to MT and less to CRIP. These data are consistent with the proposed carrier-mediated model of transcellular Zn transport (30) based on the antagonistic relationship between CRIP and MT. The two studies also indicate that high Zn intake, attributable either to overconsumption of a diet with a moderate Zn content or to normal consumption of a diet with a high Zn content, induces intestinal MT to limit Zn absorption and maintain homeostasis. Although altered CRIP synthesis also could contribute to the regulation of Zn homeostasis, it was recently shown that CRIP gene expression was not altered by short-term changes in Zn status that significantly alter MT synthesis and Zn absorption (34). Collectively, the results suggest that the down-regulation of Zn transport observed in the diabetic rats was a homeostatic response to excess Zn intake that was mediated through an effect of dietary Zn on MT gene expression and the inhibitory effect of MT on Zn binding by CRIP.

Evidence of increased intestinal MT in the diabetic rats is both new and seminal to our conclusion that, at least in these experiments, low Zn absorption was a beneficial homeostatic response to hyperphagia rather than a disease-related defect in Zn transport. It has long been recognized that tissue MT levels are positively correlated with Zn status or intake, and that MT has a role in the homeostatic regulation of Zn and other heavy metals (20, 21). Although elevated liver and kidney MT have been reported in diabetic rats previously (7, 12, 16, 22), these observations are not conclusive indicators of high Zn status, because stress, glucocorticoid hormones, interleukins, and other factors also may mediate MT synthesis in these tissues (21, 23, 24, 26). In contrast, intestinal MT gene expression is refractory to most mediators of liver MT synthesis (23, 26–28) but is closely and positively correlated with dietary Zn (20, 23).

Craft and Failla (12) reported no effect of diabetes on percent apparent (fractional) Zn and Cu absorption but, similar to our results, showed higher net absorption of Zn and Cu per unit of BW. They also reported that the rate of Zn absorption per gram of intestine from ligated duodenal segments was lower in the diabetic group. Although liver and kidney MT were elevated, the authors observed no difference in the concentration of intestinal MT, measured in this case by Hg

affinity assay. Lacking evidence of increased intestinal MT in the diabetic group, the authors concluded that the observed decrease in the rate of absorption was not the result of an intracellular mucosal block attributable to MT, but instead was the result of decreased net influx at the brush border surface. The results of the present study, however, clearly showed by both Cd-Hb affinity assay and by HPLC that intestinal MT is elevated and suggest a mucosal block mediated through a competitive interaction between MT and CRIP.

The implications of these results to human health and diabetic nutrition are unclear. Approximately 200 million people worldwide are afflicted with diabetes mellitus (39). Of the approximately 12 million diabetics in the United States, about 1 million have type I or IDDM. Although the STZ-induced diabetic rat is a model of IDDM, the effects of diabetes on Zn metabolism in human populations may differ markedly from those observed in the animal model. Most importantly, these experiments only examined the effects of short-term diabetes on Zn absorption and transcellular Zn transport; thus, the consequences of long-term diabetes and the associated metabolic complications (*e.g.* hypertension, microvascular disease, retinopathy, and inflammation) on Zn absorption and metabolism cannot be definitively extrapolated from our results. Also, although hyperphagia resulted in high Zn intake by the diabetic rats, increased Zn intake attributable to overconsumption of food probably does not represent a significant health problem to human diabetics for two reasons. First, hyperphagia is minimal in patients with IDDM who receive regular insulin therapy and who are in good glycemic control. Second, although marked hyperphagia may be observed in diabetics with significant hyperglycemia, *e.g.* noncompliant patients, homeostatic mechanisms that control absorption and excretion seem to regulate Zn and Cu status adequately over a fairly wide range of intakes.

Our evidence that diabetic rats consumed excess Zn as a result of disease-induced hyperphagia emphasizes the need to reevaluate the widely accepted premise that hyperzincuria and low Zn absorption in diabetics are necessarily indicative of increased risk of Zn deficiency. Our data clearly reiterate that, although high Zn excretion and low absorption can be a cause of Zn deficiency, they also may represent a response to high Zn intake. It should be remembered, however, that hyperzincuria and low Zn absorption may be either a cause of Zn deficiency or an effect of diabetic hyperphagia at different stages of the disease. For example, hyperphagia in uncontrolled diabetes may contribute to elevated Zn intake and up-regulation of homeostatic mechanisms to limit Zn retention. In contrast, long-term diabetes may adversely affect components of the Zn transport and excretion mechanisms, thereby increasing the susceptibility of the subject to Zn deficiency. Thus, although some studies have recommended Zn supplementation for diabetic patients because of the perceived potential for Zn deficiency (15, 17), our results indicate that food and Zn intake and Zn status should be carefully evaluated on a case-by-case basis before prescription of a Zn supplement. Incorporating these assessments into the patient's evaluation before supplementation would avoid exacerbating the potential adverse effects of a preexisting high Zn intake (18, 19).

In summary, these experiments showed that the STZ-induced diabetic rat is a useful model for the evaluation of Zn transport mechanisms and further substantiate the proposed antagonistic roles of MT and CRIP in carrier-mediated transcellular intestinal Zn transport. These data also suggest that decreased Zn absorption in the diabetic rat is a beneficial homeostatic response to hyperphagia and high Zn intake rather than a disease-related defect in Zn transport. The results showed that excess Zn intake, and the up-regulation of mechanisms to limit Zn absorption and maintain homeostasis, can result from overconsumption of a diet with a moderate Zn content. Whether this relationship between food intake and altered Zn metabolism can be extrapolated to diabetic humans, or represents a significant health concern, is uncertain. But further studies to evaluate the effects of long-term diabetes on Zn transport and the potential adverse effects of high Zn intake on Cu metabolism by diabetics seem warranted.

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