Znt7 (Slc30a7)-deficient Mice Display Reduced Body Zinc Status and Body Fat Accumulation

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In vitro studies have demonstrated that ZNT7 is involved in transporting the cytoplasmic zinc into the Golgi apparatus of the cell for zinc storage or to be incorporated into newly synthesized zinc-requiring enzymes/proteins. To evaluate the physiological role of ZNT7, we created a mouse model of Znt7 deficiency by a gene-trap approach. Znt7-deficient mice were zinc-deficient based on their low zinc content in serum, liver, bone, kidney, and small intestine. In embryonic fibroblasts isolated from Znt7-deficient mice, cellular zinc was ~50% that of wild-type controls. Znt7-deficient mice also displayed some classic manifestations of dietary zinc deficiency, such as reduced food intake and poor body weight gain. However, the mutant mice did not show any sign of hair abnormality and dermatitis that are commonly associated with dietary zinc deficiency. A radioactive feeding study suggested that Znt7-deficient mice had reduced zinc absorption in the gut resulting in decreased zinc accumulations in other organs in the body. The poor growth found in Znt7-deficient mice could not be corrected by feeding the mutant mice with a diet containing 6-fold higher zinc (180 mg/kg) than the suggested adequate intake amount (30 mg/kg). Furthermore, the reduced body weight gain of the mutant mice was largely due to the decrease in body fat accumulation. We conclude that ZNT7 has essential functions in dietary zinc absorption and in regulation of body adiposity.

Zinc plays essential roles in almost all aspects of metabolism and is required for the activity of hundreds of enzymes and DNA-binding proteins (1, 2). Zinc has a unique and extensive role in key biological processes, including immune function, growth, development, and reproduction (3, 4). Human and animal studies have shown that zinc deficiency is associated with anorexia, poor appetite, weight loss, and growth retardation (5–7). However, the molecular mechanisms that underlie the manifestations of zinc deficiency are poorly understood.

In mammals, dietary zinc is absorbed through the epithelium of the small intestine and transported into the bloodstream to the tissues and cells where zinc is needed. Body zinc homeostasis is tightly controlled, and the physiological zinc concentration is maintained largely through the regulation of zinc transporter systems for either zinc efflux/sequestration (SCL30 family) or zinc influx (SLC39 family). Eight ZNT family members (ZNT1–8) (8–15) and eight SLC39 family members (ZIP1–7 and ZIP14) (16–23) have been functionally characterized. Members in the same zinc transporter family have similar protein structures. For example, ZNT proteins have six transmembrane (TM) domains and a histidine-rich loop between TM domains IV and V except for ZIP2, which contains one histidine residue in this region (24). ZNT proteins function to decrease the cytoplasmic zinc concentration by promoting zinc export to the extracellular space or zinc sequestration into subcellular compartments when cellular zinc concentration increases with the exception of ZNT5 (isoform b) by which zinc can be transported bidirectionally (25). On the other hand, ZIP proteins function to bring up the cytoplasmic zinc concentration by enhancing zinc uptake or releasing the stored zinc from subcellular compartments to the cytoplasm when cellular zinc concentration drops (26).

We have recently identified and characterized a seventh member of the SLC30 zinc transporter family, ZNT7. Similar to the other members of the SLC30 family, ZNT7 contains six TM domains and a histidine-rich loop between TM domains IV and V. The message RNA of Znt7 is detected in many tissues, including liver, kidney, spleen, lung, and small intestine by Northern blot analysis, whereas the ZNT7 protein is only detected in lung and small intestine by Western blot analysis (14). Immunohistochemical analysis indicated that ZNT7 is abundantly expressed in the absorptive epithelium of the small intestine (14, 27). Immunofluorescence analysis revealed that ZNT7 is localized in the Golgi apparatus and plays a role in the accumulation of zinc in the Golgi apparatus (14). Moreover, electron microscopy analysis suggested that ZNT7 is dominantly localized in the cis-face or forming face of the Golgi apparatus (28). Overexpression of ZNT7 in Chinese hamster ovarian cells (14) led to an accumulation of zinc in the Golgi apparatus, indicating that ZNT7 may play important roles in

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2 The abbreviations used are: TM, transmembrane; ZNT, zinc transporter; ZIP, ZRT, IRT-like protein family; ES, embryonic stem cell; EST, expressed sequence tag; OGTT, oral glucose tolerance test; DMEM, Dulbecco’s modified Eagle’s medium; PBS, phosphate-buffered saline.
delivery of zinc into newly synthesized zinc-requiring enzymes/proteins and/or in the storage of zinc in the Golgi apparatus.

Targeted deletions of three ZNT members (ZNT1, -3, and -5) in mice have been reported (12, 29, 30). ZNT1 is the only member of the SLC30 family located primarily on the cell membrane and functions to export zinc to the extracellular space. Expression of ZNT1 is ubiquitous, and loss of ZNT1 function in mice is embryonically lethal (29). Homozygous Znt1 embryos died in the uterus by days 9–11 of the pregnancy (29). ZNT3 is localized to synaptic vesicles of glutamatergic neurons. Null mutation of the Znt3 gene resulted in a complete elimination of zinc from synaptic vesicles in brain, but Znt3−/− mice display normal electrophenochalographic activity (30). ZNT5 is the only member of the ZNT proteins having more than 6 TM domains (extra 10 TM at the N-terminal end of the protein). Loss of ZNT5 function results in poor growth, lean body composition, muscle weakness, hunched backs, and osteopenia. In addition, male Znt5−/− mice suffer from cardiac arrhythmia and die of heart block at ~15 weeks of age (12).

The lethal milk (Im) mice, an autosomal recessive mutant, arose in the strain C57BL/6J in the Jackson laboratory in 1964. Pups (0–5 days old) of any genotypes suckled on homozygous Im dams died of zinc deficiency at 5–8 days of age (6, 7, 31). A non-sense mutation in the Znt4 gene underlies the inherited zinc deficiency in the lethal milk mice (11). The defect prevents zinc from being incorporated into mother’s milk and, therefore, pups of lethal milk dams die of zinc deficiency before weaning (32–34).

To address the physiological roles of ZNT7, we generated a mouse model with an insertional mutation for the Znt7 gene and examined the effects of the null mutation of Znt7 on body zinc status, growth, and other parameters related to zinc homeostasis.

**MATERIALS AND METHODS**

*Animals and Diets*—Embryonic stem cells (ES, 129/Ola) that carried an insertional mutation in the Znt7 gene were obtained from the BayGenomics gene-trap resource (35–37). The ES cells were injected into C57BL/6 blastocysts to produce chimeric mice in the animal facility of the Murine Targeted Genomics Laboratory at University of California at Davis. The resulting two male chimeras were bred to C57BL/6 females to test for germ line transmission. Agouti offspring were genotyped by PCR using the genomic DNA isolated from tail biopsies. Animals heterozygous for the insertional mutation were intercrossed to obtain the homozygous animals and wild-type littermate controls. Pups from intercross litters were genotyped at 2 weeks of age by PCR. Congenic mice were produced by back-crossing the mutated gene into the C57BL/6 strain for ten generations using a genotype-directed breeding strategy (TaqMan® SNP Genotyping Assays, Applied Biosystems, Foster City, CA). The wild-type control (Znt7+/+) line was also established from the last back-crossing.

All mice were housed in a temperature-controlled room at 22–24 °C with a 12-h light:dark cycle and fed a standard laboratory chow diet (PicoLab® mouse diet 20) and double-distilled water ad libitum. Where indicated, mice were subjected to different diets in the study. Diets containing a defined amount of metal zinc (38) were purchased from Research Diets (New Brunswick, NJ).

Mice were fasted 16–18 h before they were killed by cardiac puncture while anesthetized by intraperitoneal injection of 250 mg/kg trimbromoethanol (Avertin®, Aldrich). Animal experiments were conducted in accordance with National Institutes of Health guidelines for the Care and Use of Experimental Animals and were approved by the Institutional Animal Care and Use Committee of the University of California at Davis.

**Isolation of Genomic DNA**—Genomic DNA was isolated from either mouse tail clips or primary cultured embryonic fibroblasts using a DNeasy Tissue kit (Qiagen, Valencia, CA).

**Genotyping**—Genomic DNA was subjected to PCR using primers designed to amplify a 383-bp fragment in the βgeo gene and a 341-bp fragment in the Znt7 gene (Fig. 1). PCR primers and cycling conditions were as follows: LacZ-F, 5′-CGTCTGACCTTGGG-3′ (in LacZ); LacZ-R, 5′-CTTCTCTGTTAGCAGCTTTCATC-3′ (in LacZ); 94 °C for 3 min, followed by 12 cycles at 94 °C for 30 min, 64.5 °C for 30 s, and 72 °C for 35 s, and then by 25 cycles at 94 °C for 30 s, 58 °C for 30 s, and 72 °C for 35 s. The final extension was at 72 °C for 3 min. DF, 5′-GAGCAGCCTACGGTTCCCTCAA-3′ (in Znt7); DR, 5′-TGCAAGAGTGGCAACCTCTCT-3′ (in Znt7); 94 °C for 3 min, followed by 35 cycles at 94 °C for 45 s, 56 °C for 45 s, and 72 °C for 45 s. The final extension was at 72 °C for 3 min. PCR products were detected by agarose-gel electrophoresis.

**Northern Blot Analysis**—Total RNA was isolated from small intestines of Znt7+/+, Znt7−/−, and Znt7−/+ mice using TRIzol reagent (Invitrogen). 10 µg of denatured RNA was loaded into each lane of a 1.0% agarose-formaldehyde gel, electrophoresed, transferred to a Hybond-N+ Nylon membrane (Amersham Biosciences), hybridized with a 32P-labeled cDNA fragment (679 bp) containing Znt7 exons 1–6. The blot was exposed to film at −80 °C overnight to get the images of the Znt7 transcripts on the blot. The blot was then stripped with 0.1% (w/v) SSC/0.1% (w/v) SDS solution at 65 °C until no 32P signal was detected. Finally, the blot was hybridized with a 32P-labeled LacZ cDNA fragment containing 1173 bp 5′-end of LacZ sequences, washed, and exposed to a film at −80 °C.

**Isolation and Culture of Primary Embryonic Fibroblasts**—Znt7+/+ (N2/F1), Znt7−/+ (congenic), or Znt7−/− (congenic) were mated, and the females were killed by cardiac puncture while anesthetized after 15.5 days of pregnancy. Uterine horns were removed and rinsed with 1× PBS, pH 7.4. Each embryo was separated from its placenta and surrounding membranes. Head and internal organs were removed from the embryos. After briefly rinsing the embryos in 1× PBS, pH 7.4, embryos were finely minced in a trypsin-EDTA solution (0.25%, 2 ml, Invitrogen) and incubated at 37 °C for 15 min with gentle shaking. The resulting cell suspensions were neutralized with 8 ml of Dulbecco’s modified Eagle’s medium (DMEM) supplemented with 10% fetal bovine serum containing 100 units/ml penicillin and 100 µg/ml streptomycin (Invitrogen). The cell suspensions were then plated in 100-mm tissue culture dishes, and cells were cultured at 37 °C for 24 h. The culture medium was changed after 24-h incubation.
Antibodies—The affinity-purified anti-ZNT7 polyclonal antibody was made as described previously (14). The Alexa 488-conjugated goat anti-rabbit antibody was purchased from Molecular Probes (Invitrogen).

Immunofluorescence Microscopy—Primary embryonic fibroblasts were cultured in a slide chamber for 48 h, fixed with 4% paraformaldehyde, and permeabilized with 0.4% saponin (Sigma). Cells were subsequently stained with an affinity-purified anti-ZNT7 antibody (1:100 dilution) followed by an Alexa 488-conjugated goat anti-rabbit antibody (1:250 dilution). Photomicrographs were obtained by a Nikon Eclipse 800 microscope equipped with a digital camera.

65Zn Accumulation, Uptake, and Efflux Assays in Embryonic Fibroblasts—Cells (passages 3–4 that were cultured over a 2- to 3-week period) were grown to 90% confluence, harvested, and washed once in a cold uptake buffer (15 mM HEPES, 100 mM KCl, 1 mM EDTA, pH 7.0) (17). Cells were resuspended in the uptake buffer, and cell numbers were determined by the standard curve method (17). Tubes containing 1 × 10^6 cells in 150 µl of uptake buffer were incubated in a shaking incubator at 37 °C for 10 min. For 65Zn accumulation and uptake assays, an equal volume of pre-warmed uptake buffer containing 14 pmol of 65ZnCl₂ (specific activity, 0.17 mCi/µg, Oak Ridge National Laboratory, Oak Ridge, TN) was added, and the mixture was incubated for the indicated time points. For 65Zn export assay, an equal volume of pre-warmed uptake buffer containing 50 pmol of 65ZnCl₂ (specific activity, 0.05 mCi/µg, Oak Ridge National Laboratory) was added, and the mixture was incubated for 15 min at 37 °C. 2.4 µl of 0.25 mM cold ZnCl₂ (final concentration, 2 µM) was added, and the mixture was incubated for the indicated time points. Reactions were stopped by adding 300 µl of ice-cold stop buffer (15 mM HEPES, 100 mM glucose, and 150 mM KCl, pH 7.0) (17). Cells were collected by filtration on glass-fiber filters (GF/C, Whatman Inc., Florham Park, NJ) and washed three times in the stop buffer (10 ml of the total wash volume). Cell-associated radioactivity was measured with a Packard Auto-Gamma 5650 γ-counter (Packard Instrument Company, Downers Grove, IL).

Oral 65Zn Administration—Male mice (N2/F3-4) of both genotypes (littermates) at 6 weeks of age were fasted for 16 h. 1.0 µCi of 65ZnCl₂ (specific activity, 0.17 mCi/µg, Oak Ridge National Laboratory) in 250 µl of 1× PBS, pH 7.4, were gavaged. Mice were kept in cages with free access to the drinking water for 4 h after oral radioisotope administration. Mice were killed by cardiac puncture while anesthetized. Whole blood was collected, and serum was isolated. Tissues, including small intestine, liver, kidney, spleen, brain, heart, and lung, were also collected. 65Zn radioactivities in serum and collected tissues were measured with a Packard Auto-Gamma 5650 γ-counter (Packard Instrument Co.).

Immunohistochemical Analysis—The duodenum (a 2-cm segment of the mouse small intestine adjacent to the pyloric sphincter) was isolated from a 5-week-old C57BL/6J mouse. Tissue was flushed twice with an ice-cold 1× PBS, pH 7.4, and once with an ice-cold 4% paraformaldehyde solution made in 1× PBS, pH 7.4. Tissue was then fixed in the ice-cold 4% paraformaldehyde solution at 4 °C overnight. Tissue embedding and sectioning were carried out as described previously (27). Immunohistochemical analysis was performed with an avidin-biotin peroxidase system (Vector Laboratories, Burlingame, CA) using an affinity-purified rabbit anti-ZNT7 antibody as described by Yu et al. (27). Expressed ZNT7 was indicated by the deposition of a brown product by using a 3’,3’-diaminobenzidine substrate kit (Vector Laboratories). Sections were counterstained briefly in hematoxylin.

Tissue Zinc Analysis—Congenic male mice, 5 weeks old, of both genotypes (Znt7+/+ and Znt7−/−) were fed with a semi-purified diet containing 30 mg/kg zinc for 3 weeks. Mice were fasted for 16–18 h before killing. Whole blood was collected by retro-orbital bleeding while mice were anesthetized. Serum was then isolated and stored at −80 °C. Mice were killed by cardiac puncture. Tissues, including liver, duodenum, kidney, spleen, brain, skeletal muscle (from the hind limb), and bone (femoral), were collected, weighed, and stored at −80 °C. For tissue zinc analysis, samples were transferred into Teflon-lined digestion vessels, and 2.5–3.0 ml of concentrated nitric acid (trace element grade) were added into the vessels. Vessels were sealed, microwaved for 10 min at 300 watts, and then held at 300 watts for an additional 30 min (115 °C) in a Microwave Accelerated Reaction System (MARS5, CEM Corp., Matthews, NC). The digested samples were then diluted 2- to 3-fold in triple filtered deionized water. The serum samples (100 µl) were digested with 2 ml of 1.0 N nitric acid at room temperature until the solution was clear. Tissue and serum zinc contents were determined by an inductively coupled plasma atomic emission spectroscopy (Varian, Palo Alto, CA).

Total RNA Isolation and cDNA Synthesis—Primary embryonic fibroblasts were grown for 48 h and harvested. The total RNA was purified by a micro total RNA purification kit (Invitrogen). The cDNA was synthesized from 3 µg of total RNA using a SuperScript Choice system (Invitrogen).

Quantitative PCR Analysis—cDNA was diluted 4-fold, and 2 µl of cDNA was added to a quantitative PCR mixture containing corresponding primer pairs and a FAM-labeled TaqMan probe (Applied Biosystems). The quantitative PCR was performed on a PRISM® ABI 7900HT Sequence Detection System (Applied Biosystems) in triplicate, and the expression of Gapdh (glyceraldehyde-3-phosphate dehydrogenase) was used for normalization. Copy numbers for the Znt1 transcript were calculated using a standard curve method and normalized to the copy numbers of the Gapdh transcripts as described previously (38).

Growth Curves—4-week-old male Znt7−/− and wild-type littermate controls were housed individually. Mice were fed ad libitum a semi-purified diet containing 5 (marginal zinc-deficient), 30 (zinc-adequate), or 180 (zinc-supplement) mg/kg zinc carbonate, for 22 weeks. Body weights were measured every other week.

Food Intake—Congenic male 5-week-old Znt7−/− mice and the wild-type controls were housed individually. Mice were fed ad libitum a semi-purified diet containing either 30 mg/kg or 5 mg/kg zinc carbonate. Food consumption was measured twice a week for 3 weeks.

X-ray Analysis—Mice were anesthetized, and the x-ray image was captured by a Faxitron Specimen Radiography System (Faxitron X-Ray Corp., Wheeling, IL).
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Body Composition—Mice were fasted for 16 h, weighed, and killed by cardiac puncture while anesthetized. Carcasses were weighed and stored at −20 °C in sealed containers until analysis. The chemical body composition analysis was done as previously described (39).

Analyses—Blood glucose levels were determined using a OneTouch Ultra Mini meter (LifeScan, Milpitas, CA). Serum or plasma insulin and leptin were measured using an ultra sensitive insulin enzyme-linked immunosorbent assay kit and a mouse leptin enzyme-linked immunosorbent assay kit, respectively (Crystal Chem Inc., Downers Grove, IL). Serum total cholesterol and triacylglycerol were determined using cholesterol-E and L-Type triglyceride H assays, respectively, from Wako (Richmond, VA).

Oral Glucose Tolerance Test—Congenic male 5-week-old Znt7+/− and Znt7+/+ mice were fed ad libitum a 30 mg/kg zinc diet for 3 weeks. OGTT was performed when mice reached 8 weeks of age. Before OGTT, mice were fasted for 16 h, weighed, and then gavage-fed 20% glucose (w/v) at 1.5 g/kg of body weight. Blood was collected from the tail vein. Blood glucose and plasma insulin were measured at 0, 15, 30, 60, and 120 min after the glucose administration.

Data Analysis—Results are presented as the mean ± S.D. or S.E. Two-way analysis of variance was used to compare the results from 65Zn uptake, accumulation, and efflux analyses between the two genotypes. Two-way repeated analysis of variance was used to compare the results from OGTT and subsequent plasma insulin analysis between the two genotypes. Longitudinal Data Analysis using linear mixed effect model (SAS PROC MIXED) was used for comparison of body weight gains and body compositions between the two genotypes. Student’s t test was used in comparisons of two test groups with a two-tailed distribution for 65Zn accumulation in mouse tissues 4 h after oral administration, tissue-associated zinc, fasting blood glucose, triglyceride, cholesterol, insulin, and leptin analyses. Differences were considered to be significant at p < 0.05.

RESULTS

Generation of Znt7-deficient Mice—Mouse ZNT7 is a 378-amino acid protein encoded by eleven exons located on the reverse strand of mouse chromosome 3. To generate Znt7 knockout mice, mouse embryonic stem cells (ES) line harboring a insertional mutation in the intron 6 of the Znt7 gene by the gene-trap technology were obtained from the BayGenomics (Fig. 1a) (36, 37). This insertional mutation truncated the ZNT7 protein at amino acid residue 221. The truncation deleted two potential transmembrane domains at the C terminus of Znt7, which was predicted to abolish the function of ZNT7. Chimeric male mice were produced by the injection of these ES cells into C57BL/6 blastocysts. The chimeras were then crossed to C57BL/6j females to generate the heterozygous offspring. Genomic DNA isolated from tail biopsies was used for genotyping (Fig. 1b). We found that the progenies from the heterozygous mating contained all three genotypes (Znt7+/+, Znt7+/−, and Znt7−/−) in the expected Mendelian ratio (data not shown). Mice of both genders with the disrupted Znt7 alleles were viable, fertile, and displayed no obvious morphological abnormalities.

Southern analysis confirmed that only one copy of the trap vector had been incorporated into the genome of the Znt7-deficient mice (data not shown). Northern blot analysis using a DNA probe against the Znt7 gene showed the absence of the Znt7 mRNA in Znt7−/− mice (Fig. 2a). Instead, a larger fusion transcript containing the mRNA sequences from Znt7 exons 1–6 and LacZ was detected in Znt7−/− mice as a result of the insertional mutation (Fig. 2a). This fusion transcript was also recognized by a DNA probe against the LacZ gene (Fig. 2b).

Lower Cellular Zinc Accumulation in the Embryonic Fibroblasts of Znt7-deficient Mice—A previous study from our laboratory demonstrated that ZNT7 played an important role in the accumulation of intracellular zinc in zinc replete conditions (21). To determine whether the loss of ZNT7 function reduced the intracellular zinc accumulation, we performed 65Zn accumulation using primary embryonic fibroblasts isolated from 15.5-day-old Znt7−/− embryos and their wild-type littermate controls. In this experiment, we first confirmed that Znt7 was expressed in the wild-type mouse embryonic fibroblasts by immunofluorescence microscopic analysis.
ZNT7 was mainly localized in the perinuclear region of the fibroblast, which was consistent with its Golgi apparatus localization reported previously (Fig. 3a) (14). Loss of both Znt7 alleles in the mutant embryonic fibroblast resulted in a reduction in cellular zinc accumulation in the cell. As shown in Fig. 3b, the cellular accumulation of $^{65}$Zn (0.09 µM) over a period of 45 min was greatly reduced (~50% reduction, p < 0.05) in Znt7$^{-/-}$ embryonic fibroblasts compared with that in Znt7$^{+/+}$ embryonic fibroblasts. The reduction in the cellular $^{65}$Zn accumulation in Znt7$^{-/-}$ embryonic fibroblasts was not due to the incompetence of zinc uptake system of the cell as the initial rates of $^{65}$Zn uptake were not significant different in both genotypes of embryonic fibroblasts (Fig. 3c).

To determine whether the block of zinc influx into the Golgi apparatus in Znt7$^{-/-}$ fibroblasts affected the zinc efflux system, zinc efflux in both Znt7$^{+/+}$ and Znt7$^{-/-}$ fibroblasts was assessed by competition for $^{65}$Zn efflux over a period of time in cells pre-loaded with $^{65}$Zn with 10-fold molar excesses of cold ZnCl$_2$. As shown in Fig. 3d, 53% $^{65}$Zn was released from Znt7$^{-/-}$ fibroblasts 2.5 min after cold substrate was added, whereas only 33% $^{65}$Zn was released from the control cells (p < 0.0001). It is known that zinc efflux is carried out by ZNT1 (8, 40, 41), and the expression of Znt1 mRNA was measured by quantitative reverse transcription-PCR with a TaqMan probe specific for the mouse Znt1. As shown in Fig. 3e, the abundance of Znt1 mRNA was increased (~40%) in the fibroblasts of Znt7$^{-/-}$ mice compared with that of Znt7$^{+/+}$ mice. Taken together, the block of zinc influx into the Golgi apparatus due to the null-mutation of Znt7 affects cellular zinc homeostasis that leads to an increase in zinc concentration in the nuclei of the cell. The increased zinc efflux of
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Znt7−/− fibroblasts is explained in part by the increased expression of ZNT1 in these cells.

Low Dietary Zinc Absorption in Znt7-deficient Mice—It has been suggested that Znt7 may play a role in dietary zinc absorption as it is highly expressed in the mouse small intestine (Fig. 4a) (14, 27). Having demonstrated that cellular zinc accumulation was reduced in the Znt7−/− embryonic fibroblasts, we hypothesized that Znt7-deficient mice would not absorb as much dietary zinc as their wild-type littermates, because Znt7 was necessary for the process of zinc absorption in the absorptive epithelial cells of the gut. To test our hypothesis, a 65Zn absorption assay was performed. Male Znt7-deficient mice (6 weeks old) and their wild-type littermates were fasted for 16 h. 1 μCi of 65ZnCl2 in 250 μl of 1× PBS, pH 7.4, was given orally. Tissues, including small intestine, liver, spleen, kidney, brain, lung, and blood, were collected and analyzed for radioactive zinc contents 4 h after administration. As shown in Fig. 4b, Znt7-deficient mice had significantly less radioactive zinc accumulation in the small intestine (∼49% of wt, p < 0.01), liver (∼58% of wt, p < 0.05), and kidney (∼55% of wt, p < 0.05), but not in the spleen when compared with the Znt7+/+ controls. Brains, lungs, and sera from both genotypes had little radioactivity 4 h after oral 65Zn dose (data not shown). Taken together, these results suggest that ZNT7 is required in dietary zinc absorption in the gut.

FIGURE 4. Oral 65Zn administration in Znt7-deficient mice. a, expression of ZNT7 in the mouse duodenum. Immunohistochemical staining of ZNT7 was performed as described under “Materials and Methods.” The control section was incubated with a preimmune serum isolated from the rabbit from which the anti-ZNT7 antibody was raised. Sections were counterstained with hematoxylin. Brown color shows the immunoreactivity. b, 65Zn radioactivity in mouse tissues 4 h after oral administration. The 65Zn radioactivity was expressed as picomoles of 65Zn per gram of tissue and plotted for both genotypes. The data points represent the mean ± S.E, n = 5. Student t test was used for statistical calculations. Small intestine, p < 0.01; liver and kidney, p < 0.05; spleen, p > 0.05.

Low Zinc Status in Znt7-deficient Mice—Having demonstrated that Znt7 is crucial for dietary zinc absorption, we next asked whether the loss of two alleles of Znt7 would affect the body zinc status. Congenic male mice (N10/F2, C57BL/6j genetic background) of both genotypes (Znt7+/+ and Znt7−/−) at 5 weeks of age were fed a semi-purified diet containing 30 mg/kg zinc carbonate (a zinc-adequate diet) for 3 weeks. Mice were killed after 16 h fasting. Blood and tissues (liver, duodenum, spleen, kidney, brain, muscle, and bone) were collected. Total tissue-associated zinc was determined by inductively coupled plasma-atomic emission spectrometry. As shown in Table 1, significant differences in tissue-associated zinc contents were observed in the serum, liver, duodenum, kidney, and bone between two genotype groups (Znt7+/+ versus Znt7−/−). The mean percent reductions in zinc contents observed in Znt7-deficient mice were 20.7%, 9%, 10.7%, 5.7%, and 17% in the serum, liver, duodenum, kidney, and bone, respectively, suggesting that Znt7-deficient mice have lower body zinc status than that in the age- and sex-matched wild-type controls.

Low Body Weight Gain in Znt7-deficient Mice—Based on the previous studies on dietary zinc deficiency (44–47), we predicted that Znt7-deficient mice would gain less body weight than the wild-type controls due to their low body zinc status. We, therefore, monitored the body weight gain in Znt7-deficient mice and compared it to that of the wild-type controls after weaning. Male mice of both genotypes (littermates) at age of 4 weeks of age were housed individually and fed a zinc-adequate diet (30 mg/kg zinc). The body weights were measured every week for 22 weeks. As shown in Fig. 5, male Znt7-deficient mice grew slower than their wild-type littermates over a period of 22 weeks (p < 0.0001). At 4 weeks of age, the percent difference in body weights between the genotypes was ∼5%, whereas the difference was ∼18% at 26 weeks of age (Fig. 5). The less body weight gain observed in Znt7-deficient mice could not be corrected by feeding the mutant mice with a zinc supplement diet (180 mg/kg zinc) for 22 weeks (p < 0.0001, Fig. 5). On the other hand, when mice were fed a marginal zinc-deficient diet (5 mg/kg zinc) Znt7-deficient mice showed less dramatic effect on the growth compared with that of the wild-type littermates (p = 0.054). Despite less body weight gain, Znt7-deficient mice remained healthy for at least 18 months and showed normal somatic development and reproductive ability (data not shown). Female Znt7-deficient mice displayed similar, but much less striking difference in the body weight (data not shown).

Table 1

| Tissues         | Genotype | p value |
|-----------------|----------|---------|
|                 | Znt7+/+  | Znt7−/− |
| Serum           | 0.87 ± 0.07 | 0.69 ± 0.04 | <0.001 |
| Liver           | 25.60 ± 0.52 | 23.30 ± 1.67 | 0.002 |
| Duodenum        | 22.01 ± 2.05 | 19.65 ± 1.58 | 0.022 |
| Spleen          | 16.29 ± 0.77 | 16.26 ± 0.51 | 0.931 |
| Kidney          | 15.68 ± 0.65 | 14.78 ± 0.71 | 0.019 |
| Brain           | 10.72 ± 0.52 | 11.12 ± 1.22 | 0.414 |
| Muscle          | 6.13 ± 1.06 | 6.49 ± 1.63 | 0.607 |
| Bone            | 75.52 ± 8.17 | 62.65 ± 7.49 | 0.005 |

a Units are milligrams/liter for serum and milligrams/kg wet tissue for all others.
To determine whether the difference in growth between Znt7-deficient mice and their wild-type controls was caused by an appetite change, food intakes of male Znt7+/− and Znt7+/+ mice over a 3-week period (5–8 weeks of age) were measured. Male Znt7-deficient mice fed either the zinc-adequate (30 mg/kg zinc) or the marginal zinc-deficient (5 mg/kg zinc) diet had less cumulative food intake than their respective wild-type controls (54.4 g ± 1.0 mutant versus 66.2 g ± 1.7 wt, n = 9 for the 30 mg/kg zinc diet group, p < 0.0001; 50.6 g ± 1.3 mutant versus 59.7 g ± 0.35 wt, n = 6–8 for the 5 mg/kg zinc diet group, p < 0.0001).

Low Fat Mass in Znt7-deficient Mice—Previous studies demonstrated that both ZNT7 and ZNT5 functioned in loading the cytoplasmic zinc into the Golgi apparatus (12, 14, 48, 49). Null-mutation of Znt5 resulted in abnormalities in bone development (12). To investigate whether Znt7-deficient mice developed a similar phenotype to the Znt5 mutant in bone development, an x-ray analysis was carried out. The radiographic image showed that Znt7-deficient mice had normal bone development compared with the wild-type control in terms of the bone length and density (Fig. 6). The x-ray image also suggested that Znt7-deficient mice might have less fat tissues accumulated in the body. As a result, body compositions of male Znt7-deficient mice and their wild-type littermate controls were investigated.

As shown in Fig. 7, in dietary groups of zinc-adequate (30 mg/kg) and zinc supplement (180 mg/kg), male Znt7-deficient mice had lower body and carcass weights than the wild-type controls (p < 0.0001). Significant differences in the body fat mass (expressed on an absolute weight of the body fat (Fig. 7a) and a percentage of the carcass weight (Fig. 7b)) were observed between Znt7+/− and Znt7+/+ mice (5.06 g ± 0.99 and 18.17% ± 2.84 mutant versus 10.18 g ± 1.49 and 29.06% ± 2.63 wt, n = 6 for the 30 mg/kg zinc diet group, p < 0.01; 4.27 g ± 0.72 and 16.65% ± 2.25 mutant versus 12.32 g ± 0.81 and 33.26% ± 0.89 wt, n = 6 for the 180 mg/kg zinc diet group, p < 0.0001). In addition, Znt7-deficient mice exhibited small reduction in the weight of the lean mass compared with the controls (11.66 g ± 0.30 mutant versus 12.28 g ± 0.37 wt, n = 6 for the 30 mg/kg zinc diet group, p < 0.01; 11.20 g ± 0.35 mutant versus 12.32 g ± 0.36 wt, n = 6 for the 180 mg/kg zinc diet group, p < 0.0001) (Fig. 7a). However, when the lean mass was expressed on a percentage basis, Znt7+/− mice displayed a higher percentage of lean mass in the body (43.46% ± 1.65 mutant versus 36.51% ± 1.59 wt for the 30 mg/kg zinc diet group, p < 0.01; 45.06% ± 1.49 mutant versus 34.40% ± 0.98 wt for the 180 mg/kg zinc diet group, p < 0.0001) (Fig. 7b), suggesting that Znt7-deficient mice were relatively lean because of the reduction of the body fat mass. Furthermore, significant differences in the weight and percentage of the ash content were observed between the two genotypes in 30 and 180 mg/kg dietary groups (1.05 g ± 0.13 wt, n = 6 for the 30 mg/kg zinc diet group, p < 0.01; 1.08 g ± 0.30 wt, n = 6 for the 180 mg/kg zinc diet group, p < 0.01). In addition, Znt7+/+ mice were relatively lean because of the reduction of the body fat mass. Furthermore, significant differences in the weight and percentage of the ash content were observed between the two genotypes in 30 and 180 mg/kg dietary groups (1.05 g ± 0.13 wt, n = 6 for the 30 mg/kg zinc diet group, p < 0.01; 1.08 g ± 0.30 wt, n = 6 for the 180 mg/kg zinc diet group, p < 0.01) (Fig. 7). No differences in the body weight, carcass weight, fat mass, lean mass, and ash content were observed between Znt7+/− and Znt7+/+ mice fed the marginal zinc-deficient diet (p > 0.05).

Taken together, the significant decrease in the body fat, lean, and ash mass in Znt7+/− mice reflected the differences in the body weight gain between Znt7+/− and Znt7+/+ mice. Znt7-deficient mice were relatively lean compared with the wild-type control mice due to the dramatic decrease in the body fat mass.

Interestingly, in wild-type mice, restriction in dietary zinc intake had a more dramatic effect on the body fat mass than on the lean mass and ash content. For example, when the fat mass was expressed either on a weight or a percentage basis, wild-type mice fed the 5 mg/kg zinc diet displayed significantly less body fat mass than that of the wild-type mice fed the 30 mg/kg zinc diet.

FIGURE 5. Postnatal growth curves of male Znt7-deficient mice and the wild-type littermate controls. Offspring (N1–6/F3) from the Znt7+/− mating was fed ad libitum a semi-purified diet containing 5, 30, or 180 mg/kg zinc carbonate from 4 to 26 weeks of age. Body weights were measured every other week. The data points represent the mean ± S.E. Seven Znt7+/− mice and nine Znt7+/+ littermate controls were in the 30 mg/kg zinc diet group. Six Znt7+/− mice and four Znt7+/+ littermate controls were in the 180 mg/kg zinc diet group. Three Znt7−/− mice and three Znt7+/− littermate controls were in the 5 mg/kg zinc diet group. Znt7−/− versus Znt7+/+, 5 mg/kg zinc diet group, p = 0.05; 30 mg/kg zinc diet group, p < 0.0001; and 180 mg/kg zinc diet group, p < 0.0001.

FIGURE 6. Radiographic image of a Znt7-deficient mouse and a wild-type littermate control. Male mice (N1/F3) of both genotypes were fed a 30 mg/kg zinc diet from 4 to 32 weeks. X-ray image was captured by a Faxitron Specimen Radiography System while mice were anesthetized.
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Serum Biochemistry and Hormones of Znt7-deficient Mice—To further investigate the biological basis for the less body fat gain phenotype observed in Znt7-deficient mice, we examined levels of serum glucose, triglycerides, total cholesterol, insulin, and leptin. 5-week-old male Znt7+/− and Znt7+/+ mice were fed a 30 mg/kg zinc diet for 3 weeks. Mice were fasted 16 h before killing. Serum was isolated and analyzed. As shown in Table 2, fasting serum leptin levels were significantly lower (p < 0.05) in Znt7+/− mice versus Znt7+/+ mice. However, fasting blood glucose, serum triglyceride, cholesterol, and insulin levels were not significantly affected by the absence of the Znt7 gene.

Glucose Metabolism and Insulin Secretion—To examine whether the low adiposity of Znt7-deficient mice was the result of altered glucose metabolism due to the defect in insulin secretion, an OGTT was performed in Znt7−/− and Znt7+/+ mice after 16 h of fasting. At 8 weeks of age after 3 weeks on the 30 mg/kg zinc diet, blood glucose at 0, 15, 30, 60, and 120 min after the oral glucose load (1.5 g of glucose/kg of body weight) in Znt7−/− mice (n = 7) was 86 ± 3, 423 ± 21, 388 ± 23, 290 ± 23, and 263 ± 32 mg/dl, respectively. (Fig. 8a). These were not significantly different (p > 0.05) from the levels observed in Znt7+/+ mice (92 ± 5, 392 ± 29, 322 ± 22, 249 ± 27, and 195 ± 17 mg/dl, respectively, n = 7) (Fig. 8a). Plasma insulin levels in Znt7−/− mice (n = 7) during the OGTT were 0.08 ± 0.03, 0.30 ± 0.06, 0.26 ± 0.03, 0.23 ± 0.02, and 0.20 ± 0.04 ng/ml, respectively, (Fig. 8b). Again, these were not significantly different (p > 0.05) from the levels observed in Znt7+/+ mice (0.06 ± 0.02, 0.27 ± 0.03, 0.18 ± 0.05, 0.17 ± 0.04, and 0.17 ± 0.04 ng/ml, respectively, n = 7) (Fig. 8b).

**DISCUSSION**

The results presented in this study demonstrate that Znt7-deficient mice accumulated less zinc in cells (embryonic fibroblasts) and tissues (blood, duodenum, liver, kidney, and bone), indicating that the mutant mice had low body zinc status. As a result, Znt7−/− mice showed the phenotypes of reduced food intake (anorexia) and poor growth, which are classic clinical manifestations of zinc deficiency (5, 50). Znt7-deficient mice were lean, a phenotype that was seen in Znt5 null-mice (12), due to a significant reduction in the body fat composition. Although Znt7-deficient mice presented low body zinc status, they did not show any sign of hair growth abnormality and dermatitis that are commonly seen in zinc-deficient animals and humans (5, 50), suggesting that one or more functionally redundant genes of Znt7 exist. In combination of the *in vivo* results presented in this study and the *in vitro* data presented previously (14, 48), it is evident that ZNT7 plays an essential role in the maintenance of homeostatic zinc levels.

Our previous study demonstrated that the ZNT7 protein is not detectable in the mouse liver regardless of abundant Znt7

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**TABLE 2**

Fasting glucose, triglyceride, cholesterol, insulin, and leptin levels in Znt7+/+ and Znt7−/− mice

| Genotype  | Glucose (mg/dl) | Triglyceride (mg/dl) | Total cholesterol (mg/dl) | Insulin (ng/ml) | Leptin (pg/ml) |
|-----------|----------------|---------------------|---------------------------|----------------|----------------|
| Znt7+/+   | 114.0 ± 3.5    | 36.6 ± 2.0          | 99.5 ± 6.3                | 0.17 ± 0.03    | 1.70 ± 0.28    |
| Znt7−/−   | 112.3 ± 4.9    | 39.4 ± 2.5          | 89.0 ± 4.0                | 0.14 ± 0.03    | 0.97 ± 0.14    |

Values are mean ± S.E., n = 10–18 for Znt7+/+ mice and n = 10–14 for Znt7−/− mice (p < 0.05 (Student’s t-test) in blood glucose, serum triglyceride, cholesterol, and insulin levels between the two genotypes; p = 0.088 (Student’s t-test) in serum leptin level between the two genotypes.
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mRNA expressed in the tissue (14), which is similar to the expression of ZNT3 in the mouse testis (10). Therefore, low levels of zinc observed in the liver of Znt7-deficient mice are an indication that supplies of zinc is limited in the mutant mice. It is known that the small intestinal epithelium is the major site for dietary zinc absorption (51–53). Dietary zinc is brought into the absorptive enterocyte from the apical side via zinc uptake proteins, mainly ZIP4 (18) and transferred through the cell to the basolateral side of the cell, from where zinc is exported to the blood stream via ZNT1 (54). The intracellular pathway that takes zinc from the apical side of the absorptive enterocyte to the basolateral side is still largely unknown. Our results suggest that the intestinal absorption of zinc is decreased in Znt7-deficient mice as shown by the decreased radioactivity in organs 4 h after oral 65Zn administration. ZNT7 is highly expressed in the epithelium of the small intestine (14, 27). Thus, in the absorptive enterocytes expressing mutant alleles of Znt7, transportation of zinc into the Golgi apparatus is decreased resulting in low level of zinc accumulation in the secretory pathway of these cells. It is reasonable to speculate that the block of zinc incorporated into the Golgi apparatus results in a reduction of zinc being transported into the circulation at the basolateral side of the absorptive enterocyte through the secretory pathway. Subsequently, delivery of dietary zinc to peripheral tissues is partially disrupted. To our knowledge, this is the first time to demonstrate that the Golgi apparatus is involved in the absorptive process of dietary zinc in the enterocyte of the gut.

The size of the adipose tissue in the body is maintained by the balance between synthesis and lipolysis of triglycerides in the adipocyte. As an adipocyte increases in size, an increase in enzyme and transporter activities involved in fatty acid influx and triglyceride synthesis is a must. As mice age, body growth decreases and adipose tissue stores normally increase due to the increase in adipocyte size and proliferation. In this study, we have observed that the difference in the body weights between Znt7-deficient and wild-type mice was ~5% at weaning (4 weeks of age). However, this difference became greater as mice age. The reduction in the adipocyte accumulation is most likely responsive for the differences in the body weights between Znt7-deficient and wild-type mice as Znt7−/− mice had only 50% of the fat mass found in the wild-type controls. The reductions in the lean body mass and mineral content in Znt7−/− mice may also contribute to the body weight differences between the two genotypes but to a smaller degree. In addition, Znt7-deficient mice fed zinc-adequate or zinc-supplemental diet showed a significant increase in percent carcass lean mass relative to wild-type controls, indicating that Znt7-null mice do not suffer from a generalized growth defect.

Although ZNT7 was found expressing in the insulin-secretion-responsive β-cells in the mouse pancreas, both blood glucose and plasma insulin levels in Znt7−/− mice throughout the OGTT were in normal ranges compared with these in Znt7+/+ mice, suggesting that an impaired function of insulin packing and secretion in the pancreas of Znt7−/− mice is unlikely. It has been demonstrated that ZNT8, a pancreas-specific zinc transporter, is a major transporter for providing zinc to insulin maturation and/or storage processes in insulin-secreting pancreatic beta-cells (15, 55). Overexpression of ZNT8 in rat insulinoma INS-1E cells enhances glucose-stimulated insulin secretion (56). Moreover, a recent genome-wide association study for type 2 diabetes revealed that a variant in ZNT8 was associated with a reduced insulin secretion in human adults (57, 58). It is possible that ZNT7 may play a role in insulin maturation, storage, and/or release in the pancreas based on its expression in the insulin-secretion β-cells. But these processes are not affected in Znt7-deficient mice possibly due to the compensation of ZNT8 for the loss of ZNT7 in β-cells. On the other hand, ZNT7 may not contribute to insulin packing and/or secretions in the β-cells of the pancreas.

ZNT7 is highly expressed in the mouse adipose tissue, and the null-mutation of Znt7 disrupts the incorporation of zinc ions into the Golgi apparatus. As a consequence, activities of enzymes and transporters associated with the triglyceride synthesis may be diminished in the adipocyte of Znt7 knockout mice. However, lipolysis of triglycerides may be enhanced in the adipocyte of Znt7 knockout mice. The finding by Eder et al. supports this correlation (59). They showed that zinc deficiency enhanced the lipolysis in the rat adipose tissue. Although Znt7-deficient mice were relatively lean, their serum triglyceride levels were not significantly changed. This resembles the phenotypes of Dgat1-deficient mice in which the serum triglyceride levels were normal, whereas their body adipose masses were

3 L. Huang, Y. Y. Yu, C. P. Kirschke, E. R. Gertz, and K. K. C. Lloyd, unpublished data.
4 L. Huang, Y. Y. Yu, C. P. Kirschke, E. R. Gertz, and K. K. C. Lloyd, unpublished observations.

![Figure 8](image-url)
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~50% less than wild-type mice (60). It suggests that the main action site of ZNT7 in triglyceride metabolism may be the adipocyte not the hepatocyte. In addition, our results show that Znt7−/− mice had less serum leptin level than the controls. This difference may be the result of either the decreased fat mass observed in Znt7−/− mice as leptin is produced and secreted by the adipose tissue or the decreased expression and secretion of leptin in adipocyte of Znt7−/− mice due to the lack of zinc in the Golgi apparatus of the cell. The exact molecular mechanisms connecting zinc and adiposity and its endocrine function remain to be explored. It will be of great interest to determine 1) how zinc deficiency in the Golgi apparatus alters protein/enzyme functions involved in fatty acid flux and triglyceride metabolism in the adipocyte, 2) whether Znt7 deficiency alters the endocrine function of the adipose tissue and energy expenditure, and 3) factors that contribute to the less adiposity in Znt7-deficient mice.

Interestingly, our data show that the disruption of zinc sequestration into the Golgi apparatus of the cell due to the defect of Znt7 resulted in an increased mRNA expression of Znt1. It is known that Znt1 mRNA expression is induced by the increased cellular zinc concentration. It is reasonable to speculate that in cells expressing mutant alleles of Znt7 zinc concentration is increased in the nucleus of the cell, resulting in an induction of Znt1 gene expression. Zinc induction of Znt1 gene expression is mediated by metal response element-binding transcription factor-1 that binds two metal response elements in the promoter of Znt1 (43). Thus, one may speculate that genes carrying the consensus metal response element sequence in their promoters, such as metallothioneins, will be affected by the null-mutation of Znt7. Further studies are required to identify the one or more metal response element-binding transcription factor-1 regulons and their function in regulation of intestinal zinc absorption and body adiposity.

Among the ZNT proteins, ZNT5 isoform a displays many similarities to ZNT7 in aspects of amino acid sequences (40), expression patterns (12, 14, 61), and cellular localization (12, 14, 61). It was demonstrated that both ZNT5 and ZNT7 participated in the activation of alkaline phosphatases, a group of glycosylphosphatidylinositol-anchored membrane proteins that require zinc for their enzymatic activities, by delivery of zinc into the lumens of the Golgi apparatus of chicken B lymphocyte-derived cells (DT40) (48). However, it appeared that ZNT5 and ZNT7 acted independently in the process of conversions of apo-alkaline phosphatases to holo-alkaline phosphatases in DT40 cells. ZNT5 exerted its function in delivery of zinc into the Golgi apparatus by partnering with ZNT6, whereas ZNT7 acted in a homo-oligomeric way (49). Furthermore, although both ZNT5 and ZNT7 contributed to the activation of alkaline phosphatases in the Golgi apparatus, ZNT5 together with ZNT6 played a major role in this process (48, 49).

Mice lacking ZNT5 or ZNT7 grew poorly and were lean as a result of reduced adiposity. One may speculate that the decreased food intake (~18% reduction) in Znt7-deficient mice might explain the poor growth. However, a major effect of a rather small decrease in food intake on the adipose mass but not on the lean mass seen in Znt7-deficient mice fed zinc-adequate diet is unlikely (Fig. 7a). Moreover, wild-type mice that were fed a diet containing 5 mg/kg zinc to induce mild zinc deficiency had a dramatic reduction in the body fat accumulation but not the lean body mass when compared with wild-type mice fed a zinc-adequate diet (Fig. 7a). This suggests that a pronounced reduction in zinc concentrations in the lumen of the Golgi apparatus in the adipocyte may be responsible for the less body weight gain and adiposity observed in Znt5- and Znt7-deficient mice. Besides poor growth and reduced adiposity, Znt5-deficient mice also exhibit muscle weakness, abnormal bone development, osteopenia, and male-specific sudden death in middle age due to bradyarrhythmia (12). These phenotypes, however, seem not to be associated with the body zinc status as Znt5-deficient mice display normal serum zinc levels (12). Our findings, together with Inoue’s (12), suggest that both ZNT7 and ZNT5 play important roles in loading zinc into the Golgi apparatus to maintain physiological function of adipocytes. The differences in manifested phenotypes between Znt7- and Znt5-deficient mice may reflect differences in their protein expression patterns in tissues, their action sites along the secretory pathway, and the proteins that they interact with. ZNT7 may be one of the key factors in modulating zinc absorption in absorptive enterocytes to maintain body zinc homeostasis, whereas ZNT5 may play a major role in modulating activities of zinc-required enzymes/proteins in the development of bone, muscle, and heart in the body.

In conclusion, we describe a detailed characterization of Znt7-deficient mice. Our findings demonstrate that transport of zinc into the Golgi apparatus in the absorptive enterocyte in the gastrointestinal tract is an essential step in the absorption of dietary zinc. Znt7 knockout mice are zinc-deficient. However, this phenotype cannot be corrected by dietary zinc supplementation in Znt7-deficient mice, indicating that ZNT7 plays an important role in keeping physiological concentrations of cellular zinc in the cell. ZNT7 may also play an essential role, either directly or via regulation of other factors, in regulation of body composition. Therefore, Znt7-deficient mice should be useful for studies involving in the regulation of dietary zinc absorption process in the gut, identification of other zinc transporters that are functionally overlapping with ZNT7, and regulation of body adiposity and composition.

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