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INORGANIC COMPOUNDS

Regulation of metal transporters by dietary iron, and the relationship between body iron levels and cadmium uptake

Dong-Won Kim · Ki-Young Kim · Byung-Sun Choi · Pilju Youn · Doug-Young Ryu · Curtis D. Klaassen · Jung-Duck Park

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Abstract Iron (Fe) plays essential roles in biological processes, whereas cadmium (Cd) is a toxic and non-essential metal. Two metal transporters, divalent metal transporter 1 (DMT1) and metal transporter protein 1 (MTP1), are responsible for Fe transport in mammals. Here, we studied the effect of dietary Fe on the expression of these metal transporters in peripheral tissues, and the uptake by these tissues of Cd. Mice were fed an Fe-sufficient (FeS: 120 mg Fe/kg) or Fe-deficient (FeD: 2–6 mg Fe/kg) diet for 4 weeks. The total Fe levels in the body were evaluated by measuring tissue Fe concentrations. Tissue Cd concentrations were determined 24 h after the mice received a single oral dose of Cd. Animals fed a FeD diet showed depletion of body Fe levels and accumulated 2.8-fold higher levels of Cd than the FeS group. Quantitative real time RT-PCR revealed that whereas DMT1 and MTP1 were both ubiquitously expressed in all FeS peripheral tissues studied, DMT1 was highly expressed in brain, kidney, and testis, whereas MTP1 was highly expressed in liver and spleen. Depletion of the body Fe stores dramatically upregulated DMT1 and MTP1 mRNA expression in the duodenum as well as moderately upregulating their expression in several other peripheral tissues. The iron response element positive isoform of DMT1 was the most prominently upregulated isoform in the duodenum. Thus, DMT1 and MTP1 may play an important role in not only maintaining Fe levels but also facilitating the accumulation of Cd in the body of mammals.

Keywords Iron (Fe) · Cadmium (Cd) · Divalent metal transporter 1 (DMT1) · Metal transporter protein 1 (MTP1) · Duodenum

Abbreviations

Cd	Cadmium
DMT1	Divalent metal transporter 1
Fe	Iron
FeD	Iron deficient
IRE	Iron response element
FeS	Iron sufficient
Pb	Lead
Mn	Manganese
MTP1	Metal transporter protein 1
RT-PCR	Reverse transcription polymerase chain reaction
UTR	Untranslated region
Zn	Zinc

Introduction

Iron (Fe) is an essential element in the body as it acts as a structural and functional component of many biological molecules in mammals. It is important to

D.-W. Kim · K.-Y. Kim · B.-S. Choi · J.-D. Park (✉)
Department of Preventive Medicine, College of Medicine, Chung-Ang University, 221 Huksukdong, Dongjakgu, Seoul, 156-756, South Korea
e-mail: jdpark@cau.ac.kr

P. Youn · D.-Y. Ryu
College of Veterinary Medicine, BK21 Program for Veterinary Science, Seoul National University, Seoul, 151-742, South Korea

C. D. Klaassen
University of Kansas Medical Center, Kansas City, KS, 66160-7417, USA

maintain appropriate levels of Fe because deficiencies or excess levels of this metal induce metabolic disorders or toxic effects (Andrews 1999; Moos and Morgan 2004). Dietary Fe absorption takes place in the first portion of the small intestine. Two metal transporters are responsible for the transport of Fe in the enterocytes of mammals. One is the apical transporter divalent metal transporter 1 (DMT1), which facilitates the uptake of Fe from the gastrointestinal tract by enterocytes (Fleming et al. 1997; Gunshin et al. 1997). The other is the basolateral transporter metal transporter protein 1 (MTP1), which exports Fe from enterocytes into the blood circulation (Abboud and Haile 2000; McKie and Barlow 2004).

Cadmium (Cd) is a toxic non-essential element that is widely found in the environment. Once absorbed, Cd can remain in the body for decades (Sugita and Tsuchiya 1995). Most people take up Cd through oral ingestion, and chronic exposure to low levels of Cd is associated with kidney and bone damage (Shigematsu 1984; Kido et al. 1989). The rate at which the intestine absorbs Cd and the tissues accumulates this metal depends on the levels of Fe in the body, as animal models have shown that the gastrointestinal absorption of Cd is inhibited in Fe-replete animals, but enhanced in Fe-deficient animals (Valberg et al. 1976; Ragan 1977). Animal models and epidemiological studies have confirmed this relationship between Fe deficiency and Cd accumulation, and have also shown that Fe deficiency increases the levels of lead (Pb) in the body (Ragan 1977; Flanagan et al. 1978; Berglund et al. 1994; Bradman et al. 2001; Park et al. 2002; Ryu et al. 2004). In vitro studies have revealed that DMT1 not only transports Fe from the gastrointestinal tract, it also transports other divalent metals, regardless of whether they are essential (Zn, Mn) or toxic (Cd, Pb) (Gunshin et al. 1997; Bannon et al. 2002; Okubo et al. 2003). Thus, it is thought that the enhanced intestinal absorption of Cd and Pb in the Fe-depleted state is due to the elevated DMT1 expression in the small intestine, which is upregulated by low body Fe levels.

The DMT1 gene produces two mRNAs, due to alternative splicing of the 3′ exon, which generates distinct C-termini of the two proteins. One of these 3′-UTRs bears an iron response element (IRE) and generates the DMT1 IRE-positive isoform, whereas the other 3′-UTR lacks the IRE and produces the DMT1 IRE-negative isoform (Lee et al. 1998; Stuart et al. 2003). It has been suggested that the DMT1 IRE-positive isoform is the functional gene in the intestine (Stuart et al. 2003). However, it remains unclear whether the functions of the DMT1 isoforms differ.

During the last few years, the localization and role of the metal transporters in several peripheral tissues of experimental animals have been analyzed (Williams et al. 2000; Ferguson et al. 2001; Canonne-Hergaux et al. 2001; Yang et al. 2002a; Zhang et al. 2004; Griffin et al. 2005). Whereas it is still not clear the function of metal transporters in peripheral tissues, we are beginning to understand the tissue distribution of metal transporters, which will facilitate understanding the distribution of metals and metal–metal interactions. This study performed to further our understanding of the roles the metal transporters DMT1 and MTP1 in Cd absorption, we here used a mouse model. Also, we analyzed the expression of the DMT1 mRNA isoforms in the duodenum, which is the tissue in the body that is most responsive to Fe deficiency.

Materials and methods

Chemicals

$^{109}CdCl_2$ was purchased from Amersham Biosciences (UK). All other chemicals were reagent grade.

Animals

Specific pathogen-free male ICR mice, aged 21 days, were purchased from Samtako BioKorea (Osan, Korea). The animals were housed at 22°C with 55% humidity and a 12 h light/dark cycle and provided with standard rodent chow and tap water ad libitum. After acclimatization to the environment, the animals were divided into two groups (12 mice per group). One was fed a Fe-deficient diet (FeD diet, 2–6 mg Fe/kg), whereas the other was fed with a Fe-supplemented diet (FeS diet, 120 mg Fe/kg). The semisynthetic FeD and FeS diets were formulated as described in a previous report (Park et al. 2002) and were purchased from Harlan (Madison, WI, USA). The animals were fed these diets for 4 weeks and their body weights were recorded twice weekly. The drinking water was replaced with distilled-deionized water during the experimental period. At the end of the fourth week, half of the animals were sacrificed and the Fe concentrations in various tissues were determined. Thus, the major organs, namely, liver, kidney, heart, lung, spleen, brain, testis, stomach, duodenum, jejunum, ileum, and colon, were collected and stored at −80°C until analysis. The remaining mice were fasted for 15 h, given a single oral administration of Cd (100 μg $^{109}CdCl_2$/kg labeled with 60 μCi) in a volume of 4 ml/kg saline, and decapitated 24 h later. The major organs were collected and snap-frozen in liquid nitrogen and stored at −80°C until use.

Assessment of body Fe status

The body Fe status was evaluated by determining the concentration of Fe in various tissues. For this, the tissues were subjected to wet digestion by using a microwave digestion system (MDS-2000, CEM, USA). Atomic absorption spectrophotometric (Perkin Elmer Model 5100, USA) analysis by the flame method was used for Fe analysis.

Quantification of Cd

Tissue ^{109}Cd content was quantified by using a Wallac 1470 gamma scintillation counter (Perkin Elmer Life Science, Finland). The total Cd body burden was calculated as the sum of the Cd contents in all the tissues collected. The relative body burden of Cd was normalized by body weight.

Analysis of the mRNA expression of DMT1, the DMT1 isoforms (IRE-positive and IRE-negative), and MTP1

DMT1 and MTP1 mRNA levels in tissues were analyzed by quantitative real time reverse transcription-polymerase chain reaction (real time RT-PCR). Briefly, total RNA was isolated from tissue using the TRI Reagent (Molecular Research Center, Cincinnati, OH, USA). Reverse transcription was performed with 2 μg of total RNA using a first strand cDNA synthesis kit from Roche (Mannheim, Germany), according to the manufacturer's instructions. DMT1, the DMT1 isoforms (IRE-positive or IRE-negative), and MTP1 were amplified in the ABI SYBR Green PCR Master Mix (Applied Biosystems, USA) from the cDNA with specific primers (Table 1). Amplification was initiated by 2 and 10 min incubations at 50 and 95°C, respectively, followed by 35–45 cycles at 95°C for 15 s and 60°C for 1 min. The amplified PCR products of DMT1 and MTP1 were normalized to the 18S rRNA levels. The DMT1 and MTP1 mRNA expression in each tissue was expressed relative to the DMT1 or MTP1 expression in the duodenum of the FeS mice.

Statistical analysis

Data were expressed as means ± standard error. The means of the FeS and FeD mice were compared by the two-tailed Student's t test. Statistical significance was set at $P < 0.05$.

Results

The body weights of the FeS- and FeD-diet-fed animals increased gradually and progressively with age (data not shown). However, the FeD mice gained less weight than the FeS mice.

After 4 weeks of being fed with the FeD or FeS diets, half of the mice ($n = 6$ per group) were sacrificed and their major organs were collected to determine their Fe levels. With regard to mice fed the FeS diet, the highest Fe concentrations were found in spleen, followed by heart, liver, and kidney. In the gastrointestinal tract, the highest Fe concentrations were found in the duodenum. With regard to mice fed with the FeD diet, almost all tissues had lower Fe levels than the equivalent tissues of the FeS mice (Fig. 1). These findings indicate that feeding mice with the FeD diet for 4 weeks depletes their body Fe stores.

The remaining mice ($n = 6$ per group) were fed with a single dose of $^{109}CdCl_2$ and their tissue concentrations of Cd were determined 24 h later (Fig. 2). In the FeS mice, the highest levels of Cd were found in the gastrointestinal tract, in particular the duodenum. The jejunum also showed high levels of Cd (15 ng/g wet

Table 1 Forward and reverse primers of the target genes used in quantitative real time RT-PCR analysis

Gene	Forward primer Reverse primer	Accession no.
DMT1	5′AGGAAGTGCGGGAAGCCAATAAGTA3′ 5′ACACGACAAAGACATTGATGATGAA3′	AF029758
DMT1 IRE(+)	5′GCAACATTAAGTAAACACTGGATCA3′ 5′TTGTGGCTATGTTCACACAGTAAA3′	AF029758
DMT1 IRE(−)	5′AGCCTGAACTCTATCTTCTGAACAC3′ 5′ACAAGCTCACCTCCGAACTAA3′	NM_008732
MTP1	5′GATGGGTCCTTACTGTCTGCTACA3′ 5′TTGTGATCGCAGTGGCAGTAC3′	NM_016917
18S rRNA	5′GGACACGGACAGGATTGAC3′ 5′TCGCTCCACCAACTAAGAAC3′	AY248756

GeneBank accession numbers (http://www.ncbi.nlm.nih.gov)

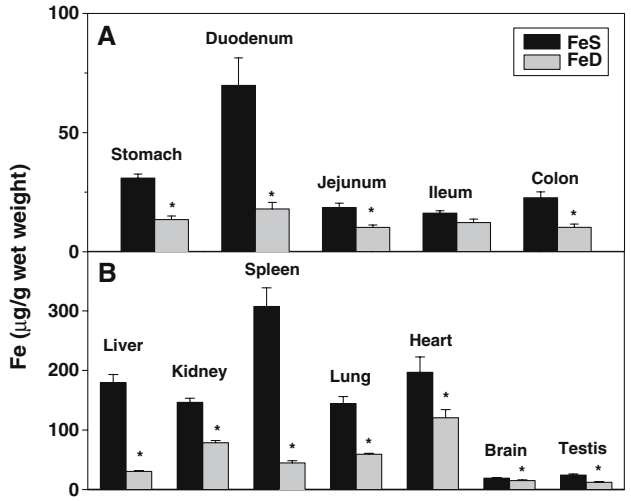

Fig. 1 Concentration of Fe in peripheral tissues of mice fed the FeS or FeD diet for 4 weeks. Fe levels in **a** stomach, duodenum, jejunum, ileum, and colon, and **b** liver, kidney, spleen, lung, heart, brain, and testis of FeS and FeD mice. Data are expressed as means ± standard error ($n = 6$). *Asterisks* (*) indicate that FeD mice differ significantly from the FeS mice ($P < 0.05$)

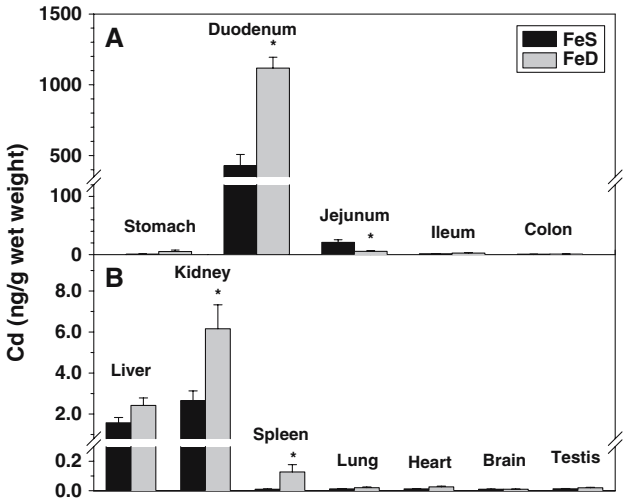

Fig. 2 Concentration of Cd in peripheral tissues of mice fed the FeS or FeD diet for 4 weeks, and then received a single oral dose of radiolabeled Cd. Cd levels in **a** stomach, duodenum, jejunum, ileum, and colon, and **b** liver, kidney, spleen, lung, heart, brain, and testis of FeS and FeD mice. Data are expressed as means ± standard error ($n = 6$). *Asterisks* (*) indicate that the FeD mice differ significantly from the FeS mice ($P < 0.05$)

weight), although they were much lower than the levels in the duodenum (600 ng/g wet weight). The kidney and liver also had relatively high Cd concentrations (2.5 and 1.8 ng/g wet weight, respectively). The other tissues had much lower Cd concentrations (>0.2 ng/g wet weight). The duodenums of the FeD mice contained 2.6-fold more Cd than those of the FeS mice (1,118 vs. 428 ng/g wet weight). Most of the other tissues of the FeD mice also had higher Cd concentrations than the equivalent FeS mouse tissues. Interestingly however, the FeS mice had higher Cd levels in their jejunums than the FeD mice. The total and relative body burden of Cd was higher in the FeD group (838 ng per mouse, 3,081 ng/100 g body weight) than in the FeS group (397 ng per mouse, 1,094 ng/100 g body weight, Fig. 3).

The expression of DMT1 and MTP1 were analyzed in tissues of the FeD and FeS mice (without Cd feeding). The DMT1 and MTP1 mRNA levels in tissues were expressed relative to their mean levels in the duodenum of the FeS mice. Both genes were expressed in all of the tissues examined in the FeS and FeD mice. In the FeS mice, the highest levels of DMT1 mRNA were found in brain, kidney, and testis, whereas the highest levels of MTP1 mRNA were observed in liver and spleen. Within the gastrointestinal tract of FeS mice, the highest levels of DMT1 and MTP1 mRNA were found in the duodenum. The FeD diet dramatically upregulated the expression of DMT1 and MTP1

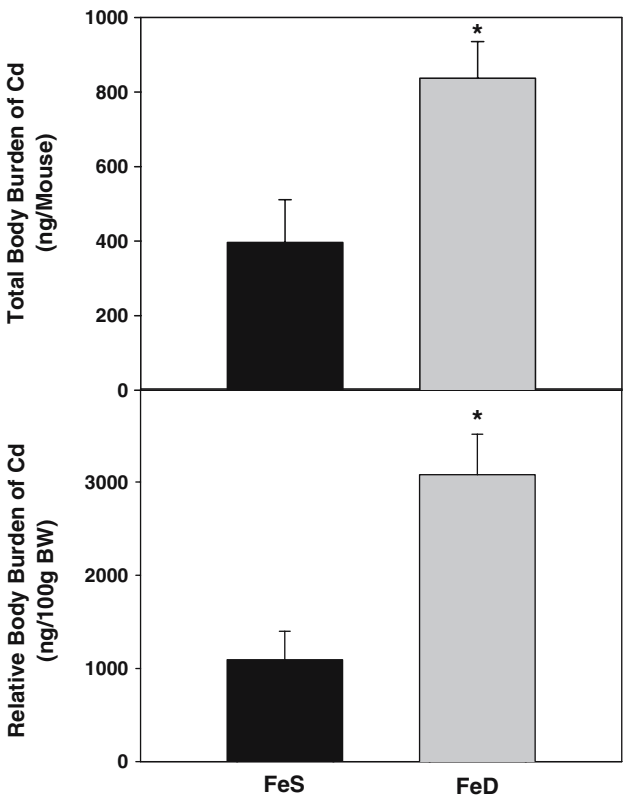

Fig. 3 Body burden of Cd in FeS and FeD mice. Total (*upper panel*) and relative (*lower panel*) body burdens of Cd in FeS and FeD mice. Data are expressed as means ± standard error ($n = 6$). *Asterisks* (*) indicate the FeD mice differ significantly from the FeS mice ($P < 0.05$)

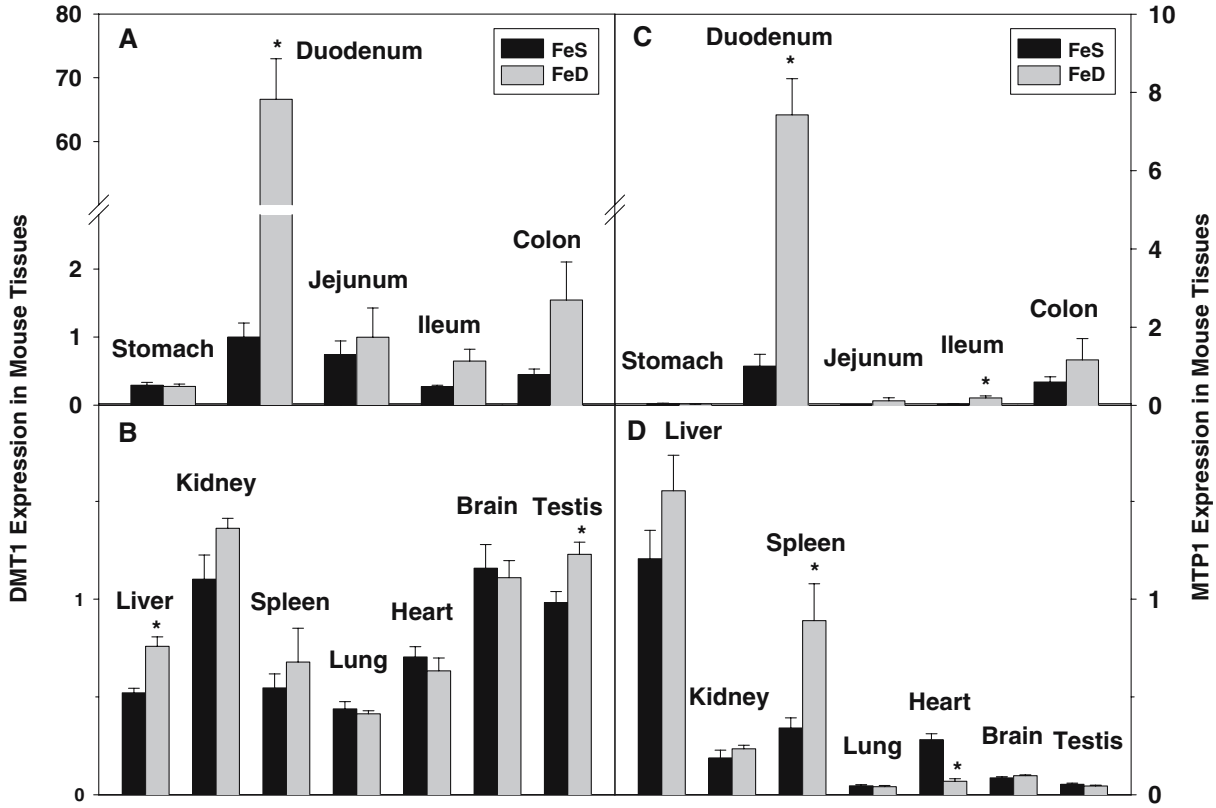

Fig. 4 Expression of DMT1and MTP1 mRNAs in FeS and FeD mice. DMT1 (**a**, **b**) and MTP1 (**c**, **d**) mRNA levels in **a**, **c** stomach, duodenum, jejunum, ileum, and colon, and **b**, **d** liver, kidney, spleen, lung, heart, brain, and testis of FeS and FeD mice. A PCR primer pair amplifying both DMT1 IRE(positive) and IRE(negative) cDNAs was used for DMT1. Data are expressed as means ± standard error ($n = 6$). *Asterisks* (*) indicate that the FeD mice differ significantly from the FeS mice ($P < 0.05$)

mRNA in duodenum by 65- and 8-fold, respectively (Fig. 4). The testis and liver also showed moderate but statistically significant upregulation of DMT1 mRNA expression in the mice fed the FeD diet (Fig. 4). Moderate but statistically significant FeD-induced increases in MTP1 mRNA were observed in spleen and ileum, whereas MTP1 expression was significantly downregulated in the heart (Fig. 4).

The tissues described above were also analyzed for the levels of IRE-positive and IRE-negative isoforms of DMT1 mRNA. As with the expression of DMT1, the duodenum of the FeD mice showed a marked increase (41-fold) in the expression of IRE-positive DMT1 compared to its expression in the FeS duodenum (Fig. 5). The IRE-negative isoform was also upregulated in the FeD duodenum, but less dramatically than the IRE-positive isoform (fivefold).

Discussion

In the present study, we confirmed that the Fe stores in mice become depleted when they are fed a Fe-deficient diet for 4 weeks, and that this depletion is associated with increased intestinal absorption of Cd. Moreover, we found that the depletion of Fe stores dramatically upregulated the expression of DMT1 and MTP1 mRNA in the duodenum. These findings are consistent with earlier studies of a rat model that suggested Cd transport in mammals is associated with DMT1 and MTP1 expression in the duodenum (Ryu et al. 2004). Thus, Cd absorption by the intestine is inversely related to the Fe stores in the body, and correlates positively with changes in DMT1 and MTP1 expression.

In mice fed the FeS diet, the Fe concentrations in various tissues were maintained at a relatively high level, whereas feeding the FeD diet for 4 weeks reduced the Fe stores in the body, and nearly all peripheral tissues studied showed decreases in Fe concentration. When mice were given oral radiolabeled Cd after 4 weeks on the FeD or FeS diets, 24 h later the vast majority of the Cd in the body was found in the gastrointestinal tract, specifically the duodenum. These intestinal Cd levels were much higher than the levels we detected previously in similarly treated rats (Ryu et al. 2004). The FeD diet increased the Cd levels in the

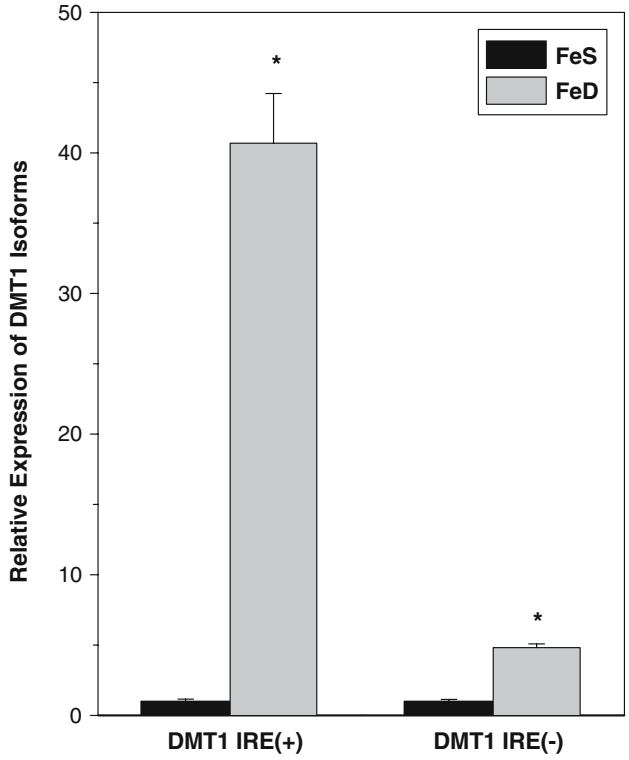

Fig. 5 Expression of the IRE(+) and IRE(−) DMT1 mRNA isoforms in the duodenum of FeD and FeS mice. Data are expressed as means ± standard error ($n = 6$). *Asterisks* (*) indicate the FeD mice differ significantly from the FeS mice ($P < 0.05$)

duodenum by 2.6-fold from 428 ng/g wet weight in FeS mice to 1,118 ng/g wet weight in FeD mice. Several other tissues showed significant increases in Cd levels upon Fe depletion, namely, the kidney and the spleen. Consequently, the total body Cd burden was about 2.8-fold higher in FeD mice (3,081 ng/100 g vs. 1,094 ng/100 g body weight). This is consistent with our previous studies in rats (Park et al. 2002; Ryu et al. 2004) as well as with human and other animal studies, which have all shown that deficiencies of essential metals, such as Fe and Zn, increase the intestinal absorption and tissue concentrations of toxic metals, such as Cd and Pb (Ragan 1977; Flanagan et al. 1978; Fox et al. 1984; Berglund et al. 1994; Park et al. 2002; Ryu et al. 2004). These findings suggest that the processes in the body that regulate the absorption and transport of essential metals are related to the processes that determine the body levels of toxic metals.

Unlike the other tissues, the jejunum of FeD mice had lower levels of Cd than the jejunum of FeS mice. A time–response study revealed that the concentration of Cd in jejunum were also lower in FeD mice than in FeS mice at 6 and 12 h after oral Cd administration (data not shown). This suggests that a single small dose of orally administered Cd in Fe-depleted mice may be almost completely absorbed by the duodenum.

We found that the metal transporters DMT1 and MTP1 were ubiquitously expressed in all tissues examined in the FeS mice. DMT1 mRNA was particularly highly expressed in brain, kidney, and testis, whereas within the gastrointestinal tract, DMT1 mRNA expression was highest in the duodenum. This DMT1 mRNA distribution pattern was similar to that in rats (Gunshin et al. 1997). The prominent expression of DMT1 in brain, kidney, and testis has been noted previously, and the biological significance of this has received particular attention (Williams et al. 2000; Ferguson et al. 2001; Canonne-Hergaux and Gros 2002; Knutson et al. 2004; Griffin et al. 2005).

MTP1 is responsible for cellular Fe efflux at the basolateral portion of the intestinal epithelium and may be involved in Fe recycling in the reticuloendothelial system (Yang et al. 2002a). We found that MTP1 mRNA was highly expressed in liver and spleen of FeS mice. These observations are consistent with previous studies that show high levels of MTP1 in reticuloerythroid cells (Abboud and Haile 2000; Yang et al. 2002a), the red pulp of spleen, and the hepatic stellate and Kupffer cells of liver (Yang et al. 2002a; Zhang et al. 2004). With regard to the intestine, we found that the duodenum has the highest expression of MTP1.

Fe depletion elevated the expression of DMT1 and MTP1 mRNA in some of the tissues studied, most notably in the duodenum, which showed 67- and 8-fold higher expression of these genes in the FeD mice, respectively. Most of the increase in DMT1 mRNA levels was due to upregulation of the expression of the IRE-positive isoform. This supports previous reports that have suggested that in situations of Fe depletion, the IRE-positive isoform rather than the IRE-negative type may be important in regulating the intestinal Fe balance (Canonne-Hergaux et al. 1999; Tchernitchko et al. 2002; Stuart et al. 2003). Notably, it has been found that IRE-negative is the major DMT1 mRNA isoform in the spleen (Canonne-Hergaux et al. 2001; Tchernitchko et al. 2002). However, the functional roles the DMT1 isoforms play in the peripheral tissues remain unclear. Nevertheless, the present findings that the FeD diet upregulates duodenal expression of metal transporters, and that this tissue contains the highest levels of Cd after a single oral administration of Cd, suggests that these transporters may be involved in the absorption and distribution not just of Fe, but also Cd. The present results support previous in vitro and in vivo studies (Gunshin et al. 1997; Canonne-Hergaux et al. 1999; Park et al. 2002; Okubo et al. 2003; Ryu et al. 2004).

As mentioned above, DMT1 and MTP1 were expressed in all tissues and were upregulated in several peripheral tissues by Fe depletion. These findings suggest that DMT1 and MTP1 are responsible for the maintenance of Fe levels and the biological roles Fe plays in distribution to tissues as well as for the absorption of Fe by the intestine. In addition, it has been observed that Fe overexposure leads to upregulation of DMT1 and MTP1 in the lung, which suggest that these metal transporters may also play a role in the detoxification of excess Fe (Wang et al. 2002; Yang et al. 2002b). However, the expression and biological functions of these metal transporters are likely to vary between the various tissues.

The fact that the FeD diet increased the uptake and deposition of Cd in the body by elevating the duodenal expression of DMT1 and MTP1, has important ramifications for public health. There are about two billion people in the world that suffer from Fe deficiency (WHO 2003). Our observations suggest that these people may be significantly more susceptible to toxic metals such as Cd and Pb.

In summary, the FeD diet induced the systemic depletion of body Fe stores in mice. This in turn upregulated the expression of the IRE-positive isoform of DMT1 by the apical portion of the intestine, which is likely to increase the influx of Fe from the gastrointestinal tract into the intestinal cells. MTP1, which encodes a basolateral Fe exporter in intestine, was also upregulated by the FeD diet; its elevated expression is likely to increase the efflux of Fe from the intestine to the blood circulation, thereby reducing cellular Fe in the intestine. The increased uptake and transport of Cd coincided with low body Fe levels, and the increased deposition of Cd in the peripheral tissues can be attributed to the increased Cd absorption by the intestine. Upregulated expression of metal transporters in tissues other than the intestine may also play a role in the accumulation of Cd in the various tissues. Thus, DMT1 and MTP1 play important roles in not only maintaining Fe levels, but also facilitating the accumulation of Cd in the body of mammals.

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References

Abboud S, Haile DJ (2000) A novel mammalian iron-regulated protein involved in intracellular iron metabolism. J Biol Chem 275:19906–19912

Andrews NC (1999) Disorders of iron metabolism. N Engl J Med 341:1986–1995

Bannon DI, Portnoy ME, Olivi L, Lees PS, Culotta VC, Bressler JP (2002) Uptake of lead and iron by divalent metal transporter 1 in yeast and mammalian cells. Biochem Biophys Res Commun 295:978–984

Berglund M, Akesson A, Nermell B, Vahter M (1994) Intestinal absorption of dietary cadmium in women depends on body iron stores and fiber intake. Environ Health Perspect 102:1058–1066

Bradman A, Eskenazi B, Sutton P, Athanasoulis M, Goldman LR (2001) Iron deficiency associated with higher blood lead in children living in contaminated environments. Environ Health Perspect 109:1079–1084

Canonne-Hergaux F, Gros P (2002) Expression of the iron transporter DMT1 in kidney from normal and anemic mk mice. Kidney Int 62:147–156

Canonne-Hergaux F, Gruenheid S, Ponka P, Gros P (1999) Cellular and subcellular localization of the Nramp2 iron transporter in the intestinal brush border and regulation by dietary iron. Blood 12:4406–4417

Canonne-Hergaux F, Zhang AS, Ponka P, Gros P (2001) Characterization of the iron transporter DMT1 (NRAMP2/DCT1) in red blood cells of normal and anemic mk/mk mice. Blood 98:3823–3830

Ferguson CJ, Wareing M, Ward DT, Green R, Smith CP, Riccardi D (2001) Cellular localization of divalent metal transporter DMT-1 in rat kidney. Am J Physiol Renal Physiol 280:F803–F814

Flanagan PR, McLellan JS, Haist J, Cherian G, Chamberlain MJ, Valberg LS (1978) Increased dietary cadmium absorption in mice and human subjects with iron deficiency. Gastroenterology 74:841–846

Fleming MD, Trenor CC III, Su MA, Foernzler D, Beier DR, Dietrich WF, Andrews NC (1997) Microcytic anaemia mice have a mutation in Nramp2, a candidate iron transporter gene. Nat Genet 16:383–386

Fox MR, Tao SH, Stone CL, Fry BE Jr (1984) Effects of zinc, iron and copper deficiencies on cadmium in tissues of Japanese quail. Environ Health Perspect 54:57–65

Griffin KP, Ward DT, Liu W, Stewart G, Morris ID, Smith CP (2005) Differential expression of divalent metal transporter DMT1 (Slc11a2) in the spermatogenic epithelium of the developing and adult rat testis. Am J Physiol Cell Physiol 288:C176–C184

Gunshin H, Mackenzie B, Berger UV, Gunshin Y, Romero MF, Boron WF, Nussberger S, Gollan JL, Hediger MA (1997) Cloning and characterization of a mammalian proton-coupled metal-ion transporter. Nature 388:482–488

Kido T, Nogawa K, Yamada Y, Honda R, Tsuritani I, Ishizaki M, Yamaya H (1989) Osteopenia in inhabitants with renal dysfunction induced by exposure to environmental cadmium. Int Arch Occup Environ Health 61:271–276

Knutson M, Menzies S, Connor J, Wessling-Resnick M (2004) Developmental, regional, and cellular expression of SFT/UbcH5A and DMT1 mRNA in brain. J Neurosci Res 76:633–641

Lee PL, Gelbart T, West C, Holloran C, Beutler E (1998) The human Nramp2 gene: characterization of the gene structure, alternative splicing, promoter region and polymorphisms. Blood Cells Mol Dis 24:199–215

McKie AT, Barlow DJ (2004) The SLC40 basolateral iron transporter family (IREG1/ferroportin/MTP1). Pflügers Arch 447:801–806

Moos T, Morgan EH (2004) The metabolism of neuronal iron and its pathogenic role in neurological disease: review. Ann NY Acad Sci 1012:14–26

Okubo M, Yamada K, Hosoyamada M, Shibasaki T, Endou H (2003) Cadmium transport by human Nramp 2 expressed in *Xenopus laevis* oocytes. Toxicol Appl Pharmacol 187:162–167

Park JD, Cherrington NJ, Klaassen CD (2002) Intestinal absorption of cadmium is associated with divalent metal transporter 1 in rats. Toxicol Sci 68:288–294

Ragan HA (1977) Effects of iron deficiency on the absorption and distribution of lead and cadmium in rats. J Lab Clin Med 90:700–706

Ryu DY, Lee SJ, Park DW, Choi BS, Klaassen CD, Park JD (2004) Dietary iron regulates intestinal cadmium absorption through iron transporters in rats. Toxicol Lett 152:19–25

Shigematsu I (1984) The epidemiological approach to cadmium pollution in Japan. Ann Acad Med Singapore 13:231–236

Stuart KA, Anderson GJ, Frazer DM, Powell LW, McCullen M, Fletcher LM, Crawford DH (2003) Duodenal expression of iron transport molecules in untreated haemochromatosis subjects. Gut 52:953–959

Sugita M, Tsuchiya K (1995) Estimation of variation among individuals of biological half-time of cadmium calculated from accumulation data. Environ Res 68:31–37

Tchernitchko D, Bourgeois M, Martin ME, Beaumont C (2002) Expression of the two mRNA isoforms of the iron transporter Nrmap2/DMTI in mice and function of the iron responsive element. Biochem J 363:449–455

Valberg LS, Sorbie J, Hamilton DL (1976) Gastrointestinal metabolism of cadmium in experimental iron deficiency. Am J Physiol 231:462–467

Wang X, Ghio AJ, Yang F, Dolan KG, Garrick MD, Piantadosi CA (2002) Iron uptake and Nramp2/DMT1/DCT1 in human bronchial epithelial cells. Am J Physiol Lung Cell Mol Physiol 282:L987–L995

Williams K, Wilson MA, Bressler J (2000) Regulation and developmental expression of the divalent metal-ion transporter in the rat brain. Cell Mol Biol 46:563–571

World Health Organization (2003) Micronutrient deficiencies: battling iron deficiency anemia

Yang F, Liu XB, Quinones M, Melby PC, Ghio A, Haile DJ (2002a) Regulation of reticuloendothelial iron transporter MTP1 (Slc11a3) by inflammation. J Biol Chem 277:39786–39791

Yang F, Wang X, Haile DJ, Piantadosi CA, Ghio AJ (2002b) Iron increases expression of iron-export protein MTP1 in lung cells. Am J Physiol Lung Cell Mol Physiol 283:L932–L939

Zhang AS, Xiong S, Tsukamoto H, Enns CA (2004) Localization of iron metabolism-related mRNAs in rat liver indicate that HFE is expressed predominantly in hepatocytes. Blood 103:1509–1514