

Phlebotomy Increases Cadmium Uptake in Hemochromatosis

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The intestinal absorption of the nephrotoxic environmental pollutant cadmium increases markedly when iron stores are depleted. This may be mediated by an up regulation of the recently identified mucosal transporter DMT1 (Nramp2 or DCT1) for divalent cations. We tested whether the highly increased iron absorption in hereditary hemochromatosis (HH) was accompanied by an enhanced absorption of cadmium and lead. Cadmium and lead in blood and iron status markers were determined in 21 nonsmoking subjects with HH genetically tested for the *HFE* mutations and in 21 nonsmoking controls matched for age and sex. In subjects with HH on maintenance phlebotomy treatment, blood concentrations of cadmium, but not lead, were significantly higher than in paired controls. There was a strong age-independent positive association between blood cadmium and the number of years of phlebotomy treatment. Blood lead showed a similar but less pronounced consequence of treatment. All HH subjects with lower blood cadmium than the corresponding controls had either no mutation in the *HFE* gene, were not phlebotomized, or were phlebotomized for only a limited time. Our findings indicate that the treatment rather than the disease increased the cadmium uptake in homozygous HH. Further studies are needed to confirm whether the disease decreased cadmium absorption and whether the absorption was dependent on the genotype. **Key words:** absorption, cadmium, DCT1, DMT1, hemochromatosis, *HFE*, human, iron, lead, Nramp2. *Environ Health Perspect* 108:289–291 (2000). [Online 15 February 2000] <http://ehpnet1.niehs.nih.gov/docs/2000/108p289-291akesson/abstract.html>

Cadmium, a widespread environmental pollutant, remains a threat to human health because there is little or no margin between existing exposure levels and the level that causes the first signs of tubular damage in the general population (1). Recent work shows a possible role for cadmium in the development of osteoporosis (2). The intestinal absorption of cadmium increases when iron stores are depleted but the mechanism of cadmium uptake is not known (3,4).

Both the size of the iron stores and the rate of erythropoiesis determine the intestinal iron absorption (5). The mechanisms behind this regulation, as well as those behind the inappropriately increased mucosal iron transfer in the iron-loading disorder hereditary hemochromatosis (HH), have largely remained unknown. During the last 3 years, however, two important discoveries, a transmembrane iron transporter (DMT1) (6,7) and the gene responsible for HH, known as *HFE* (8), have led to significant progress. Nevertheless, the complexity of iron homeostasis leaves much of the intestinal iron absorption in both health and disease obscure.

DMT1 (9), previously called Nramp2 (6,10–13) or DCT1 (7), is a duodenal metal transporter with affinity for several divalent cations. It is present in the brush border membrane at the site where most of the iron is absorbed (7). DMT1 is up regulated by iron deficiency (7,11,14) and may be responsible for the increased absorption of iron and cadmium observed at depleted iron stores. The *HFE* protein is involved in the

down regulation of iron absorption that normally occurs with increasing iron status (9,11,15–22). The *HFE* gene has a mutation with cysteine substituted by tyrosine at amino acid position 282 (C282Y) in approximately 85% of the about 0.5% Caucasians affected by HH (9,23). The loss of normal *HFE* function may impair transferrin-receptor-mediated uptake of transferrin-bound serum iron in the intestinal crypt cells, leading to iron-deficient cells and increased expression of DMT1 in mature villus enterocytes (14,24). If this is the case, increased cadmium absorption could be expected in HH, as was previously shown for lead (25), and we tested this hypothesis.

Materials and Methods

We determined cadmium and lead in blood and iron status markers in 21 nonsmoking subjects with HH (18 men and 3 women) and conducted blood sampling in parallel with phlebotomies. We compared the results to those for 21 nonsmoking healthy controls matched for age (\pm 8 years) and sex.

HH diagnosis was based on family history, increased serum ferritin and transferrin saturation, and liver histology. We conducted genetic testing for the *HFE* mutations; 17 of 21 subjects were homozygous for the C282Y mutation, and 1 was homozygous for the H63D mutation (histidine changed to aspartate at amino acid position 63) (26). None of the patients had any history of previous blood transfusion, alcohol overconsumption ($>$ 30 g ethanol/day), or dietary

iron supplementation. Serologic tests for hepatitis B and C infection were negative in all of the patients. Of the 21 subjects, 3 were recently diagnosed cases in whom intensive phlebotomy treatment had not yet started or had just begun, and the remaining 18 patients were under maintenance treatment with two to six phlebotomies per year. The aim of the treatment was to maintain serum ferritin at 30–60 μ g/L. Phlebotomy treatment totaled 0–10 years (mean 6 years), the total number of phlebotomies was 0–75 (mean 38), and 0–34 L blood was removed (mean 17 L).

Cadmium and lead in blood were used as measures of absorbed dose (27,28). They were determined by graphite furnace atomic absorption spectrometry with appropriate quality control (29). We used the Wilcoxon signed-ranks test for paired differences, and cadmium and lead concentrations were logarithmically transformed in the linear regression analysis (SPSS version 9; SPSS Inc., Chicago, IL). We obtained protocol approval from the ethical committee at Huddinge University Hospital (Huddinge, Sweden).

Results

The significantly higher hemoglobin and transferrin saturation in subjects with HH as compared to controls agrees with the laboratory manifestations of the disease (Table 1). Serum ferritin was lower in the phlebotomized group because of the treatment. The phlebotomized subjects were not considered to have depleted iron stores or iron deficiency anemia at the time of blood sampling. Although two subjects on a maintenance phlebotomy treatment had serum ferritin $<$ 20 μ g/L (14 and 16 μ g/L), they had hemoglobin within the reference interval. The blood concentrations of cadmium and lead in the healthy controls were similar to those of middle-aged Swedes (Table 1) (30). Blood cadmium concentration, but not lead, was significantly higher in the subjects

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with HH on maintenance phlebotomy treatment than in the paired controls (Table 1). In the three nonphlebotomized subjects both cadmium and lead concentrations were lower than in paired controls, but we did not perform statistical testing for differences because of the small number of samples.

Concentrations of cadmium, but not lead, increased with increasing age in both controls and in phlebotomized HH subjects, although the increase was more pronounced in the latter group. To control for this increase and for sex, the ratio between HH subjects and controls was expressed in relation to phlebotomy and to *HFE* mutations (Figure 1). On average, blood cadmium concentrations were 2 times higher in phlebotomized subjects than blood cadmium concentrations in the controls, whereas nonphlebotomized subjects had concentrations that were 60% of that in the controls. The phlebotomized subjects with substantially lower blood cadmium concentrations than the corresponding age- and sex-matched control (cadmium ratio < 0.5 in Figure 1; $n = 3$) had either no mutation in the *HFE* gene ($n = 1$), or were phlebotomized for short periods of time (< 1.7 years; $n = 2$). No such pattern was seen for blood lead.

In HH subjects, blood cadmium was highly related to the number of years of treatment by phlebotomy (Figure 2), the total amount of blood removed by phlebotomy, and the total number of phlebotomies. When we controlled for covariance through a stepwise multiple regression analysis, only years of treatment (adjusted $r^2 = 0.48$) and age, but not the amount of blood loss or the number of phlebotomies, were significantly correlated to blood cadmium. The number of years of phlebotomy and age together explained > 70% of the variation in cadmium

concentrations (adjusted $r^2 = 0.71$; $p < 0.001$). In addition, lead in the blood was significantly correlated to the total number of years of treatment (adjusted $r^2 = 0.35$; $p = 0.03$), but not to the total amount of blood loss or to the number of phlebotomies. Adjustment of cadmium and lead to differences in hemoglobin concentration did not alter the results.

Discussion

To our knowledge, this is the first study of cadmium in subjects with pathologically increased iron absorption. Our main finding was the marked increase in blood cadmium concentrations with the increasing number of years of treatment by phlebotomy in subjects with HH. This increase occurred independently of age and despite the approximately 5–10% reduction of the amount of cadmium in blood with each phlebotomy treatment. Thus, the treatment rather than the disease caused the increase in blood cadmium in these subjects as compared to controls.

Blood lead showed a similar but less pronounced consequence of treatment, but was not higher in HH subjects than in controls. This finding was in contrast with previously reported higher lead concentrations in HH patients than in healthy controls (25). Barton et al. (25) found no difference in blood lead between phlebotomized and nonphlebotomized subjects, and no correlation between blood lead and the number of phlebotomies. However, they did not investigate an association between blood lead and the number of years of treatment, which was the only association with lead in the present study.

The absorption of iron increases after hemorrhage. The present results indicate that the repeated withdrawal of blood also led to enhanced absorption of cadmium and,

to some extent, also lead. Depleted iron stores increase the cadmium absorption of both a single dose of cadmium and of dietary cadmium (3,4), whereas similar studies on lead are inconsistent (31). In the present study, where no subjects with HH were considered clinically iron deficient, it seems likely that the prolonged elevated erythropoiesis, perhaps in combination with temporarily reduced iron stores immediately after the phlebotomy, caused an elevated intestinal absorption of cadmium and lead. The increased absorption of cadmium and lead may be mediated by up regulation of the duodenal metal-transporter DMT1. We found that the effect of phlebotomy was more pronounced for cadmium than for lead, which is in accordance with an indicated higher affinity of cadmium than of lead for the DMT1 protein (7). In the phlebotomized group, one subject had none of the mutations in the *HFE* gene and showed no signs of increased absorption due to the phlebotomies (Figure 1). Whether this reflects a different absorption in subjects lacking a homozygote mutation as compared to the homozygous mutations needs to be proven.

Unfortunately, few nonphlebotomized HH subjects were available in this study. Still, the concentrations of cadmium and lead in these three subjects were consistently lower and close to one-half that of their respective controls. These findings further support the conclusion that the treatment rather than the disease caused increased cadmium uptake. Because of the limited number of nonphlebotomized subjects, it is not possible to

Table 1. Age, iron status markers, and cadmium and lead in blood in nonsmoking subjects with hereditary hemochromatosis and in nonsmoking healthy controls matched for age and sex.

	Hemochromatosis		Controls median (range)	<i>p</i> -Value
	Subject ^a	Median (range)		
Age	All	58 (31–80)	53 (28–77)	NS
Hemoglobin (g/L)	All	155 (131–167)	147 (131–160)	0.006
	Phlebotomized	156 (131–167)	149 (134–160)	0.021
	Nonphlebotomized	142 (141–144)	138 (131–140)	–
Serum ferritin (µg/L)	All	54 (14–1,314)	129 (30–301)	0.039
	Phlebotomized	44 (14–168)	104 (30–218)	NS
	Nonphlebotomized	1,030 (780–1,314)	262 (162–301)	–
Transferrin saturation (%)	All	78 (36–98)	29 (6–43)	< 0.001
	Phlebotomized	78 (36–98)	29 (6–43)	< 0.001
	Nonphlebotomized	89 (51–95)	22 (18–32)	–
Blood cadmium (µg/L)	All	0.31 (0.06–1.2)	0.26 (0.05–0.92)	NS
	Phlebotomized	0.34 (0.06–1.2)	0.25 (0.05–0.92)	0.020
	Nonphlebotomized	0.15 (0.12–0.19)	0.26 (0.21–0.27)	–
Blood lead (µg/L)	All	25 (8–125)	26 (13–65)	NS
	Phlebotomized	26 (8–125)	26 (13–65)	NS
	Nonphlebotomized	13 (11–19)	31 (14–34)	–

NS, not significant.

^aAll subjects ($n = 21$), phlebotomized subjects ($n = 18$), and nonphlebotomized subjects ($n = 3$). No statistical testing was performed on the nonphlebotomized group because of the small number of samples.

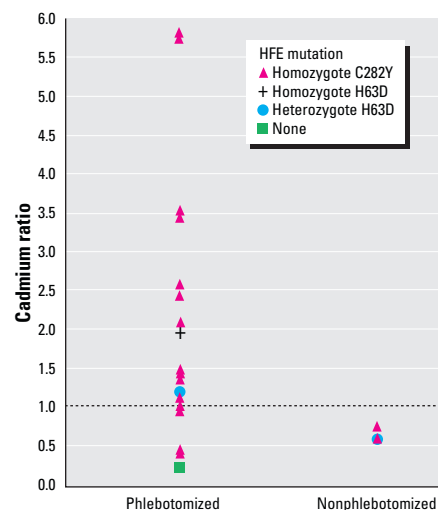


Figure 1. The ratio between concentrations of blood cadmium in subjects with HH and the concentrations in the age- and sex-matched paired controls. Values above 1.0 (the dotted line) indicate a higher value in subjects with HH than in controls, whereas values below 1.0 indicate the opposite. The ratio is calculated for phlebotomized subjects ($n = 18$; mean = 2.0) and nonphlebotomized subjects ($n = 3$; mean = 0.6), respectively.

evaluate whether the disease decreased cadmium uptake. If this were the case, it does not seem to be mediated by a down regulation of DMT1. Recent studies showed increased expression of DMT1 mRNA in both phlebotomized and nonphlebotomized HH subjects and in *HFE* knockout mice (14,32).

Thus, other mechanisms may explain our results. The approximately 1,000 times higher iron concentration than cadmium and lead concentrations in the intestine (3,29) speak against a competition between the toxic metals and iron at the binding site of the transporter. However, although both cadmium and lead exist in the divalent form, which is the form that binds to DMT1, Fe(III) is the most abundant form of environmental iron and requires reduction to Fe(II) for binding (33,34). If there is not enough ferrous iron available, it is possible for cadmium and lead to bind to the transporter. Interestingly, there is an increased reduction of ferric to ferrous iron in the mucosal surface of both phlebotomized and nonphlebotomized HH subjects as compared to controls (34). Thus, it is possible that both the iron reduction (34) and the DMT1 expression are increased during the untreated stage of the disease (14,32), whereas the phlebotomy treatment increases the DMT1 expression without any further increase in the reduction of ferric iron. This might cause a slight advantage for cadmium, and maybe for lead, as compared to before the phlebotomy, resulting in an increased absorption of these toxic metals.

Cadmium is accumulated in the kidney with a half-life of 10–30 years. Even though

the concentration of cadmium in blood mainly reflects recent exposure, it is also influenced by the body burden (3,28). Therefore, the observed increase in blood cadmium after several years of treatment most likely reflects an increase in body burden. Thus, despite the fact that the concentration of cadmium in phlebotomized subjects in this study was relatively low, it is apparent that repeated phlebotomy over a long period of time leads to substantially increased cadmium body burden.

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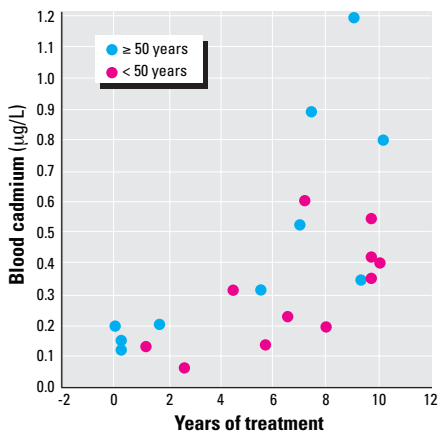


Figure 2. Blood cadmium in relation to the number of years of treatment (phlebotomy) of hereditary hemochromatosis. The median age of the subjects younger than 50 years of age = 38 years of age; the median age of the subjects older than 50 years of age = 64 years of age. The median and (range) of blood cadmium in controls were 0.20 (0.05–0.30) and 0.28 (0.09–0.92) µg/L for those below and above 50 years of age, respectively.