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In vitro analysis of intestinal absorption of cadmium and calcium in rainbow trout fed with calcium- and cadmium-supplemented diets

B. BALDISSEROTTO*†, M. J. CHOWDHURY‡§ AND C. M. WOOD‡

**Departamento de Fisiologia e Farmacologia, Universidade Federal de Santa Maria, 97105.900, Santa Maria, RS, Brazil and ‡Department of Biology, McMaster University, Hamilton, ON, L8S 4K1, Canada*

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The protective effects of dietary Ca^{2+} supplementation against Cd accumulation in rainbow trout *Oncorhynchus mykiss* fed with Cd-contaminated food were evaluated in relation to chronic changes in intestinal absorption rates. The changes were measured '*in vitro*'. The control diet contained *c.* 20 mg Ca^{2+} g^{-1} food and 0·25 μg Cd g^{-1} food; the experimental diets were supplemented with $CaCO_3$ and $Cd(NO_3)_2{\cdot}4H_2O$ to levels of 50 mg Ca^{2+} g^{-1} food and 300 μg Cd g^{-1} food, alone and in combination. The Ca^{2+} and Cd absorption rates were measured using radiotracers (^{45}Ca, ^{109}Cd) at total Ca^{2+} and Cd concentrations of 3·0 and 0·12 mmol l^{-1}, respectively in the intestinal saline. Chronically elevated dietary Cd caused a significant increase in Cd absorption rate by up to 10-fold at 30 days in the mid-intestine. The high Ca^{2+} diet prevented this up-regulation of Cd transport rate. Conversely, intestinal Ca^{2+} absorption was significantly increased by two- to five-fold by the Ca^{2+}-supplemented diet at 30 days in both the mid- and posterior intestine, and this effect was eliminated when Cd was simultaneously elevated in the diet. Ca^{2+} and Cd probably interact at common pathways and transport mechanisms in the intestine, though independent pathways may also exist.

Key words: dietary cadmium; dietary calcium; fish; intestinal absorption.

INTRODUCTION

Drinking rates of freshwater fishes are very low, and therefore the contribution of the intestine to Ca^{2+} uptake is restricted to absorption of dietary Ca^{2+}. This participation is more important when waterborne Ca^{2+} concentration (and consequently gill Ca^{2+} uptake) is low (Flik *et al.*, 1995). Studies of the mechanisms of Ca^{2+} transport in fish enterocytes have revealed the existence in the apical membrane of L-type Ca^{2+} channels (Larsson *et al.*, 1998) and a P_2 purinoceptor-mediated Ca^{2+} uptake through Ca^{2+} channels or carriers (Klaren *et al.*,

†Author to whom correspondence should be addressed. Tel.: +55 55 3220 9382; fax: +55 55 3220 8241; email: bernardo@smail.ufsm.br
§Present address: USEPA-MED, 6201 Congdon Boulevard, Duluth, MN 55804, U.S.A.

1997). Transport of Ca^{2+} from the enterocytes into the circulatory system occurs *via* the Na^{+}/Ca^{2+} exchanger and, to a lesser extent, *via* Ca^{2+}-ATPase located on the basolateral membranes (Flik *et al.*, 1990).

Cadmium is a metal, which originates from mining and other anthropogenic processes. Contamination of freshwater fishes with this metal can occur from the water, by the gills, or from the diet, by the gastrointestinal tract (Handy, 1992, 1993). Waterborne Cd uptake occurs *via* apical Ca^{2+} channels in the branchial chloride cells, leading to an inhibition of the basolateral Ca^{2+}-ATPase (Verbost *et al.*, 1987). The mechanism of dietary Cd uptake in fishes has not been characterized, though it is known that dietary Cd inhibits the basolateral Ca^{2+}-ATPase and the Na^{+}/Ca^{2+} exchanger in fish intestine (Schoenmakers *et al.*, 1992). Indeed, both waterborne and dietary Cd exposure results in a decrease in Ca^{2+} uptake (Verbost *et al.*, 1987; Zohouri *et al.*, 2001; Baldisserotto *et al.*, 2004*a*, *b*, 2005). Crespo *et al.* (1986) reported that dietary Cd caused an increase of intestinal mucous cell activity, and a disruption of intestinal brush border membranes, but did not change the transepithelial fluxes of Na^{+} and Cl^{-} in the intestine of rainbow trout *Oncorhynchus mykiss* (Walbaum). In mammals, while some studies support the hypothesis that intestinal Cd absorption involves competition with Ca^{2+}, chloro-complexes and a non-specific metal transporter (divalent metal transporter 1, DMT1 or Nramp2) may also participate in this process (Jumarie *et al.*, 1997, 2001; Elisma & Jumarie, 2001; Park *et al.*, 2002).

Cadmium toxicity due to waterborne and dietary Cd contamination is reduced by an elevation of waterborne (Carroll *et al.*, 1979; Pratap *et al.*, 1989; Meinelt *et al.*, 2001 Hansen *et al.*, 2002) or dietary Ca^{2+}, but dietary Ca^{2+} supplementation did not prevent the decreases in waterborne Ca^{2+} uptake caused by exposure to waterborne and dietary Cd (Zohouri *et al.*, 2001; Baldisserotto *et al.*, 2004*a*, *b*).

Most of these investigations on Ca^{2+} *v.* Cd interactions have been whole-animal studies with the effects deduced indirectly from differences in whole-body accumulations of Ca^{2+} and Cd. The objective of the present study was to use an approach, which involved whole-animal dietary pre-exposures followed by *in vitro* gut sac transport studies so as to focus exclusively on the effects on uptake of Ca^{2+} and Cd in the intestinal tract. Specifically, the effects of prior dietary Ca^{2+} and Cd supplementation (alone and in combination) for 15 and 30 days on the intestinal absorption of Ca^{2+} and Cd in rainbow trout were examined. The goal was to verify whether or not the protective effects of dietary Ca^{2+} supplementation on Cd accumulation in fish fed with Cd-contaminated food are related to long-term changes in intestinal absorption rates, independent of any acute effects due to simple competition or increased substrate availability in the metal-supplemented diets. Thus, all transport rate comparisons were performed under uniform conditions in the mucosal and serosal bathing solutions of the gut sac preparation.

MATERIAL AND METHODS

EXPERIMENTAL ANIMALS

Rainbow trout (200–300 g) were purchased from Humber Springs Fish Hatchery (Orangeville, Ontario, Canada). Fish were randomly separated into four aerated 200 l

polypropylene tanks supplied with *c.* 2 l min^{-1} of dechlorinated Hamilton tap water: [Na] = 0·6 mmol l^{-1}, [Ca] = 1·0 mmol l^{-1}, [Cl] = 0·7 mmol l^{-1}, [Cd] = 0·006 μmol l^{-1}, pH = 8·0, hardness = 140 mg l^{-1} as $CaCO_3$, alkalinity = 95 mg l^{-1} as $CaCO_3$, temperature = 10–12° C. Fish were fed once a day with commercial trout food (Debut Corey Starter Fish Feed, Corey Feed Mills Ltd, Fredericton, New Brunswick, Canada) at a ration of 2% of body mass per day. Photoperiod was maintained at 12L:12D.

After an acclimation period of 1 week, fish in each tank (30 fish each) received a different specific diet once a day for 15 or 30 days at a ration of 2% body mass per day. Uneaten food and faeces were siphoned off daily. Dead fish were removed daily and mortality was recorded. This cleaning regimen, in addition to the flow-through experimental design, ensured that excess Ca^{2+} and Cd from Ca^{2+}- and Cd-supplemented diets did not accumulate in the water. Changes in water Ca^{2+} levels were undetectable, whereas waterborne Cd levels, in the tanks of fish fed with Cd-supplemented diets, were 0·004–0·025 μmol l^{-1} relative to control levels *c.* 0·006 μmol l^{-1} throughout the 30 days of the experiment.

DIET PREPARATION

All diets were prepared with Debut Corey Starter Fish Feed (manufacturer's specifications: extruded granulated feed, [P] = 1·2%; crude protein = 57%; crude fat = 14%; crude fibre = 2%). The food was ground to a fine powder in a blender, followed by hydration with *c.* 40% v/w deionized water so as to form a paste. To prepare the treatment diets, the control diet (20 mg Ca^{2+} g^{-1} food) was supplemented with $CaCO_3$ and $Cd(NO_3)_2{\cdot}4H_2O$ to yield three different experimental diets with 50 mg Ca^{2+} g^{-1} food, or 300 μg Cd g^{-1} food, or 50 mg Ca^{2+} g^{-1} food + 300 μg Cd g^{-1} food. $CaCO_3$ and $Cd(NO_3)_2{\cdot}4H_2O$ were dissolved in the deionized water and added to the food paste. The paste was then thoroughly mixed and extruded through a pasta maker, air-dried and broken into small pellets by hand. The control diet was prepared by the same method but with the addition of deionized water only.

Actual measured Ca^{2+} mean ± S.E. concentrations in the control, 50 mg Ca^{2+} g^{-1} food, 300 μg Cd g^{-1} food and 50 mg Ca^{2+} g^{-1} food + 300 μg Cd g^{-1} food diets were 20·78 ± 3·66, 21·67 ± 1·57, 51·27 ± 0·98 and 55·72 ± 5·37 mg g^{-1} food (n = 3), respectively. Mean ± S.E. measured Cd concentrations were 0·25 ± 0·003, 293·92 ± 23·84, 0·22 ± 0·002 and 298·88 ± 19·69 μg g^{-1} food (n = 3), respectively.

IN VITRO INTESTINAL UPTAKE OF $^{45}Ca^{2+}$ AND ^{109}Cd

After 15 or 30 days of exposure to experimental diets, seven fish (200–300 g) from each group were collected and sacrificed with a blow to the head, and the mid-intestine (the portion between the pyloric cecae and posterior intestine) and the posterior intestine (distinguished from mid-intestine by the former's larger diameter, annular rings and darker colour, as described by Buddington *et al.*, 1987) were separated. The remaining fish received their daily feeding only after withdrawal of the specimens that were used in the experiments. After removal of the gut contents for ionic analyses, both portions were rinsed vigorously with Cortland saline (Table I).

The data obtained from the analysis of the fluid phase of the intestinal contents of the present study for Na^+, K^+, Mg^{2+} and Cl^- levels (unpubl. data) and those from Baldisserotto *et al.* (2005), for intestinal fluid Ca^{2+} and Cd levels of fish fed the same diets, were used to prepare the intestinal salines for the *in vitro* studies. Cortland saline was used on the serosal side of all experiments, and the intestinal saline–Ca^{2+} was used on the mucosal side in all Ca^{2+} intestinal absorption experiments independent of dietary treatment. A different intestinal saline (intestinal saline–Cd; lower Ca^{2+}, Mg^{2+}, SO_4^{2-} and PO_4^{3-}, no HCO_3^-) was used on the mucosal side in all experiments on Cd intestinal absorption because the addition of $Cd(NO_3)_2{\cdot}4H_2O$ to the regular saline provoked precipitation. The only way to avoid this problem was to lower the concentration of some ions in the intestinal saline–Cd (Table I).

TABLE I. Composition of salines (pH 7·8) used in the experiments. Cortland saline was used on the serosal side. Intestinal saline–Ca^{2+} and intestinal saline–Cd, the salines used on the mucosal side in experiments measuring Ca^{2+} and Cd intestinal absorption, respectively

	Cortland saline (mmol l^{-1})	Intestinal saline–Ca^{2+} (mmol l^{-1})	Intestinal saline–Cd (mmol l^{-1})
Na^+	139·3	125·3	125·7
Cl^-	130·6	121·8	128·4
K^+	5·1	1·48	1·48
Ca^{2+}	1·6	3·0	1·9
Mg^{2+}	0·9	2·3	1·1
HCO_3^-	11·9	5·0	—
SO_4^{2-}	0·9	2·3	1·1
PO_4^{3-}	3·4	3·0	0·7
Glucose	5·6	5·6	5·6
Cd	—	—	0·12

The intestinal absorption rates of Ca^{2+} and Cd were determined in separate preparations from an adaptation of the methodology of Bury *et al.* (2001). Briefly, one end of the intestinal segment was sealed tight with surgical thread, and into the other end a 5 cm piece of heat-flared PE-50 polyethylene (PE) tubing was inserted and tied to allow administration and sampling of intestinal saline. These sacs received 0·3–0·5 ml of the appropriate radiolabelled intestinal saline (0·5 μCi ml^{-1} ^{109}Cd or 0·3 μCi ml^{-1} $^{45}Ca^{2+}$, from New England Nuclear, Boston, MA, U.S.A.), and a 50 μl sample was taken for initial counting. The PE tubing was then sealed and the sacs incubated for 3 h in 20 ml of Cortland saline as the serosal bathing solution, which was bubbled with air. After the incubation, another 50 μl sample was taken from the mucosal fluid and a 10 ml sample of the serosal saline was also collected for counting. The sacs were cut open and all mucosal fluid removed. The sacs were then washed with intestinal saline–Ca^{2+} (Table I), blotted dry and weighed. The mucosal epithelium was scraped from the muscle layers using a glass slide, placed into a pre-weighed vial and weighed. Intestinal Ca^{2+} or Cd absorption (A) were calculated as follows: $A = T\,(SMt)^{-1}$, where T = total cpm of mucosal epithelium *or* sum of the rest of the preparation (muscular layer + serosa + the serosal saline), S = specific activity of the intestinal saline (cpm $nmol^{-1}$), M = mass of the mucosal epithelium (g) and t = duration of the incubation (3 h). It was assumed that the Ca^{2+} or Cd accumulation in the mucosal epithelia represented the amount that had entered the enterocytes as well as that tightly bound to any remaining mucus, while Ca^{2+} or Cd accumulation in the rest of the preparation (muscle + serosa + the outside serosal saline) represented the amount which went through mucous layer and mucosal epithelia into the underlying tissues. For simplicity, these have been designated as mucosal accumulation and tissue accumulation. Note that both are expressed on a common basis, per unit mass of the mucosal epithelium, so they can be directly compared.

Radioactivity in the intestinal tissues and saline samples containing ^{109}Cd was measured on a Canberra-Packard Minaxi Auto-Gamma 5000 series gamma counter (Canberra-Packard Instruments, Meriden, CT, U.S.A.). There was no quenching for ^{109}Cd. For counting $^{45}Ca^{2+}$, intestinal tissues were digested in 1N HNO_3 for 24–48 h at 60° C, and then 5 ml of Ultima Gold (Perkin Elmer, Wellesley, MA, U.S.A.) was added to this mixture. Samples were counted on a liquid scintillation counter (LKB Wallac 1217 Rackbeta, Pharmacia-LKB AB, Helsinki, Finland). Counting efficiencies for $^{45}Ca^{2+}$ were determined by internal standardization, *i.e.* by addition and recovery of known amounts of $^{45}Ca^{2+}$.

STATISTICAL ANALYSES

Data are reported as means ± S.E. Homogeneity of variances among groups was tested with the Levene test. Data for Ca^{2+} and Cd absorption into the mucosal epithelia were submitted to a square root transformation to obtain homogeneity among groups. Comparisons among different diets and times of exposure to these diets were made using pooled data by multivariate ANOVA and Tukey test. Analyses were performed using the software Statistica (version 5.1), and the minimum significance level was set at $P < 0{\cdot}05$.

RESULTS

Overall mortality of all treatments was 6·6 ± 3·0% over the 30 days of the experiment and there was no significant difference among treatments.

Mucosal and tissue Ca^{2+} accumulation rates were generally similar to each other, and also similar between mid- and posterior intestinal segments in the various treatments at both 15 and 30 days (Fig. 1). One exception was the two-fold higher Ca^{2+} accumulation in the posterior intestine than in the mid-intestine for both mucosal and tissue rates in rainbow trout fed control food at 15 days. This difference was not seen at 30 days. The most obvious treatment effect was a two- to five-fold elevation of both mucosal and tissue Ca^{2+} accumulation rates in rainbow trout fed high Ca^{2+} diet for 30 days. These differences were significant relative to the comparable rates at 15 days and relative to the other treatment groups at 30 days (except in the mid-intestine tissue compartment). Thus, this stimulatory effect of a high Ca^{2+} diet on Ca^{2+} accumulation was not seen in the same treatment at 15 days, and was prevented at 30 days if high Cd was present in the diet, either alone or in combination with high Ca^{2+} (Fig. 1).

In contrast to Ca^{2+} accumulation (Fig. 1), mucosal Cd accumulation rates were generally lower than tissue rates at 15 days and generally higher than tissue rates at 30 days in the mid-intestine (Fig. 2). These differences were not apparent in the posterior intestine (Fig. 2). Mucosal Cd accumulation in the mid-intestine was not significantly affected by the various treatment diets after 15 days of feeding. By 30 days, however, mucosal Cd accumulation in the mid-intestine was elevated in rainbow trout fed the high Cd diet. (Fig. 2). This difference was significant relative to the comparable rate at 15 days, as well as relative to both diets containing high Ca^{2+}. Thus, this stimulatory effect of a high Cd diet on Cd accumulation was not seen in the same treatment at 15 days, and was prevented at 30 days if high Ca^{2+} was present in the diet, either alone or in combination with high Cd. Indeed, tissue Cd accumulation was significantly reduced at 30 days in rainbow trout, which had been fed a high Ca^{2+} diet alone (Fig. 2).

The posterior intestine presented a different pattern of Cd accumulation relative to treatment (Fig. 2). At 15 days, both mucosal and tissue Cd uptake rates were significantly elevated in rainbow trout, which had been fed either a high Ca^{2+} diet or a high Ca^{2+} + high Cd diet, but not in those fed a high Cd diet alone. These differences were no longer seen at 30 days, though mucosal Cd uptake rate had increased relative to the comparable 15 day value in rainbow trout fed a high Cd diet alone.

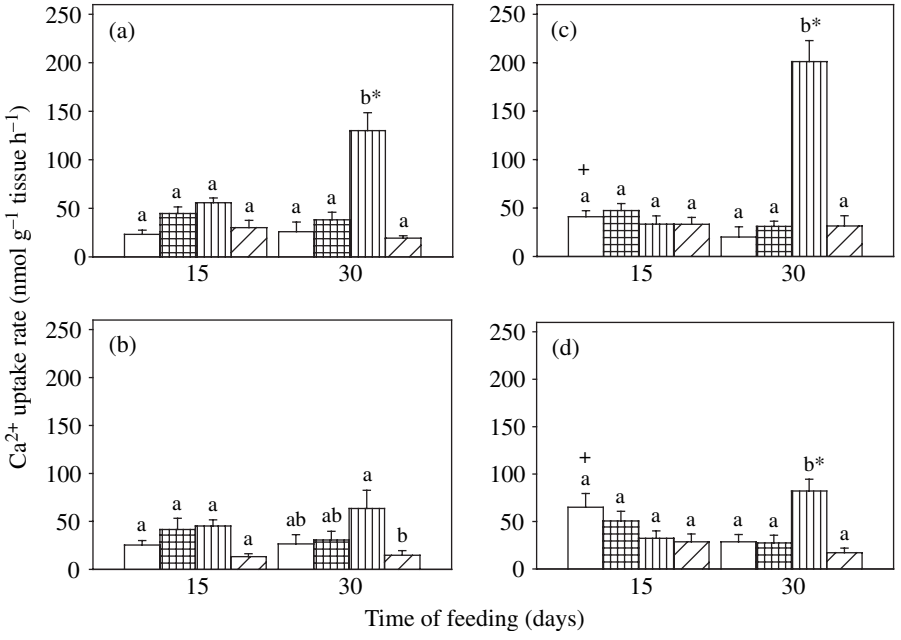

FIG. 1. Ca^{2+} uptake rates into the mucosal and tissue compartments in the (a), (b) mid- and (c), (d) posterior intestine of rainbow trout exposed to diets with different Ca^{2+} and Cd concentrations: □, the control food treatment; ▦, the Cd food treatment; ▥, the Ca^{2+} food treatment; ▨, the Ca^{2+} + Cd food treatment. Values are means ± S.E. (n = 6–7). For groups exposed to different dietary treatments, but within time point, means with different lower case letters are significantly different ($P < 0{\cdot}05$) as determined by multivariate ANOVA and by the Tukey test. *, significantly different from group submitted to the same treatment at 15 days ($P < 0{\cdot}05$); +, significantly different from group submitted to the same treatment in the mid-intestine within time point ($P < 0{\cdot}05$).

DISCUSSION

The two different measurements of intestinal Ca^{2+} and Cd absorption used in the present study (mucosal and tissue uptake rates) showed different absolute values, but the effect of the treatment diets was similar in all of them, except for Cd absorption in the mid-intestine. In addition, the use of these different measurements demonstrated that after 3 h, both Ca^{2+} and Cd passed through the mucosal epithelia to the underlying tissues, which include the vascular compartment. The results for Ca^{2+} are in agreement with an earlier study (Baldisserotto *et al.*, 2004*b*), which detected a sharp rise in plasma Ca^{2+} concentration within 2 h after first feeding of the high Ca^{2+} diet to rainbow trout. Similarly, the results for Cd absorption are in agreement with a previous study (Chowdhury *et al.*, 2004), which demonstrated that within 2 h after infusion of radiolabelled Cd into the gastrointestinal tract *in vivo* it was possible to detect this metal in the plasma of rainbow trout. Most Cd absorbed by the gastrointestinal tract of this species, however, accumulates in the gut tissues themselves (Handy, 1992). In addition, the amount of Ca^{2+} and Cd that passed through the mucosal epithelia into the underlying gut tissues in the present study is probably lower than would occur *in vivo*. Notably Foulkes (1996), commenting

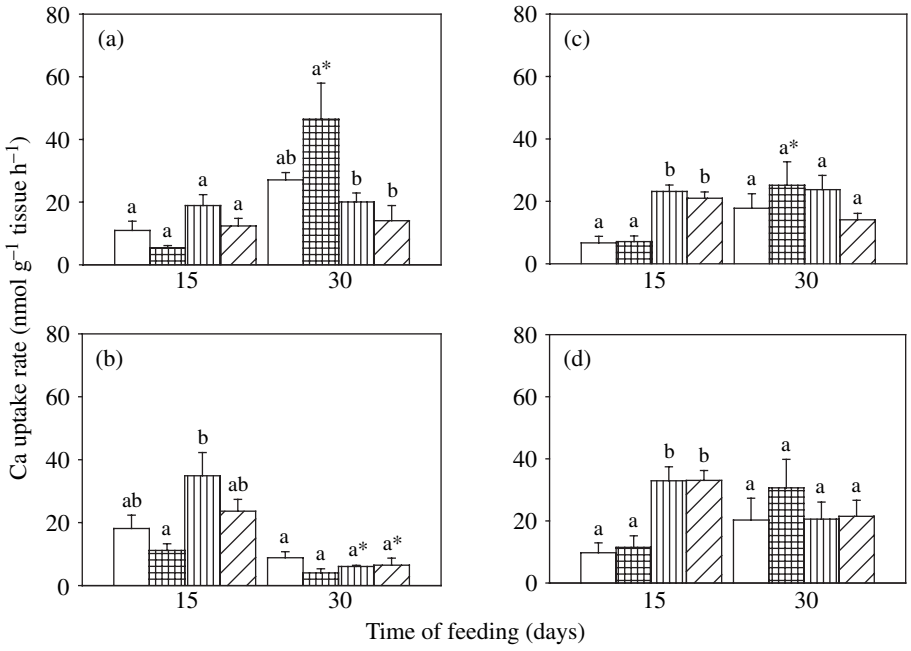

FIG. 2. Cd uptake rates into the mucosal and tissue compartments in the (a), (b) mid- and (c), (d) posterior intestine of rainbow trout exposed to diets with different Ca^{2+} and Cd concentrations (see Fig. 1). Values are means ± S.E. (n = 7–8).

on the use of gut sacs in mammals, pointed out that there is no blood perfusion in the epithelium and that an extensive metal binding in the submucosal tissues may therefore occur, which would diminish transmural movements.

It is important to emphasize that the uptake measurements in the present study were always performed under uniform conditions for the control and three experimental groups, *i.e.* with identical Ca^{2+} levels in the mucosal saline for the Ca^{2+} uptake rate measurements, and with identical Ca^{2+} and Cd levels in the mucosal saline for the Cd uptake rate measurements. Thus, the present study examined changes in transport rates of Ca^{2+} and Cd resulting from chronic feeding of high Ca^{2+} and high Cd diets and not competition effects in themselves. In other words, changes in uptake rate of the system at constant substrate concentration were examined, on top of which the effects of differences in substrate concentration and competition effects would be superimposed *in vivo*.

A surprising finding of the present study was the up-regulation of Ca^{2+} transport in both the mid- and posterior intestine in rainbow trout fed the high Ca^{2+} diet, though only at 30 days (Fig. 1). It had been anticipated that chronic high Ca^{2+} feeding would down-regulate intestinal Ca^{2+} transport as the post-feeding surges of plasma Ca^{2+} *in vivo* attenuated with time (Baldisserotto *et al.*, 2004*b*). The increase of intestinal Ca^{2+} absorption (present study) and whole-body waterborne Ca^{2+} uptake (Baldisserotto *et al.*, 2005) provoked by the Ca^{2+}-supplemented diet after 30 days of feeding indicates that both systems were up-regulated instead of exhibiting the anticipated compensatory actions. This increase of intestinal Ca^{2+} absorption after 30 days of feeding was not

observed when the fish received the diet supplemented with Ca^{2+} + Cd (Fig. 1). Therefore, chronically elevated dietary Cd does not reduce intestinal Ca^{2+} absorption when it is at control levels (whatever is the diet), but inhibits any increase of intestinal Ca^{2+} absorption rate due to dietary Ca^{2+} supplementation. This suggests that the transporter system related to this increase of intestinal Ca^{2+} absorption rate is probably blocked or down-regulated to some extent by chronically elevated dietary Cd.

An important finding of the present study was that intestinal mucosal Cd accumulation increased after 30 days of feeding with the Cd-supplemented diet, at least in comparison to fish fed with the same diet for 15 days in the posterior intestine, as well as relative to several other simultaneous treatments in the mid-intestine (Fig. 2). *A priori*, a compensatory down-regulation might have been expected. The result, however, is in complete accord with the finding of Chowdhury *et al.* (2004) that the plasma appearance of radiolabelled Cd infused into the gastrointestinal tract is enhanced rather than inhibited in rainbow trout, which have been chronically fed with a high Cd diet. Apparently, Cd transport is increased by chronic high Cd feeding. Dietary Ca^{2+} supplementation for 30 days (either alone or in combination with high Cd), however, prevented this increase in Cd absorption in the mid-intestine (Fig. 2) and could explain why rainbow trout fed with a Ca^{2+} + Cd-supplemented diet showed lower Cd accumulation in the tissues than fish fed with Cd-supplemented diet (Baldisserotto *et al.*, 2005).

The higher Cd absorption in the posterior intestine of fish fed with the Ca^{2+}- and Ca^{2+} + Cd-supplemented diets for 15 days compared to fish fed with control diet (Fig. 2) indicates that dietary Ca^{2+} supplementation induced a transient effect on Cd absorption, which was overturned in the chronic presence of high Ca^{2+} in the diet, as this effect was no longer observed after 30 days.

Studies with Caco-2 cells indicated that Cd transport may be due to diffusion, by a co-transport with Cl^{-}, or *via* other transport systems bound to thiol-containing peptides, but not the Ca^{2+} channel pathway (Pigman *et al.*, 1997; Jumarie *et al.*, 2001). In addition, there is some evidence that the promiscuous intestinal metal transporter DMT1 may mediate the transport of Cd (Elisma & Jumarie, 2001; Park *et al.*, 2002; Bressler *et al.*, 2004), and stimulation of Ca^{2+} binding protein transcription by vitamin D_3 enhances Cd transport (Pigman *et al.*, 1997). Similar studies have not been done with fish intestine, but Cd that has entered the fish enterocyte may be shuttled across the basolateral plasma membrane by the Na^{+}/Ca^{2+} exchanger (Schoenmakers *et al.*, 1992). The present study demonstrated that Ca^{2+} and Cd interact in their effects on one or more intestinal transport pathways in the rainbow trout because chronically elevated dietary Cd prevented the increase of intestinal Ca^{2+} absorption provoked by elevated dietary Ca^{2+} (Fig. 1) and chronically elevated dietary Ca^{2+} prevented the increase of intestinal Cd absorption provoked by elevated dietary Cd. (Fig. 2). It is likely, however, that there are also transport mechanisms, which are not changed by chronically elevated dietary Ca^{2+} or Cd, since Ca^{2+} absorption in fish fed with the Cd-supplemented diet was not lower than in fish fed with control diet (Fig. 1). Conversely, the lack of a significant inhibitory effect of dietary Ca^{2+} supplementation on Cd absorption in the posterior intestine after 30 days of feeding indicated that Cd absorption might occur at least

partially by a Ca^{2+}-independent transport pathway (possibly DMT1) in this segment. Clearly, future studies measuring the activities and protein levels of Ca^{2+}-ATPase, Na^{+}/Ca^{2+} exchange, and DMT1, as well as histological analyses, will be useful in addressing these possibilities.

In conclusion, the present study demonstrated that the protective effects of elevated dietary Ca^{2+} against dietary Cd absorption observed by Baldisserotto *et al.* (2005) after 30 days of feeding are probably due in part to the fact that the high Ca^{2+} diet prevents the increase of Cd transport rate that would otherwise occur. Furthermore, chronically elevated dietary Cd does not reduce intestinal Ca^{2+} absorption rate when it is at control level, but inhibits any increase of intestinal Ca^{2+} absorption rate due to dietary Ca^{2+} supplementation.

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