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Renal function in the freshwater rainbow trout after dietary cadmium acclimation and waterborne cadmium challenge

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Abstract

Renal function was examined in adult rainbow trout (*Oncorhynchus mykiss*) after chronic exposure to a sublethal level of dietary Cd (500 mg/kg diet) for 52 d and during a subsequent challenge to waterborne Cd (10 μg/L) for 72 h. Dietary Cd had no major effects on UFR (urine flow rate) and GFR (glomerular filtration rate) but caused increased renal excretion of glucose, protein, and major ions (Mg^{2+}, Zn^{2+}, K^+, Na^+, Cl^- but Ca^{2+}). However, dietary Cd did not affect any plasma ions except Na^+ which was significantly elevated in the Cd-acclimated trout. Plasma glucose and ammonia levels fell by 25% and 36% respectively, but neither plasma nor urine urea was affected in Cd-acclimated fish. Dietary Cd exposure resulted in a remarkable increase of Cd load in the plasma (48-fold, ~22 ng/mL) and urine (60-fold, 8.9 ng/mL), but Cd excretion via the kidney was negligible on a mass-balance basis. Clearance ratio analysis indicates that all ions, Cd, and metabolites were reabsorbed strongly (58–100%) in both naïve and dietary Cd exposed fish, except ammonia which was secreted in both groups. Mg^{2+}, Na^+, Cl^- and K^+ reabsorption decreased significantly (3–15%) in the Cd-exposed fish relative to the control. Following waterborne Cd challenge, GFR and UFR were affected transiently, and only Mg^{2+} and protein excretion remained elevated with no recovery with time in Cd-acclimated trout. Urinary Ca^{2+} and Zn^{2+} excretion rates dropped with an indication of renal compensation towards plasma declines of both ions. Cadmium challenge did not cause any notable effects on urinary excretion rates of metabolites. However, a significant decrease in Mg^{2+} reabsorption but an increase in total ammonia secretion was observed in the Cd-acclimated fish. The study suggests that dietary Cd acclimation involves physiological costs in terms of renal dysfunction and elevated urinary losses.

Keywords: Acclimation; Fish; Kidney; Metal; Plasma ions; Renal function; Toxicity

1. Introduction

Cadmium (Cd) is nephrotoxic to fish (Larsen and Perkins, 2001) and other animals (Zalups and Ahmad, 2003). As a primary target organ, the kidney of freshwater fish accumulates substantial amounts of Cd during both waterborne (Giles, 1988; Harrison and Klaverkamp, 1989; Glynn et al., 1992; Chowdhury et al., 2003) and dietary (Harrison and Klaverkamp, 1989; Kraal et al., 1995; Szebedinszky et al., 2001; Chowdhury et al., 2004a, 2005) Cd exposure. Chronic exposure to waterborne Cd often leads to renal pathologies with moderate changes like vacuolization and granulation of tubular cells to severe changes like tubular necrosis and glomerular collapse (Oronsaye, 1989; Gill et al., 1989; Singhal and Jain, 1997; Thophon et al., 2003). In contrast to these well-documented histological changes, the effects of Cd-induced renal damage on renal physiology in fish are not yet well known. However, alterations of enzyme activity, cortisol production, and glucose metabolism have been reported (Srivastava, 1982; Gill et al., 1991; Kinne-Saffran et al., 1993; Hontella et al., 1996). Giles (1984) reported proteinuria and urinary ion losses during chronic exposure to waterborne Cd, but concluded that electrolyte imbalances were not caused by impairment of renal function. To the best of our knowledge, the effects of chronic dietary Cd on renal handling of ions or other components in fish have not been studied yet.

On the contrary, both the structural and functional effects of Cd on the mammalian kidney have been studied extensively (see recent review by Zalups and Ahmad, 2003). Similar kidney lesions occur mainly in the proximal tubules and glomeruli, and

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the effects on the distal tubule are relatively much less. The changes in renal function involve abnormalities of tubular reabsorption and secretion manifested by renal dysfunction in electrolyte handling together with proteinuria, glucusuria, aminoaciduria, and phosphaturia (Friberg et al., 1974; Kendall et al., 1983; Piscator, 1986; Robinson et al., 1993). The endogenous metal binding protein metallothionein (MT) is known to play a special role in Cd nephrotoxicity by providing Cd a vehicle for transport to kidney, and aiding in cellular uptake. The circulating Cd-MT complexes, released during liver damage or formed by binding plasma Cd, are freely filtered through the renal glomeruli and efficiently taken up by the tubular epithelial cells, where they are rapidly degraded by lysosomal enzymes. The liberated Cd binds to endogenous MT but excess Cd interacts with intracellular machinery to elicit toxicity (Dudley et al., 1985; Chan et al., 1993; Zalups and Ahmad, 2003). The existence and induction of MT in fish tissues including the liver and kidney after Cd exposure are commonly observed (Roesijadi and Robinson, 1994; Olsson et al., 1995; Zhang and Schlenk, 1995; Chowdhury et al., 2005), although the involvement of piscine MT in mammalian-type renal toxicity has not been established.

The kidney, second only to the gills, plays a vital role in ionoregulation, acid–base regulation, and nitrogenous waste excretion in fish (Wood, 1993, 1995). It is the primary organ for elimination of water and particularly important for freshwater fish in which efficient ion reabsorption mechanisms in the kidney minimize the accompanying loss of ions (Hickman and Trump, 1969; Larsen and Perkins, 2001). Chronic exposures to waterborne or dietary Cd have been shown to manifest ionoregulatory and other types of physiological disturbances in fish (Haux and Larsson, 1984; Giles, 1984; Pratap et al., 1989; Fu et al., 1990; Pratap and Wendelaar Bonga, 1993; McGeer et al., 2000; Lacroix and Hontela, 2004; Baldisserotto et al., 2005). Recently, we described the respiratory, acid–base, ionoregulatory, hematological, and stress responses in adult rainbow trout (*Oncorhynchus mykiss*) after an exposure to a sublethal level of dietary Cd (500 mg/kg diet) for 45 d (Chowdhury et al., 2004b). We also examined the same parameters in these fish during a subsequent challenge to waterborne Cd (10 μg/L) for 72 h to understand if pre-exposure to dietary Cd exerted any impacts (e.g., acclimation or sensitization) on acute responses to waterborne Cd exposure (Chowdhury et al., 2004b).

Here, we report renal function (glomerular filtration rate, urine flow rate, and urine composition) studied consecutively with a parallel group of identically treated fish. Following 52 days of exposure to dietary Cd, renal function in the Cd exposed fish was compared to that of non-exposed fish, by examining plasma and urine samples collected via arterial and urinary bladder catheters. The preceding study revealed that chronic exposure to dietary Cd produced acclimation with increased protection against the effects of waterborne Cd on arterial blood P_{aCO2} and pH, plasma ions and stress indices (Chowdhury et al., 2004b). Therefore, the same dietary Cd concentration (500 mg/kg diet) was used in this study to induce acclimation, and both the dietary Cd-acclimated (pre-exposed) and non-acclimated (non-exposed) fish were challenged with the same level of waterborne Cd (10 μg/L) for 72 h. This challenge concentration is approximately 50% of the 96-h LC50 of waterborne Cd for juvenile trout (22 μg Cd/L; Niyogi et al., 2004). A higher concentration was not chosen to avoid lethality. The primary goal of Cd challenge was to understand if there is any renal compensation or dysfunction related to acclimatory changes in the fish.

2. Materials and methods

2.1. Acclimation to dietary Cd

The experimental methods used in this study for laboratory conditioning and dietary acclimation to Cd were the same as those described in Chowdhury et al. (2004b), since both studies were consecutively conducted with groups of fish held in the same control and treatment tanks. In brief, adult rainbow trout were purchased from a local hatchery and held for at least 2 weeks in the laboratory flow-through set-up of dechlorinated Hamilton City tapwater (Lake Ontario water, 12±1 °C, pH: 8.0±0.2) for laboratory conditioning. Afterwards, fish (~165 g) in one holding tank were fed a 1.5% daily ration (dry feed/wet body wt) of a prepared Cd-enriched diet (nominal 500 mg Cd/kg dry feed, actual measured concentration: 461.2±4.1 mg Cd/kg feed or 6.9 mg Cd/kg fish/day) for 52 d for dietary Cd acclimation. Another holding tank was used as control tank in which fish (~165 g) were fed a prepared control diet (feed with no added Cd, actual measured concentration: 0.212±0.013 mg Cd/kg feed) at the same ration for 52 d. Diets were prepared by mixing a solution of Cd $(NO_3)_2{\cdot}4H_2O$ (6.86 g/L; Fisher Scientific, ON, Canada; Cd diet) or only double distilled water (control diet) into the commercial trout chow as described in Chowdhury et al. (2004a). Fish were not fed for 48 h prior to cannulation and during acute Cd challenge. The measured concentrations of waterborne Cd in both tanks were minimal (<0.35 μg/L).

Our results reported in Chowdhury et al. (2004b) suggest that dietary Cd exposure produced physiological acclimation in fish used in this study. Therefore, Cd-exposed fish are hereafter called the "Cd-acclimated" group and the fish on control diet are the "non-acclimated" group.

2.2. Cannulation

Two catheters were surgically implanted in fish from non-acclimated (258.6±31.1 g) and Cd-acclimated groups (251.4±35.3 g). One was an arterial catheter (PE-50 polyethylene tubing, Intramedic, Becton Dickinson, Sparks, MD, U.S.A) fitted in the dorsal aorta as described by Soivio et al. (1972) for sampling arterial blood. The other was a urinary catheter (heat molded PE-60 polyethylene tubing, Intramedic) fitted in the urinary bladder as described by Curtis and Wood (1991) and Wood and Patrick (1994) for gravimetrical collection of urine as it is produced from the ureters. Both catheters were implanted simultaneously while fish were anaesthetized with MS-222 (0.075 g/L) and artificially ventilated on a surgery table. After surgery, fish were individually

transferred to darkened plexiglas chambers (2.5 L) supplied with a constant water flow (250 mL/min) and aeration and allowed to recover from surgery for 36 h. Urine flow was continuously collected with the catheter emptying into a vial approximately 3.0 cm below the water level of the chamber.

2.3. Waterborne Cd challenge and sampling

The sampling protocol following dietary Cd exposure and during waterborne Cd challenge has been presented in Fig. 1. After 24 h of post-surgical recovery, [^{3}H]-polyethyleneglycol-4000 (PEG-4000, Wood and Patrick, 1994) was injected into the circulatory system of the fish as a marker to determine glomerular filtration rate (GFR). Each fish received 17 μCi of [^{3}H] PEG-4000 (Perkin-Elmer; specific activity 1.56 mCi g^{-1}) via the arterial catheter. The radiotracer was delivered in 0.66 mL of Cortland saline (Wolf, 1963) and allowed to equilibrate within the fish for 12 h (i.e., until 36 h of recovery) prior to the collection of urine and blood for analysis (−12 to 0-h pre-challenge period, Fig. 1). After 36-h post-surgery recovery, blood and urine samples were collected from the dietary Cd-exposed and non-exposed fish via catheters at 24-h intervals for blood and 12-h intervals for urine. Blood samples (~1 mL) were instantaneous but urine samples were collections of urine flow over 12-h periods. Immediately after the 24-h sample time, both treatment groups were "challenged" with a nominal waterborne Cd concentration of 10 μg/L (89 nmol/L) and exposure continued until 96 h (72-h post-challenge). The first blood (0 h) and urine (0–12 h) samples were taken to document the effects of chronic dietary Cd exposure on renal function, and the second ones taken at 24 h for blood and 12–24 h for urine to confirm any difference which could be attributed to the termination of feeding. The fish chambers were connected to a Mariotte bottle exposure system with an acidified stock solution of $Cd(NO_3)_2{\cdot}4H_2O$ to maintain the nominal Cd concentration in the water (Chowdhury et al., 2004b). Actual dissolved Cd concentration as measured by graphite furnace atomic absorption spectrophotometry (GFAAS; SpectrAA-220, Varian, Australia) after passing the water through 0.45 μm filters was 11.8±0.2 μg/L (105.0±1.5 nmol/L, n=40).

Blood samples were centrifuged at 13,000 ×g for 1 min and separated plasma was used to determine [^{3}H]-PEG-4000 cpm for GFR calculations and plasma concentrations of ions (Ca^{2+}, Mg^{2+}, K^+, Na^+, Cl^-, Zn^{2+}), Cd^{2+}, metabolites (total ammonia, urea, glucose, lactate) and protein. Erythrocytes were carefully resuspended in the appropriate volume of Cortland saline (Wolf, 1963) and reinjected into the fish via the catheter. The plasma samples were immediately frozen in liquid nitrogen and stored at −80 °C. Prior to analysis, the samples were thawed and sonicated on ice for 5 s at 5 W (Microson, Misonix Inc, NY, USA) to ensure homogeneity. One aliquot of the plasma sample was deproteinized in two volumes of ice-cold 6% perchloric acid for the determination of lactate and glucose. Urine samples were also frozen at the end of every 12-h collection and stored at −80 °C for later analysis of the same parameters.

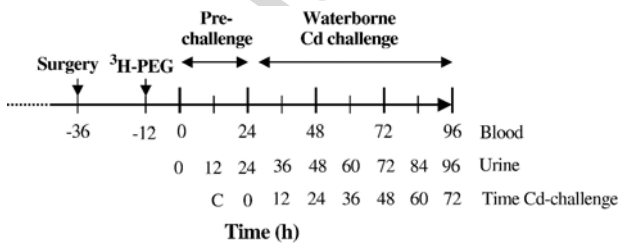

Fig. 1. Schematic of the sampling protocol for blood and urine, and actual time of sampling during Cd-challenge for both. "C" indicates the "pre-challenge" mean calculated for all ions and metabolites from two data points (0 and 24 h for plasma, 0–12 and 12–24 h for urine) recorded before waterborne Cd challenge. ^{3}H-PEG: ^{3}H-polyethyleneglycol-4000.

To determine GFR, 25 μL of plasma was counted (1217 Rackbeta; LKB Wallac, Turku, Finland) along with 4.975 mL of double-distilled water and 10 mL of scintillation fluid (ACS; Amersham) for β radioactivity. Urine samples (0.5 mL aliquots) were similarly processed by addition of 4.5 mL of double-distilled water and 10 mL of scintillation fluid prior to β counting. Tests demonstrated that quench was uniform, so no correction was necessary. Since blood samples were taken only at 24-h intervals, the two consecutive blood counts were averaged to generate an intermediate value, thereby yielding a complete set of blood counts at every 12 h from 0 h pre-challenge through 96 h of the experimentation. Blood and urine counts for every 12-h period were used to determine GFR (see below for calculation).

2.4. Analytical measurements of plasma and urine parameters

The concentrations of Na^+, K^+, Ca^{2+}, Mg^{2+} and Zn^{2+} were measured by flame atomic absorption spectrophotometry (FAAS; SpectrAA-220FS, Varian, Australia). Cd concentrations were determined by GFAAS as described above. Cl^- concentrations were measured by the mercuric thiocyanate spectrophotometric method (Zall et al., 1956). Plasma and urine protein concentrations were assayed using Bradford reagent and bovine serum albumin standards (Sigma-Aldrich). Total ammonia (T_{amm}) concentrations were determined enzymatically (glutamate dehydrogenase/NADP at 340 nm; Sigma-Aldrich) as described by Neeley and Phillipson (1988). Urea concentrations were measured using the diacetyl monoxime method of Rahmatullah and Boyde (1980). Glucose and lactate concentrations were measured enzymatically on deproteinized samples (6% perchloric acid) using commercially available reagent kits for glucose (hexokinase/glucose-6-phosphate dehydrogenase at 340 nm; ThermoDMA kit, Louisville, CO, USA) and lactate (lactate dehydrogenase/NADH at 340 nm; Sigma-Aldrich).

2.5. Calculation of renal parameters

GFR (mL/kg/h) is the clearance rate of [^{3}H]PEG-4000 via the urinary system and calculated as the excretion of ^{3}H counts in the urine relative to its concentration in the blood plasma:

$$GFR = \{(cpm_u)(UFR)\}/(cpm_p) \quad (1)$$

where (cpm_u) is the counts per minute per milliliter of urine and (cpm_p) is the average of two plasma counts (cpm/mL) bracketing the 12-h urine collection period. UFR is the urine flow rate (mL/kg/h) relative to the body weight of fish over this collection period.

The urinary excretion rates (UER_x) of all ions, Cd and metabolites were calculated as:

$$UER_x = [X]_u(UFR) \quad (2)$$

where $[X]_u$ is the concentration of a substance in urine collected from a kilogram of fish over a 12-h period.

From the above relationships the clearance ratios (CR_x) of ions and metabolites were calculated according to the following equation:

$$CR_x = \{[X]_u(UFR)\}/\{[X]_p(GFR)\} \quad (3)$$

where $[X]_p$ is the concentration of a substance in the blood plasma. Thus, CR_x relates the clearance rate of a substance (X) to the clearance rate of the non-reabsorbed, non-secreted marker [^{3}H]-PEG 4000 (i.e. GFR). Values of CR_x greater than 1.0 indicate that X is secreted on a net basis by the renal system, while values of CR_x less than 1.0 indicate that X is reabsorbed on a net basis (see Wood and Patrick, 1994; Wood, 1995). For example, a CR_x value of 0.1 would indicate 90% net reabsorption of the filtered load of X, while a CR_x value of 2.0 would indicate that the rate of excretion of X was twice as large as that attributable to glomerular filtration alone — i.e. net secretion of X must have occurred.

The clearance rate (mL/kg/h), which allows for estimation of the efficiency of renal clearance of plasma constituents (Wood and Patrick, 1994), was calculated for Cd from UER_{Cd} and plasma Cd concentration:

$$\text{Clearance rate} = (UER_{Cd})/[Cd]_p. \quad (4)$$

2.6. Data presentation and statistical analysis

All data values are presented as mean ± standard error of the mean (SE, n = number of fish). The two data points (0 and 24 h for plasma, 0–12 and 12–24 h for urine, Fig. 1) recorded before waterborne Cd challenge were never significantly different and therefore have been presented as the "pre-challenge" mean for

Table 1
Plasma and urine parameters in non-acclimated and Cd-acclimated rainbow trout after exposure to dietary Cd (500 mg/kg) for 52 d (pre-challenge), and 72 h after a waterborne Cd challenge (10 μg/L)

Parameters	Pre-challenge		Cd-challenge	
	Non-acclimated	Cd-acclimated	Non-acclimated	Cd-acclimated
UFR (mL/kg/h)	1.68±0.14	1.54±0.08	1.33±0.06	1.30±0.16
GFR (mL/kg/h)	3.76±0.48	3.92±0.19	3.80±0.63	3.92±0.51
Plasma				
Ca^{2+} (μmol/mL)	2.34±0.04	2.45±0.05	1.61±0.03$^+$	2.14±0.12*
Mg^{2+} (μmol/mL)	0.96±0.04	1.01±0.04	0.98±0.03	1.14±0.06
K^+ (μmol/mL)	2.64±0.05	2.79±0.06	3.24±0.05$^+$	2.72±0.05*
Na^+ (μmol/mL)	138.7±3.5	148.5±2.1*	142.8±1.4	164.2±2.8*$^+$
Cl^- (μmol/mL)	124.3±2.0	125.2±1.8	123.0±1.0	137.0±1.8*$^+$
Cd^{2+} (ng/mL)	0.45±0.08	21.89±2.33*	1.28±0.19$^+$	23.0±3.3*
Zn^{2+} (ng/mL)	6733±623	8856±1021	3449±549$^+$	5017±982$^+$
T_{amm} (μmol/mL)	0.085±0.003	0.054±0.003*	0.119±0.008$^+$	0.060±0.001*
Urea (μmol/mL)	1.43±0.03	1.42±0.05	1.43±0.13	1.63±0.10
Glucose (μmol/mL)	10.41±0.50	7.86±0.48*	12.51±0.45$^+$	8.68±0.54*
Lactate (μmol/mL)	0.89±0.15	0.88±0.14	1.60±0.09$^+$	0.78±0.08*
Protein (mg/mL)	21.45±0.27	23.58±0.36*	20.21±0.34	22.51±0.39*
Urine				
Ca^{2+} (μmol/mL)	1.49±0.20	1.48±0.22	0.78±0.04$^+$	0.99±0.17
Mg^{2+} (μmol/mL)	0.29±0.07	0.67±0.11*	0.10±0.006$^+$	0.66±0.10*
K^+ (μmol/mL)	0.76±0.05	1.71±0.27*	0.82±0.06	1.25±0.14*
Na^+ (μmol/mL)	12.04±1.68	25.95±2.19*	14.82±2.08	16.7±4.66
Cl^- (μmol/mL)	8.60±1.17	20.44±1.78*	12.15±1.80	12.96±2.82
Cd^{2+} (ng/mL)	0.15±0.01	8.90±1.13*	1.09±0.07$^+$	11.48±2.65*
Zn^{2+} (ng/mL)	54.6±4.2	123.8±12.9*	27.8±8.7$^+$	35.8±6.0$^+$
T_{amm} (μmol/mL)	0.93±0.08	0.93±0.09	1.16±0.03$^+$	1.19±0.03$^+$
Urea (μmol/mL)	1.32±0.04	1.48±0.09	0.96±0.13$^+$	1.27±0.12
Glucose (μmol/mL)	ND	0.92±0.18*	ND	0.03±0.009$^+$
Lactate (μmol/mL)	ND	ND	ND	ND
Protein (μg/mL)	ND	118.7±7.0*	ND	122.6±19.5*

(*) indicates significant difference ($p<0.05$) between non-acclimated and Cd-acclimated fish for pre-challenge and Cd-challenge groups; (+) indicates significant difference ($p<0.05$) between the pre-challenge and Cd-challenge groups for non-acclimated or Cd-acclimated fish; ND: not detected.

all ions and metabolites. Time-dependent responses in both non-acclimated and dietary Cd-acclimated groups during Cd challenge were tested against their respective "pre-challenge" means by a one-way ANOVA followed by a posthoc multiple comparison (Newman–Keuls test). In addition, each mean for the acclimated group was also compared to that of the non-acclimated group at the same time by an unpaired Student's *t*-test. All statistical tests were performed using the computer software InStat (GraphPad, San Diego, CA, USA). Statistical significance was accepted at $p \leq 0.05$ in all cases.

3. Results

3.1. Mortality

No mortality was recorded during chronic exposure (52 days) of trout to dietary Cd (500 mg/kg). There was also no mortality in either the non-acclimated or Cd-acclimated treatment groups during waterborne Cd challenge (10 μg/L). However, urinary catheters in two non-acclimated fish failed during Cd challenge, resulting in a decrease from a pre-challenge $n=7$ to a post-challenge $n=5$.

3.2. Renal water handling

3.2.1. Effects of dietary Cd exposure

UFR and GFR in non-acclimated and Cd-acclimated fish were not significantly different before waterborne Cd challenge (Table 1), suggesting that renal water handling by trout was not affected by chronic exposure to dietary Cd for 52 days. However, the clearance ratio of water in Cd-acclimated fish (0.40, Fig. 2a) was slightly but significantly smaller relative to non-acclimated fish (0.46, Fig. 2a), indicating that water reabsorption in Cd pre-exposed fish was greater (60% vs 54%).

3.2.2. Effects of waterborne Cd challenge

Cd-acclimated fish showed significantly greater UFR and GFR than non-acclimated fish in the first 24 h of Cd challenge (Fig. 3). Only in the naïve, non-acclimated fish, UFR significantly decreased (~20–30%) relative to the pre-challenge level (1.68 mL/kg/h) but with recovery by 72 h during the Cd challenge (Fig. 3a). CRs indicated that water reabsorption in the Cd pre-exposed fish was not significantly different at 72 h after Cd challenge (Fig. 2b).

3.3. Renal handling of ions

3.3.1. Effects of dietary Cd exposure

Prior to waterborne Cd challenge, urine concentrations of most ions (Mg^{2+}, K^+, Na^+, Cl^-, and Zn^{2+}, Table 1) and their urinary excretion rates (UER, Figs. 4 and 6) were approximately twice as high in the Cd-acclimated fish relative to the naïve fish, suggesting that renal regulation of ions by trout was impacted by prolonged exposure to dietary Cd, such that Cd-exposed fish were experiencing increased renal loss of ions. However, dietary Cd did not affect any plasma ions except Na^+ which was

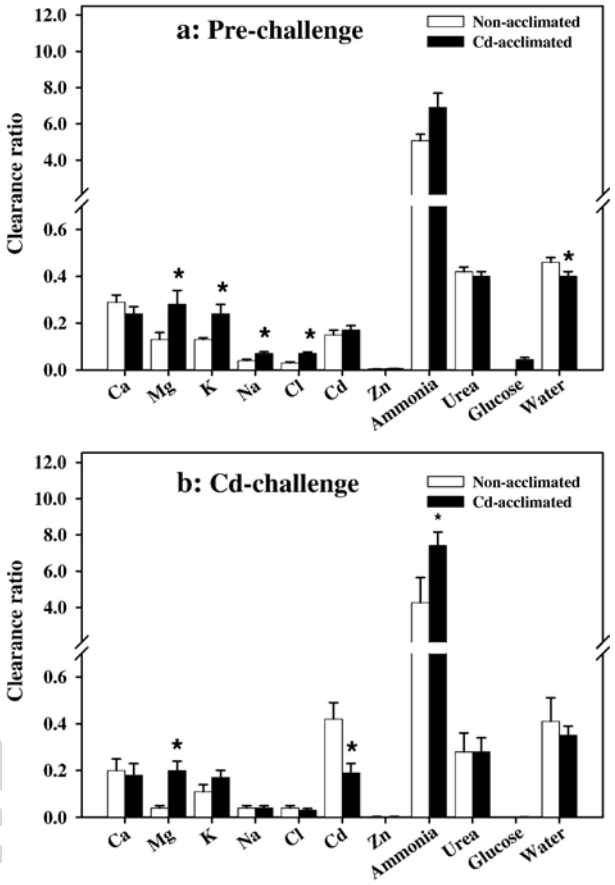

Fig. 2. Clearance ratios (CR, see Eq. (3)) of urinary ions and metabolites in non-acclimated and Cd-acclimated rainbow trout after exposure to dietary Cd (500 mg/kg) for 52 d (a: Pre-challenge), and 72 h after a challenge exposure to 10 μg/L of waterborne Cd (b: Cd challenge). Results are presented as mean±SE ($n=5$–7). Means with "*"indicate significant difference ($p<0.05$) between non-acclimated and Cd-acclimated fish during simultaneous sampling.

significantly elevated in the Cd-acclimated trout (Table 1). In the case of Ca^{2+}, no effect of dietary Cd was observed as plasma and urine concentrations (Table 1) and UERs (Fig. 4a) between the treatment groups were not significantly different. In general, all ions were reabsorbed strongly (71–99.6%) in both naïve and dietary Cd exposed fish (CRs: 0.004–0.29, Fig. 2a) and reabsorption of ions was greater in the order Zn^{2+} (CRs: 0.004–0.006)>Na^+ and Cl^- (0.03–0.07)>K^+ and Mg^{2+} (0.13–0.28)>Ca^{2+} (0.24–0.29). In the case of Mg^{2+}, Na^+, Cl^- and K^+, clearance ratios increased significantly in Cd-acclimated fish relative to the control (Fig. 2a), suggesting a decrease in the relative reabsorption of these ions caused by dietary Cd.

3.3.2. Effects of waterborne Cd challenge

During waterborne Cd challenge, UERs of Ca^{2+} were still not significantly different between the treatment groups but dropped significantly over time only in non-acclimated fish (Fig. 4a). The decrease of UER from the pre-challenge level (~2.5 μmol/kg/h) was approximately 60% by 72 h, which was relatively greater than the decrease (31%) in the plasma Ca^{2+} (Table 1). Unlike Ca^{2+}, UERs of Mg^{2+} were significantly greater in Cd-acclimated fish throughout as observed before Cd

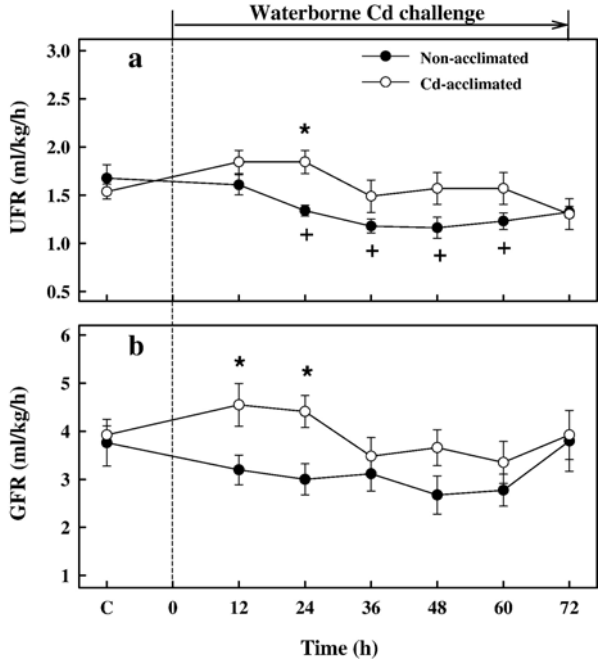

Fig. 3. Renal water handling in adult rainbow trout before and during waterborne Cd challenge (10 μg/L). UFR (a): Urine flow rate; GFR (b): Glomerular filtration rate. Before experimental sampling, the fish had been fed either a control diet (0 mg Cd/kg dry diet, non-acclimated) or a Cd diet (500 mg Cd/kg dry diet, Cd-acclimated) for 52 d. Urine was collected via a urinary bladder catheter. Results are presented as mean±SE (n=5–7). Means with "∗"indicate significant difference (p<0.05) between non-acclimated and Cd-acclimated fish during simultaneous sampling. Cd-challenge means with "+" indicate significant difference (p<0.05) from the "pre-challenge" mean (indicated by "C" on x-axis) in the same group.

challenge. Similar to Ca^{2+}, Mg^{2+} excretion also diminished significantly at 72 h (~75%) from the pre-challenge level (~0.53 μmol/kg/h) only in non-acclimated fish (Fig. 4b). However, plasma Mg^{2+} was not affected by waterborne Cd (Table 1). Monovalent ions showed similar trends during waterborne Cd challenge: the significant differences in UERs between non-acclimated and Cd-acclimated groups attributable to dietary Cd were abolished gradually by 12 h (Na^+, Fig. 4d), 36 h (Cl^-, Fig. 4e), or 72 h (K^+, Fig. 4c) of the challenge. Neither of the monovalent ions varied significantly in UER relative to the pre-challenge levels in both groups. Similar to Ca^{2+}, urinary Zn^{2+} excretion also dropped significantly over time following Cd challenge by 62% and 75% in non-acclimated and Cd-acclimated fish respectively at 72 h (Fig. 6c). At this time, plasma Zn^{2+} levels declined by 50% and 43% respectively (Table 1). The pre-challenge difference in UER_{Zn} between the two groups was not consistently observed during the challenge period.

Similar to the pre-challenge effects, the challenge CR of Mg^{2+} ($CR_{non\text{-}acclimated}$=0.04; $CR_{Cd\text{-}acclimated}$=0.20; Fig. 2b), but not of other divalent ions (Ca^{2+}, Zn^{2+}), was significantly greater in the Cd-acclimated fish, suggesting that dietary and waterborne Cd affected the reabsorption of Mg^{2+}, but not of Ca^{2+} and Zn^{2+} in the kidney. Among monovalent ions (K^+, Na^+ and Cl^-), none showed a significant difference in CRs between non-acclimated and Cd-acclimated fish, Fig. 2b).

3.4. Renal handling of metabolites

3.4.1. Effects of dietary Cd exposure

The effects of Cd exposure on plasma and urine metabolites (total ammonia, urea and glucose) are presented in Table 1 and Fig. 5. Prolonged exposure to dietary Cd resulted in a significant decrease of plasma total ammonia (T_{amm}, 36%, Table 1) without any effect on urinary excretion (Table 1, Fig. 5a). However, plasma glucose level fell significantly (25%, Table 1) in the Cd-acclimated fish with an elevation of urinary glucose from a non-detectable level in the naïve fish to 0.92±

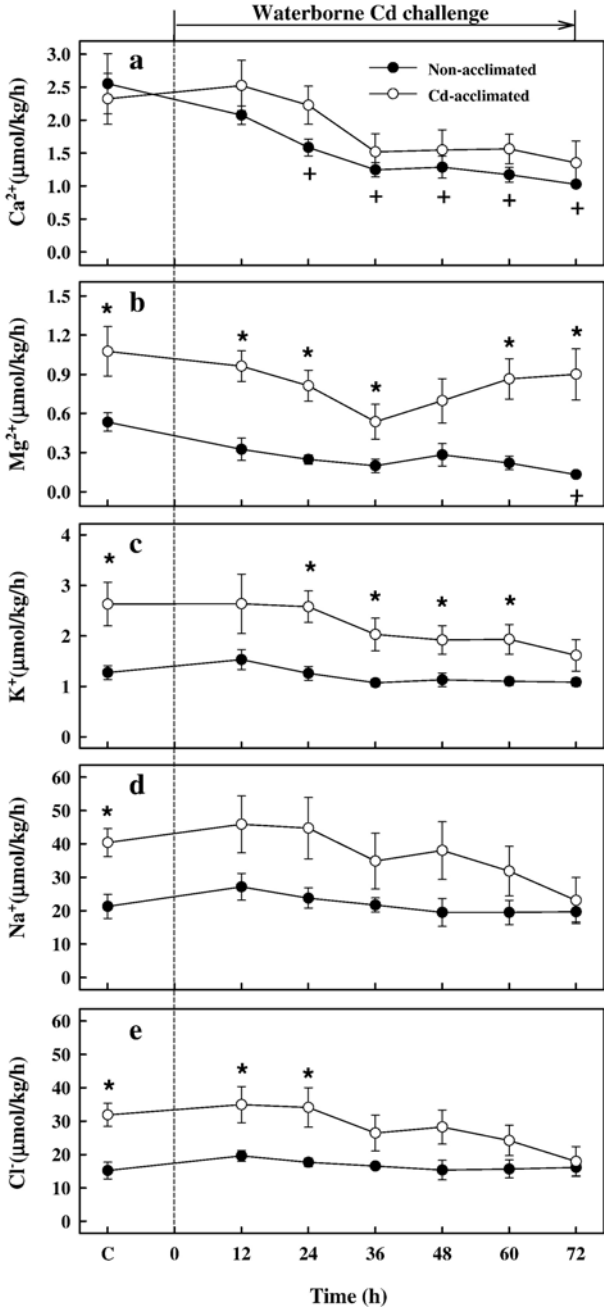

Fig. 4. Urinary excretion rates of Ca^{2+} (a), Mg^{2+} (b), K^+ (c), Na^+ (d), and Cl^- (e) in adult rainbow trout before and during waterborne Cd challenge (10 μg/L). Format as per Fig. 3.

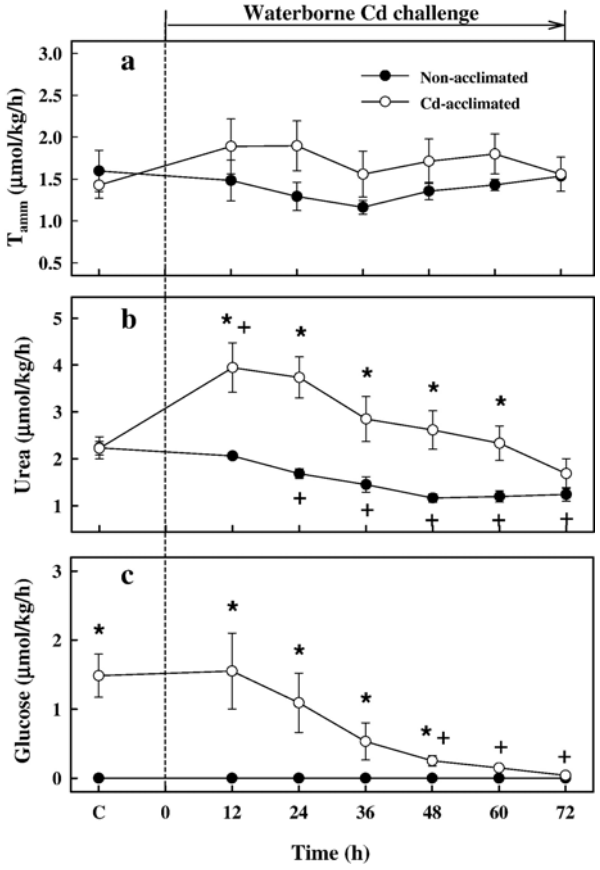

Fig. 5. Urinary excretion rates of metabolites in adult rainbow trout before and during waterborne Cd challenge (10 μg/L). (a) Total ammonia (T_{amm}), (b) Urea, (c) glucose. Format as per Fig. 3.

0.18 μmol/mL (Table 1; $UER_{glucose}$ = 1.5 ± 0.3 μmol/kg/h, Fig. 5c) in Cd-acclimated fish, suggesting that dietary Cd caused glucose loss via the kidney. No significant effects of prolonged dietary Cd exposure were observed on plasma and urine urea concentrations (Table 1) or UER_{urea} (Fig. 5b). Clearance ratios indicate that ammonia was secreted (CR_{Tamm} = 5–7, Fig. 2a), while urea and glucose were reabsorbed substantially in the kidney of control and Cd exposed fish (CR_{urea} = 0.40–0.42; $CR_{glucose}$ = 0–0.04; Fig. 2a), showing no significant difference between the treatments.

3.4.2. Effects of waterborne Cd challenge

Cadmium challenge did not cause any notable effects on urinary excretion rates of metabolites (Fig. 5) but resulted in a temporary elevation (~2-fold) of urea excretion rate in Cd acclimated fish, which gradually fell to the level observed in non-acclimated fish at 72 h (Fig. 5b). However, plasma ammonia and glucose were significantly lower (43% and 30% respectively, Table 1) in the Cd-acclimated fish at 72 h after Cd challenge, when only ammonia secretion ($CR_{ammonia}$) was affected in these fish (Fig. 2b). The elevated glucose excretion attributable to dietary Cd also did not persist over time in Cd pre-exposed fish (Fig. 5c), which exhibited significantly smaller CRs at 72 h (0.001) relative to the pre-challenge value (0.045, Fig. 2). Lactate was not detectable in urine in either non-acclimated or Cd-acclimated fish during pre-or post-challenge period, although it was available in the plasma (Table 1).

3.5. Urinary excretion of protein

3.5.1. Effects of dietary Cd exposure

Prolonged exposure of trout to dietary Cd resulted in protein loss via the kidney, as a notable amount of urinary protein was observed in the Cd pre-exposed trout while it was not detectable in the control group (Table 1). Plasma protein concentration was also slightly but significantly higher in the Cd pre-exposed trout (Table 1). The urinary excretion rate of protein in the Cd pre-exposed trout was 183 ± 14 μg/kg/h before waterborne Cd challenge. (Fig. 6a).

3.5.2. Effects of waterborne Cd challenge

The UERs (Fig. 6a) and plasma levels (Table 1) of protein remained more or less unchanged from pre-challenge levels in both groups except that a transient increase in excretion was observed at 12-h post-challenge time in the non-exposed control group ($UER_{protein}$ = 123 ± 24 μg/kg/h).

3.6. Renal handling of Cd

3.6.1. Effects of dietary Cd exposure

Dietary Cd exposure resulted in remarkable increase of Cd load in the blood plasma (48-fold) and consequently in urine

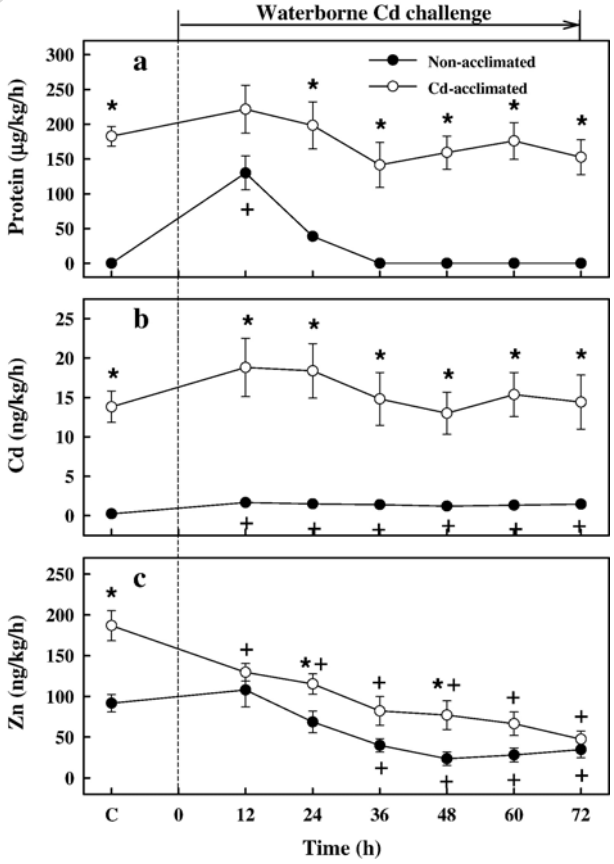

Fig. 6. Urinary excretion rates of protein (a), cadmium (b), and zinc (c) in adult rainbow trout before and during waterborne Cd challenge (10 μg/L). Format as per Fig. 3.

(60-fold) in Cd-acclimated fish relative to the background concentration in non-acclimated trout (Table 1). The UER of Cd in the pre-challenge, Cd-exposed trout was 13.8±2.0 ng/kg/h which was approximately 58 times greater than that in the non-exposed group (Fig. 6b). The clearance ratios in both groups were low and non-significantly different (~0.15), suggesting that a substantial and identical fraction (~85%) of filtered Cd load was reabsorbed in the kidney during dietary Cd exposure (Fig. 2a).

3.6.2. Effects of waterborne Cd challenge

After Cd challenge, the pre-challenged UER_{Cd} and plasma Cd level in the Cd-acclimated fish remained more or less unchanged over time, maintaining significantly higher values as compared with non-acclimated fish through 72 h (Table 1, Fig. 6b). On the other hand, the pre-challenge UER_{Cd} and plasma Cd in the non-acclimated fish was elevated slightly but significantly after Cd challenge (Table 1, Fig. 6b). Waterborne Cd resulted in 3-fold increase in CR_{Cd} in non-acclimated fish relative to the pre-challenge value (0.15) and 2-fold difference in CRs between the two experimental groups (Fig. 2), suggesting that the non-exposed group had relatively less capacity to reabsorb Cd originating from acute waterborne exposure.

4. Discussion

4.1. Effects on renal water handling

GFR and UFR are strong indicators of renal water handling, and freshwater fish tend to maintain a tight relationship between these two functional parameters over a wide range of values (GFR: ~2–9 mL/kg/h, UFR: ~1.0–4.5 mL/kg/h; Larsen and Perkins, 2001), allowing approximately 50% reabsorption of filtered water. GFR (3.8–3.9 mL/kg/h) and UFR (1.5–1.6 mL/kg/h) in the present study agree with the values commonly observed for freshwater fish and were not impacted before waterborne Cd challenge, suggesting that chronic exposure to dietary Cd did not affect water handling in the kidney (Table 1, Fig. 3). A 6% increase of pre-challenge water reabsorption in Cd-acclimated fish (CR_{water}=0.46 vs 40, Fig. 2a), but not a matching increase in Na^+ (CR_{Na}=0.04 vs 0.07,) or Cl^- (CR_{Cl}=0.03 vs 0.07,) reabsorption, was likely due to increased permeability of renal epithelia to water. Possibly, dietary Cd might mobilize arginine vasotocin, the major anti-diuretic hormone in lower vertebrates (Hickman and Trump, 1969; Larsen and Perkins, 2001).

The major effects after waterborne Cd challenge were the greater GFR and accordingly the UFR in the dietary Cd pre-exposed trout relative to the naïve non-acclimated trout (Fig. 3) and a decrease of UFR (20–30%) only in the non-acclimated fish relative to the pre-challenge value (Fig. 3a). Both effects appear to be related to the acute response of fish to waterborne Cd with limited or no acclimatory benefit as they were transient in nature.

4.2. Renal Cd burden and excretion

In our previous study (Chowdhury et al., 2005), Cd burden in the trout kidney exposed to dietary Cd (500 mg/kg diet) for 30 days under the same experimental conditions was 6.0±0.9 μg/g, which was a 116-fold increase from the control level. Assuming that kidney burden of Cd increases linearly with time (Dudley et al., 1985; Harrison and Klaverkamp, 1989; Thomann et al., 1997), the kidney concentration of Cd in the Cd-acclimated trout exposed to the same dietary Cd level but for 52 days in the present study, would have been approximately 10 μg/g.

The remarkable increase (58-fold relative to non-acclimated fish) in pre-challenge Cd excretion in the Cd-acclimated trout (UER_{Cd}=13.8±2.0 ng/kg/h, Fig. 6b) was largely a reflection of the filtered load as plasma Cd level also increased almost 48-fold at the same time (Table 1). Some Cd was also probably added to the luminal compartment of nephrons from necrotic or apoptotic tubular cells as observed in mammalian studies (Zalups et al., 1992). Given that only 6.6% of dietary Cd dose is internalized beyond the gastrointestinal tract in trout (Chowdhury et al., 2004a), the internally bioavailable Cd in the present study was ~455 μg/kg/d in trout exposed to dietary Cd (500 mg/kg diet, dose: 6.9 mg/kg fish/d, see Section 2.1). Then, the pre-challenge excretion of Cd (~14 ng/kg/h=0.336 μg/kg/d, Fig. 6b), which remained unaltered even after waterborne Cd challenge, would represent only 0.07% of the dietary Cd internalized daily in the Cd-exposed trout (~455 μg/kg). Thus, despite the fact that urinary excretion increased many fold during dietary Cd exposure, Cd loss via the kidney was negligible on a mass-balance basis. Similarly, the clearance rates of Cd (see Eq. (4)) calculated from the pre-challenge UER_{Cd} (Fig. 6b) and plasma Cd (Table 1) for the control (0.54±0.06 mL/kg/h) and Cd-exposed trout (0.67±0.08 mL/kg/h) were only approximately 30–40% of pre-challenge UFR (1.5–1.7 mL/kg/h, Table 1). Since UFR is essentially a measure of the clearance rate of water, the urinary route appears to be a poor means of Cd excretion, with Cd being cleared from the blood plasma less efficiently than water in the kidney. Interestingly again, the urinary clearance (0.54–0.67 mL/kg/h, see above) of plasma Cd is <2% of the overall plasma clearance rate of Cd in rainbow trout (~30 mL/kg/h, Chowdhury et al., 2004a), which includes disposition of plasma Cd into fish tissues including the kidney. The poor renal clearance of Cd in rainbow trout has been reported previously for waterborne and dietary Cd (Giles, 1988; Harrison and Klaverkamp, 1989).

The inefficiency of renal Cd clearance may be attributed to the high binding capacity of Cd to cellular proteins like metallothionein (MT) in fish including rainbow trout (Roesijadi and Robinson, 1994; Chowdhury et al., 2005) and to strong Cd reabsorption as observed in the present study (~85%, CR_{Cd}=0.15, Fig. 2a). In mammals, the conjugates of Cd with thiol-containing molecules such as albumin, glutathione and cysteine as well as Cd-MT have been shown to be filtered into the luminal tubule and are implicated in the absorptive transport of Cd by endocytosis in the proximal tubule; fractional reabsorptions of various forms of Cd as high as 82% have been observed (Felley-Bosco and Diezi, 1987; Zalups and Ahmad, 2003). The likely involvement of MT or other proteins in Cd transport to kidney and tubular uptake is probably the reason why non-acclimated trout showed a relatively smaller

reabsorption capacity (increased CR_{Cd}, Fig. 2b) when plasma total Cd level was elevated after waterborne Cd challenge (Chowdhury et al., 2004b). Indeed, basal tissue MT levels in naïve trout were 2–19 times smaller in comparison to the trout pre-exposed to dietary Cd for 30 days (Chowdhury et al., 2005).

4.3. Effects of chronic dietary Cd on renal ion handling

For homeostatic regulation, freshwater fish mostly reabsorb and sometimes secrete ions in the proximal tubule and later segments of the nephron (Larsen and Perkins, 2001). The overall CRs for ions (0.004–0.29, Fig. 2a) suggest that all ions (Ca^{2+}, Mg^{2+}, Zn^{2+}, Na^{+}, Cl^{-}, K^{+}) were reabsorbed strongly in both the control and Cd exposed trout (71–99.6%), in general agreement with the reabsorptions observed in control trout (37–98%) by Wood (1995), McDonald and Wood (1998), Pane et al. (2005), and Patel et al. (2006). There was no evidence of net secretion of any ions in the control and dietary or waterborne Cd exposed trout as CRs were never > 1 (Fig. 2). This is in complete agreement with Pane et al. (2005) who studied the renal effects of waterborne Ni in trout, but not with Patel et al. (2006) who observed a net secretion of Mg^{2+} (CR = 1.3) but not other ions in the trout exposed to waterborne Pb for 96 h. Thus, it appears that a stimulatory effect of metals on tubular secretion of ions in fish is not a common phenomenon, and it depends on the particular metal and probably the route of exposure (dietary vs waterborne).

One of the major effects of chronic dietary Cd in the present study was the elevated urinary excretion of all major ions (Mg^{2+}, Zn^{2+}, Na^{+}, Cl^{-}, K^{+}) except Ca^{2+} (Table 1, Figs. 4 and 6), suggesting that ion handling by the kidney was impacted by dietary Cd. To our knowledge, there are no previous studies demonstrating the effects of dietary Cd or other metals on urinary excretion of ions in fish. However, during chronic sublethal exposure of rainbow trout to waterborne Cd (6.4 μg/L), urinary excretion of K^{+} and Cl^{-} were unaffected, but Na^{+} and Zn^{2+} were elevated and Ca^{2+} and Mg^{2+} reduced (Giles, 1984, 1988). The increased excretion of most of the ions in the present study but not all ions in the work of Giles might be related to different exposure routes (dietary vs waterborne), doses, and durations.

The increased excretion of ions in response to dietary Cd was associated with the reduced tubular reabsorption of the ions as shown by increased CRs for Cd acclimated trout (Fig. 2a). Although, in general, an efficient reabsorption of ions was observed in both the control and dietary Cd exposed fish (see above), relative reabsorption was reduced sufficiently (75–133% increase in CRs, Fig. 2a) for Mg^{2+}, K^{+}, Na^{+} and Cl^{-} to account for most or all of the increases observed in urinary concentrations (115–137%, Table 1) and excretion rates (90–100%, Fig. 4) of these ions. The increased excretion is apparently not associated with plasma ion status which remained more or less unchanged in both the present study (Table 1) and in our preceding study under the same experimental conditions (Chowdhury et al., 2004b). Since GFR was not impacted before waterborne Cd challenge (Table 1, Fig. 3), the possibility of increased filtered load of the ions is also not likely.

The mechanism of interactions between Cd and other nutrient ions in the fish kidney is not known. In the gills, Cd follows Ca^{2+} uptake pathways and exerts strong inhibition on transepithelial Ca^{2+} uptake by competitively interacting with Ca^{2+} transporters (Ca channel, Ca^{2+}-ATPase; Verbost et al., 1987, 1989), but has little or no effect on the uptake or plasma levels of Mg^{2+}, Na^{2+}, Cl^{-}, and K^{+} (Giles, 1984; Reid and McDonald, 1988; Verbost et al., 1989; Pratap et al., 1989; Wood, 2001; Chowdhury et al., 2004b). Cd is also known to interact competitively with Zn^{2+} for uptake and binding to a biological site in fish gills (Van Ginneken et al., 1999) as well as in various other transporting epithelia including the kidney in mammals (Brzoska and Moniuszko-Jakoniuk, 2001; Zalups and Ahmad, 2003). In the present study, Cd did not affect the reabsorption of either Ca^{2+} or Zn^{2+} (Fig. 2), the two major "competing" ions for Cd, but affected other ions non-selectively. Thus the mechanism of reduced reabsorption appears to be different from that observed in the gills. Rather, the effects may have occurred due to the tubular damage caused by Cd. Cadmium is known to be nephrotoxic and can cause substantial histopathological damage with tubular necrosis, apoptosis, and increased urinary losses of many substances in the mammalian (Friberg, 1984; Dudley et al., 1985; Zalups et al., 1992; Zalups and Ahmad, 2003) and fish kidney (Gill et al., 1989; Singhal and Jain, 1997).

4.4. Effects of waterborne Cd challenge on renal ion handling

During waterborne Cd challenge, urinary excretion of Ca^{2+} was gradually reduced until 72 h in both the non-acclimated (60%) and Cd-acclimated fish (40%) although the reduction was significant only in non-acclimated fish (Fig. 4a). At the same time, plasma Ca^{2+} level had fallen by 30% in the naïve fish but only 13% in the dietary Cd acclimated fish (Table 1). Similar effects (44% and 14% decline in plasma Ca) were also observed in our earlier study (Chowdhury et al., 2004b). We suggested that chronic exposure to dietary Cd was protective against plasma hypocalcaemia during waterborne Cd challenge through acclimatory or adaptive responses in the gills or gut (Chowdhury et al., 2004b). The reduced urinary excretion of Ca^{2+} appears to be directly associated with decreased plasma Ca^{2+} levels, hence filtered load. Furthermore, the kidney also helped resist plasma Ca^{2+} decline by reducing urinary loss in both groups, as urinary declines (60% and 40%) were greater than those observed for plasma (30–44% and 13–14%). Similar to Ca^{2+}, Zn^{2+} excretion during waterborne Cd challenge declined gradually in the non-acclimated (62%) and Cd-acclimated fish (75%) by 72 h (Fig. 6c), when plasma Zn^{2+} levels declined to 50% and 43% respectively, from the pre-challenge levels (6733 and 8856 ng/mL, Table 1).

Among the measured urinary ions, only Mg^{2+} excretion remained elevated with no recovery with time in Cd-acclimated trout after exposure to chronic dietary Cd and acute Cd challenge (Fig. 4b). Since plasma Mg^{2+} was not affected either by dietary or waterborne Cd at this time (Chowdhury et al., 2004b), the reduced reabsorption (greater CR_{Mg}, Fig. 2) is likely the reason of elevated Mg^{2+} loss via the kidney, which occurred

without acclimatory attenuation. From the significantly reduced excretion (Fig. 4b) together with an efficient reabsorption in the non-acclimated trout at 72-h post-challenge (96%, CR_{Mg}=0.04, Fig. 2b), it seems likely that the naïve kidney can handle Mg^{2+} reabsorption more efficiently than the dietary Cd impacted kidney during acute challenge to waterborne Cd. On the contrary, a strong inhibition of Mg^{2+} reabsorption during acute exposure of naïve trout to waterborne Ni and Pb, but an attenuation of inhibition in trout chronically pre-exposed to waterborne Ni was observed in other studies in our laboratory (Pane et al., 2005; Patel et al., 2006). Giles (1984) observed a reduced Mg^{2+} excretion in response to waterborne Cd exposure over 50 days, but not thereafter. Thus, it appears that pre-exposure to waterborne Cd or Ni produces acclimatory protection to Mg^{2+} handling but pre-exposure to dietary Cd does not. Despite the differences in the nature of effects, urinary Mg^{2+} is clearly sensitive to metal poisoning in fish, as observed in mammals (Kendall et al., 1983; Leffler et al., 1990, Shore et al., 1995). Further studies are necessary to understand effects on urinary Mg^{2+} during chronic and acute exposure to waterborne and dietary Cd and other metals.

Unlike Mg^{2+}, the UERs of all three monovalent ions (K^+, Na^+, Cl^-) in the non-acclimated trout were well conserved and those in Cd-acclimated fish showed a decrease with a complete recovery by 72 h of challenge (Fig. 4c,d,e). Since more or less similar trends were observed in CRs (Fig. 2b), but different in plasma ion levels in the non-acclimated (K^+ increase) and Cd-acclimated fish (Na^+ and Cl^- increase) (Chowdhury et al., 2004b), we can suggest that: (1) monovalent ions in the kidney were less sensitive to acute exposure to waterborne Cd, as similarly observed for waterborne Ni and Pb (Pane et al., 2005; Patel et al., 2006); (2) renal responses associated with the dysfunction of K^+, Na^+ and Cl^- regulation during chronic dietary Cd exposure are reversible, probably through nephrogenesis and tubular regeneration (Larsen and Perkins, 2001); and (3) effects of acute waterborne Cd challenge on plasma K^+, Na^+ and Cl^- are not primarily related to kidney dysfunction, but, as we explained before, to acute Cd stress and acclimatory or protective changes at the gill site (Chowdhury et al., 2004b).

4.5. Effects on renal metabolite handling

Among metabolites, the most significant effect of prolonged dietary Cd was the elevation of urinary glucose excretion (Table 1), which, however, returned to the control level during waterborne Cd challenge (Fig. 5c). The increase in the glucose excretion was not probably caused by an increase in filtered load, because the plasma glucose level did not increase but decreased in the Cd-acclimated fish during this time (Chowdhury et al., 2004b). Glucose is freely filtered at the glomerulus and almost completely reabsorbed (Bucking and Wood, 2005), which agrees with $CR_{glucose}$ in the present study (0–0.04, Fig. 2). Despite an efficient reabsorption, the detection of a small amount of glucose in the urine of the Cd-exposed trout (~12% of the plasma level, Table 1) might be attributed to a small effect on the Na^+ coupled glucose transporter (Freire et al., 1995) caused by Cd induced tubular damage, as discussed above for urinary ions. The decline of glucose excretion to the control level during waterborne Cd challenge suggests that dietary Cd, but not waterborne, affects glucose excretion, and that the effect of dietary Cd on glucose is not persistent.

While other urinary components showed reabsorption, urinary ammonia showed net secretion (CRs>1, Fig. 2), as usually observed for this primary end product of N-metabolism in fish (Wood, 1993). The increased ammonia secretion in the Cd-exposed fish only after waterborne Cd exposure (CR_{Tamm}, Fig. 2b) suggests that the effect was acute in nature, as observed for waterborne Pb in rainbow trout (Patel et al., 2006). Unlike ammonia, urea, another end product of N-metabolism, is known to be actively reabsorbed by the trout kidney (McDonald and Wood, 1998) and exhibited approximately 60% reabsorption of the filtered load after chronic exposure to dietary Cd (pre-challenge CR_{urea}=40–42, Fig. 2a). However, urea excretion was also unaltered before waterborne Cd challenge, indicating that dietary Cd did not have any chronic effects on renal regulation of both N-metabolites. The transient increase of UER_{urea} in the Cd-acclimated fish and a decrease in non-acclimated fish after waterborne Cd challenge are again acute in nature and probably associated with more or less similar changes in plasma urea (results not shown) and GFR (Fig. 3b), and a transiently decreased urea reabsorption in the Cd-acclimated fish (results not shown).

4.6. Effects on protein excretion

The fish chronically exposed to dietary Cd developed proteinuria with a daily protein loss of approximately 4.4 mg/kg in the urine ($UER_{protein}$=0.183 mg/kg/h) and a similar effect was seen in naïve fish during the first 12 h of waterborne Cd challenge (Fig. 6a). Similarly, an elevation of urinary protein excretion relative to resting levels were observed in rainbow trout exposed acutely or chronically to waterborne Cd (~6–13 mg/kg/d, Giles, 1984) and Ni (~2–7 mg/kg/d, Pane et al., 2005) and inorganic Hg (Fletcher and White, 1986), but not in trout exposed to waterborne Pb (Patel et al., 2006). Although urinary protein concentration (~0.119 mg/mL) represents only 0.5% of the plasma protein concentration in the Cd-acclimated fish (Table 1), chronic protein loss might be energetically costly for fish, and proteinuria is a hallmark of damage to the glomerular filtration barrier resulting in renal dysfunction (Haraldsson and Sörensson, 2004). Protein loss caused by Cd-induced damage to glomerular filtration barrier and proximal tubule are commonly observed in mammals (Dudley et al., 1985; Tang et al., 1998; Zalups and Ahmad, 2003). Dietary Cd might have caused proteinuria in trout in a similar way, but further study on kidney histopathology and protein composition will provide more information on the true reason.

4.7. Conclusions

The results in the present study demonstrate that chronic exposure to dietary Cd at the level tested (500 mg/kg) causes increased renal excretion of several ions (Mg^{2+}, Zn^{2+}, K^+, Na^+, Cl^-), glucose, and protein in rainbow trout. Dietary Cd does not

affect urinary Ca^{2+} and renal responses appear to be compensatory towards plasma hypocalcaemia caused by waterborne Cd. In our earlier study we have shown that chronic exposure to the same level of dietary Cd develops acclimation in trout with increased protection against the effects of waterborne Cd on acid–base status, plasma ions and stress responses (Chowdhury et al., 2004b). For example, arterial blood CO_2 tension and pH, and plasma Ca^{2+} and K^+ are much less affected in dietary Cd pre-exposed fish than in naïve fish when both groups are challenged with waterborne Cd; plasma cortisol rises in the non-exposed fish but not in the dietary Cd-exposed fish. Similar to this physiological evidence, toxicological evidence of acclimation (elevated 96 h LC50) in dietary Cd exposed trout has also presented previously (Szebedinszky et al., 2001). Thus, chronic exposure to dietary Cd has benefits of acclimatory protection in trout against additional stress caused by waterborne Cd but such acclimation also involves some physiological costs in terms of renal dysfunction and elevated urinary losses.

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