ELSEVIER

Comparative Biochemistry and Physiology, Part C 139 (2004) 163–173

CBP

www.elsevier.com/locate/cbpc

Physiological effects of dietary cadmium acclimation and waterborne cadmium challenge in rainbow trout: respiratory, ionoregulatory, and stress parameters

M.J. Chowdhury*, E.F. Pane, C.M. Wood

Department of Biology, McMaster University, 1280 Main Street West, Hamilton, Ontario, Canada L8S 4K1

Received 5 August 2004; received in revised form 8 October 2004; accepted 12 October 2004

Abstract

A suite of respiratory, acid–base, ionoregulatory, hematological, and stress parameters were examined in adult rainbow trout (*Oncorhynchus mykiss*) after chronic exposure to a sublethal level of dietary Cd (500 mg/kg diet) for 45 days and during a subsequent challenge to waterborne Cd (10 μg/L) for 72 h. Blood sampling via an indwelling arterial catheter revealed that dietary Cd had no major effects on blood gases, acid–base balance, and plasma ions (Ca^{2+}, Mg^{2+}, K^+, Na^+, and Cl^-) in trout. The most notable effects were an increase in hematocrit (49%) and hemoglobin (74%), and a decrease in the plasma total ammonia (43%) and glucose (49%) of the dietary Cd-exposed fish relative to the nonexposed controls. Dietary Cd resulted in a 26-fold increase of plasma Cd level over 45 days (~24 ng/mL). The fish exposed to dietary Cd showed acclimation with increased protection against the effects of waterborne Cd on arterial blood P_{aCO2} and pH, plasma ions, and stress indices. After waterborne Cd challenge, nonacclimated fish, but not Cd-acclimated fish, exhibited respiratory acidosis. Plasma Ca^{2+} levels declined from the prechallenge level, but the effect was more pronounced in nonacclimated fish (44%) than in Cd-acclimated fish (14%) by 72 h. Plasma K^+ was elevated only in the nonacclimated fish. Similarly, waterborne Cd caused an elevation of all four traditional stress parameters (plasma total ammonia, cortisol, glucose, and lactate) only in the nonacclimated fish. Thus, chronic exposure to dietary Cd protects rainbow trout against physiological stress caused by waterborne Cd and both dietary and waterborne Cd should be considered in determining the extent of Cd toxicity to fish.

Keywords: Acclimation; Acid–base; Cadmium; Chronic; Diet; Hematology; Plasma ions; Respiration; Stress; Trout

1. Introduction

Cadmium (Cd) is a biologically nonessential metal that can be toxic to aquatic animals. The freshwater quality criteria values of Cd for the protection of aquatic life in North America are less than 1 μg/L at low hardness (USEPA, 2001; CCME, 2002). Freshwater fish exposed to waterborne Cd at total concentrations well below 100 μg/L exhibit substantial pathophysiology (Wood, 2001). Some of the physiological effects of chronic exposure to waterborne Cd at sublethal levels are manifested in the form of disturbances in respiration (Majewski and Giles, 1981; Shaffi et al., 2001), disruption in whole-body or plasma ion regulation (Haux and Larsson, 1984; Giles, 1984; Pratap et al., 1989; McGeer et al., 2000; Baldisserotto et al., 2004b), and changes in hematology (Haux and Larsson, 1984; Gill and Epple, 1993; Zikic et al., 2001) and other blood parameters, such as cortisol and glucose, that reveal the stress response in fish (Fu et al., 1990; Pratap and Wendelaar Bonga, 1990; Gill et al., 1993; Brodeur et al., 1998; Lacroix and Hontela, 2004). At sublethal concentrations, most of the effects were transient with recovery during extended exposures (Haux and Larsson, 1984; Giles, 1984; Fu et al., 1990; McGeer et al., 2000), suggesting the ability of fish to acclimate to waterborne Cd. During

* Corresponding author. Present address: USEPA-MED, 6201 Congdon Boulevard, Duluth, MN 55804, USA. Tel.: +1 218 529 5221; fax: +1 218 529 5003.

E-mail address: chowdhury.jasim@epa.gov (M.J. Chowdhury).

1532-0456/$ - see front matter © 2004 Elsevier Inc. All rights reserved.
doi:10.1016/j.cca.2004.10.006

acclimation, fish undergo changes to achieve a new physiological steady state and develop an increased resistance and/or tolerance to acute Cd challenges (McDonald and Wood, 1993; Stubblefield et al., 1999; Hollis et al., 1999).

Dietary Cd (either in contaminated live food or prepared diet) has been found to contribute greatly to metal accumulation in trout and other fish (Harrison and Klaverkamp, 1989; Harrison and Curtis, 1992; Kraal et al., 1995; Chowdhury et al., 2004b). Szebedinszky et al. (2001) showed that chronic exposure to dietary Cd can also produce acclimation in fish expressed as an increased tolerance to waterborne Cd. We have shown that there is a significant effect of chronic dietary Cd exposure on gastrointestinal uptake and disposition of Cd in gut as well as nongut tissues (Chowdhury et al., 2004b). However, despite the fact that the transfer of metals through food chains can be high enough to cause toxicity to fish in a contaminated aquatic system (Dallinger and Kautzky, 1985; Farag et al., 1994), and that dietary Cd can alter the nature of gill–metal interactions (Szebedinszky et al., 2001), there is only a small body of information related to the respiratory, ionoregulatory, hematological, and stress-related responses of fish chronically exposed or acclimated to dietary Cd. A limited number of studies suggest that chronic exposure to dietary Cd causes ionoregulatory disturbances in plasma and structural changes in the gills similar to those induced by waterborne Cd (Pratap et al., 1989; Pratap and Wendelaar Bonga, 1993) and induces de novo synthesis of the metal-binding protein metallothionein in the gills and other tissues (Dang et al., 2001; Berntssen et al., 2001, Chowdhury et al., 2004a).

Therefore, in the present study, a suite of physiological parameters were examined in rainbow trout after chronic exposure to a sublethal level of dietary Cd (500 mg/kg diet) for 45 days. The concentration was selected based on previous studies mentioned above (Szebedinszky et al., 2001; Chowdhury et al., 2004a,b), in which similar dietary Cd concentrations (500–800 mg/kg) were used. Fish were arterially cannulated after dietary exposure and blood samples were collected repeatedly over a 96-h experimental period with minimal disturbance. Parameters were mostly selected based on previous studies examining physiological impacts in freshwater fish acutely or chronically exposed to waterborne Cd (see above) or other metals, such as aluminum, zinc, copper, and nickel (Spry and Wood, 1984; Wood et al., 1988c; Dethloff et al., 1999; Pane et al., 2003). Our goal was to evaluate respiratory and acid–base parameters (blood gases, ventilation rate, blood pH and HCO_3^-, and metabolic acid load), hematology (hematocrit, hemoglobin, and plasma proteins), plasma ions (Ca^{2+}, Mg^{2+}, K^+, Na^+, and Cl^-), and stress indices (plasma cortisol, glucose, ammonia, and lactate) in dietary-exposed and nonexposed trout both before and during waterborne Cd challenge.

One of the criteria for acclimation to a toxicant is the reduction of the magnitude of physiological disturbances when challenged with that toxicant (McDonald and Wood, 1993). Therefore, after recovery from surgery, a blood sample was taken to document the effects of chronic dietary Cd exposure and a second one taken at 24 h to confirm any difference. Immediately thereafter, both dietary Cd-exposed and nonexposed fish were "challenged" with waterborne Cd (10 μg/L) for 72 h in order to discern whether pre-exposure to dietary Cd is protective against physiological perturbations caused by waterborne Cd. This challenge concentration is approximately 50% of the 96-h LC50 of waterborne Cd for juvenile trout (19–22 μg Cd/L; Hollis et al., 1999; Niyogi et al., 2004).

2. Materials and methods

2.1. Experimental fish

Adult rainbow trout (*Oncorhynchus mykiss*; ~150 g) were obtained from Humber Springs Trout Hatchery (Mono Mills, Ontario, Canada) and held under laboratory conditions for at least 2 weeks before experimental use. Fish were held in 500-L tanks supplied with a minimum of 2.5 L/min flow-through of moderately hard dechlorinated Hamilton City tap water (from Lake Ontario) at a temperature and pH of 12±1 °C and 8.0±0.2, respectively, and fed a daily 1% ration (relative to body weight) of commercial trout pellets (Martin's Feed Mills, Ontario, Canada). A 12/24-h photoperiod was maintained throughout the study. Water composition was Ca ≅ 1 mM, Na ≅ 0.6 mM, Cl ≅ 0.7 mM, Mg ≅ 0.2, Cd ≅ 0.3 nM, Cu ≅ 47 nM, Zn ≅ 270 nM, dissolved organic carbon (DOC) ≅ 3 mg/L, and total hardness (as $CaCO_3$) approximately 140 mg/L.

2.2. Chronic exposure to dietary Cd

For chronic dietary Cd exposure, 40 fish in one holding tank were fed a 1.5% daily ration (dry feed/wet body weight) of a prepared Cd-enriched diet containing nominally 500 mg Cd/kg dry feed for 45 days (actual measured concentration: 461.2±4.1 mg Cd/kg food or 6.9 mg Cd/kg fish/day). Another holding tank was used simultaneously as a control tank in which 40 fish were fed a prepared control diet with no added Cd (actual measured concentration: 0.212±0.013 mg Cd/kg) at the same ration. Fish ate all food immediately after feeding. The results indicated that the phenomenon of acclimation occurred, so for convenience, the Cd-exposed fish are hereafter termed the "Cd-acclimated" group and the fish on control diet are called the "nonacclimated" group. The average initial weight of fish, determined at day zero from the bulk weight of 20 fish selected randomly from both tanks, was 165.4 g.

Diets were prepared by mixing a concentrated stock 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.

(2004b). The Cd concentrations in the tank water measured after passing through a 0.45-μm filter were minimal for both nonacclimation (0.125±0.013 μg/L or 1.11±0.12 nmol/L) and Cd-acclimation groups (0.251±0.033 μg/L or 2.24±0.29 nmol/L). The average terminal body weight after dietary exposure was 252.5±12.2 g (n=18) for nonacclimated fish and 265.8±15.6 g (n=18) for Cd-acclimated fish. Fish were fasted for 48 h prior to arterial cannulation and during acute Cd challenge.

2.3. Cannulation

Fish from nonacclimation and Cd-acclimation groups (262.6±21.5 and 245.1±16.8 g, respectively, n=9–10) were randomly selected from the tanks and cannulated by surgical implantation of a catheter (PE-50 polyethylene tubing, Intramedic, Becton Dickinson, Sparks, MD, USA; internal diameter 0.58 mm, external diameter 0.97 mm, 30 cm long) in the dorsal aorta for sampling arterial blood (Soivio et al., 1972). During cannulation, fish were anaesthetized with MS-222 (0.075 g/L) and placed on a surgery table, where the gills were continuously irrigated with water. After cannulation, each fish was placed in an aerated experimental chamber (2.5 L) supplied with flowing water (250 ml/min) and allowed to recover from surgery for at least 36 h.

2.4. Experimental procedures

After recovery from surgery, blood samples from both treatment groups were collected at 0, 24, 48, 72, and 96 h for different respiratory and blood parameters. Immediately after 24-h sampling, both fish groups were exposed to a nominal waterborne Cd concentration of 10 μg/L (89 nmol/L) and exposure continued until 96 h (72 h postchallenge). For Cd exposure, the fish chambers received water from a head tank via a mixing container that was constantly fed with an acidified stock solution of $Cd(NO_3)_2 \cdot 4H_2O$ (161 mg/L; Fisher Scientific) from a Mariotte bottle to maintain a nominal Cd concentration of 10 μg/L. Water samples from the chambers were taken daily and analyzed for Cd concentration after passing through 0.45-μm filters and acidification with HNO_3 (1%). Actual measured dissolved Cd concentration was 11.8±0.2 μg/L (105.0±1.5 nmol/L, n=40).

Daily, each fish was sampled following a protocol similar to that of Wood et al. (1996). First, ventilation rate was counted visually from opercular movements, and then water samples from in front of each fish's mouth were taken for inspired O_2 tension (P_{IO2}) and pH_I. One milliliter of blood was drawn anaerobically via the arterial catheter into an ice-cold, Li-heparanized (50 i.u. ml^{-1}; Sigma-Aldrich), gas-tight Hamilton syringe for analysis of blood pH (pH_a), O_2 tension (P_{aO2}), plasma total CO_2 (C_{aCO2}), hematocrit (Ht), blood hemoglobin (Hb), and plasma levels of Na^+, K^+, Ca^{2+}, Mg^{2+}, K^+, Cd^{2+}, lactate, glucose, protein, cortisol, and total ammonia. Plasma was separated by centrifugation at 13,000×g for 1 min and erythrocytes were gently 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, NY, USA). One aliquot of the plasma sample was deproteinized in two volumes of ice-cold 6% perchloric acid for the determination of lactate and glucose, while the remainder of the plasma was used for the determination of protein, cortisol, total ammonia, electrolytes, and Cd concentrations.

2.5. Analytical measurements and calculations

Arterial blood pH (pH_a), P_{aO2}, inspired water pH (pH_I) and O_2 tension (P_{IO2}) were measured with Radiometer electrodes and meters as described by Wood et al. (1988a). Hb was determined by the colorimetric cyanmethemoglobin method (Sigma-Aldrich reagents). Plasma for total CO_2 (C_{aCO2}) was obtained by centrifuging whole blood (5000×g for 10 min) in ammonium-heparinized microhematocrit tubes in duplicate. Ht was read directly from the tubes, while total CO_2 was analyzed on true plasma using a Corning 965 CO_2 analyzer. After measuring Ht, the tubes were broken to allow aspiration of the plasma into a Hamilton syringe for transfer to the CO_2 analyzer. Lactate was measured enzymatically (lactate dehydrogenase/NADH at 340 nm; Sigma-Aldrich) on deproteinized plasma, while glucose was measured enzymatically (hexokinase/glucose-6-phosphate dehydrogenase at 340 nm; ThermoDMA kit, Louisville, USA) on the same acid extracts neutralized with 1 M K_2CO_3. Plasma total protein was determined by the Bradford method using Sigma-Aldrich Bradford reagents. Plasma cortisol was determined using an ^{125}I-radioimmunoassay (ICN Biomedicals, Montreal, Quebec) with γ-radioactivity measured in a Minaxi-γ Auto-gamma 5530 counter (Canberra Packard, Mississauga, ON, Canada). Plasma total ammonia (T_{amm}) concentration was determined enzymatically (glutamate dehydrogenase/NADP at 340 nm) as described by Neeley and Phillipson (1988) and with reagents from Sigma-Aldrich. Electrolyte and metal concentrations were measured by atomic absorption spectrophotometry (SpectrAA-220, Varian, Mississauga, ON, Canada) with flame atomization for Na^+, K^+, Ca^{2+}, and Mg^{2+} and with graphite furnace atomization for Cd. Fisher-certified standards (Fisher Scientific) were used to determine Cd concentrations in unknown samples. Plasma Cl^- concentration was measured by the mercuric thiocyanate spectrophotometric method (Zall et al., 1956).

Mean cellular hemoglobin concentration (MCHC) was calculated as the ratio of Hb to Ht and is expressed as gram Hb per ml red blood cell (RBC). Arterial CO_2 tension (P_{aCO2}) was calculated using the Henderson–Hasselbalch equation in the form:

$$P_{aCO2} = C_{aCO2} / \left[{}_{\alpha}CO_2 \cdot \{1 + \text{antilog}(pH_a - pK')\} \right] \quad (1)$$

where C_{aCO2} and pH_a were measured directly and values for CO_2 solubility ($_\alpha CO_2$) and apparent pK (pK′) at the appropriate temperature were taken from Boutilier et al. (1984). The concentration of plasma HCO_3^- was calculated by

$$\left[HCO_3^-\right] = C_{aCO2} - \left({}_\alpha CO_2 \cdot P_{aCO2}\right) \quad (2)$$

The concentration of metabolic H^+ load (ΔH_m^+) was calculated from the following equation (McDonald et al., 1980):

$$\left[\Delta H_m^+\right] = \left[HCO_3^-\right]_1 - \left[HCO_3^-\right]_2 - \beta(pH_1 - pH_2) \quad (3)$$

where the subscripts 1 and 2 refer to plasma bicarbonate and pH values at daily intervals, and the slope of the true plasma buffer capacity (β) was calculated from the regression relationship between measured whole-blood Hb and β as determined for rainbow trout plasma at this temperature by Wood et al. (1982).

2.6. Data presentation and statistical analysis

Data are expressed as mean±1 SE (n) where n is the number of fish. The data collected before waterborne Cd challenge are presented as "prechallenge" means of individual values for 0 h and 24 h. Time-dependent "postchallenge" responses in both nonacclimated and dietary Cd-acclimated groups were tested against their respective "prechallenge" means by a one-way ANOVA followed by a post hoc multiple comparison (Newman–Keuls test), and each mean for the acclimated group was compared to that of the nonacclimated 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. The significance limit of $p \leq 0.05$ was used throughout.

3. Results

3.1. Fish health and mortality

Exposure of trout to 500 mg/kg of Cd in the diet resulted in no mortality during the 45-day feeding period. Routine daily observations did not show any apparent differences in appetite or swimming activity between control and dietary Cd-exposed fish. No mortality occurred in either the nonacclimated or Cd-acclimated groups after waterborne Cd challenge (10 μg/L). No grossly visible changes were observed in the gills of any Cd-exposed fish.

3.2. Blood gases and acid–base status

The arterial O_2 tension (P_{aO2}, Fig. 1a) and CO_2 tension (P_{aCO2}, Fig. 1b) in nonacclimated and Cd-acclimated fish were not significantly different before waterborne Cd challenge, suggesting that gas exchange was not affected by chronic exposure of trout to dietary Cd. However, with

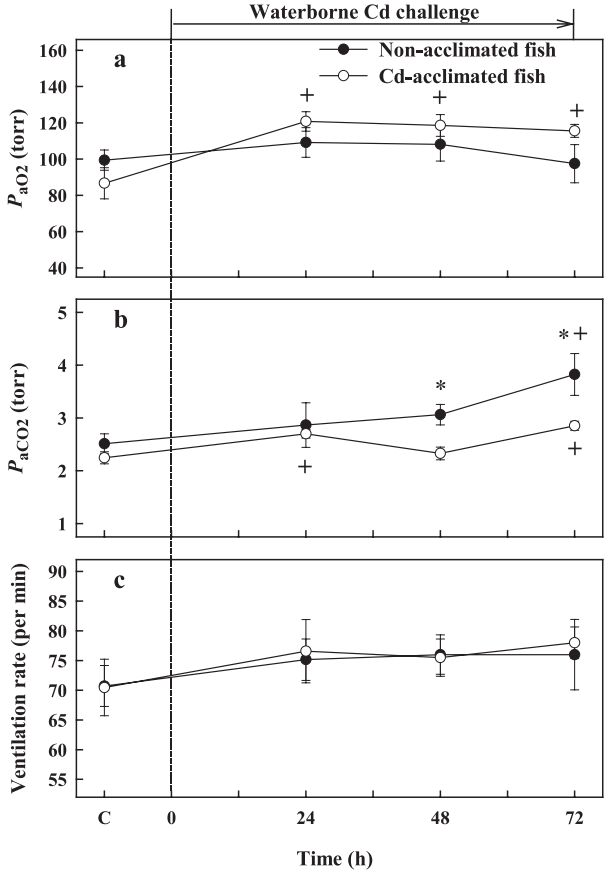

Fig. 1. Blood gas tensions (a and b) and ventilation rates (c) in rainbow trout before and after waterborne Cd challenge (10 μg/L). Before experimental sampling, the fish had been fed either a control diet (0 mg Cd/kg dry diet, nonacclimated) or a Cd diet (500 mg Cd/kg dry diet, Cd-acclimated) for 45 days. Blood samples were collected via an intra-arterial catheter. P_{aO2}: arterial oxygen tension; P_{aCO2}: arterial plasma carbon dioxide tension. Results are presented as mean±S.E. (n=6–9). Means with "*" indicate significant difference (p<0.05) between nonacclimated and Cd-acclimated fish during simultaneous sampling. Postchallenge means with "+" indicate significant difference (p<0.05) from the "prechallenge" mean (indicated by "C" on x-axis) in the same group.

exposure to waterborne Cd, P_{aO2} significantly increased in the Cd-acclimated fish (33–39%) relative to the prechallenge O_2 tension (87 Torr, Fig. 1a). By 72 h postchallenge, P_{aCO2} increased in both nonacclimated (52%) and Cd-acclimated fish (27%) from the prechallenge levels (2.5 and 2.2 Torr, respectively, Fig. 1b). At 48 and 72 h, P_{aCO2} levels in nonacclimated fish were significantly greater than those in Cd-acclimated fish. Neither dietary Cd nor waterborne Cd challenge affected ventilation rates (Fig. 1c).

Similar to blood gases (Fig. 1), arterial acid–base status also did not show any significant differences between nonacclimated and dietary-acclimated groups before challenge (Fig. 2). As P_{aCO2} rose (Fig. 1b), arterial blood pH (pH_a) dropped slightly but significantly in both groups after Cd challenge (Fig. 2a). Only at 72 h, however, was the pH_a in nonacclimated fish significantly (0.092 unit) lower than that in Cd-acclimated fish. Waterborne Cd did not affect plasma HCO_3^- (Fig. 2b). No notable trend of blood acid

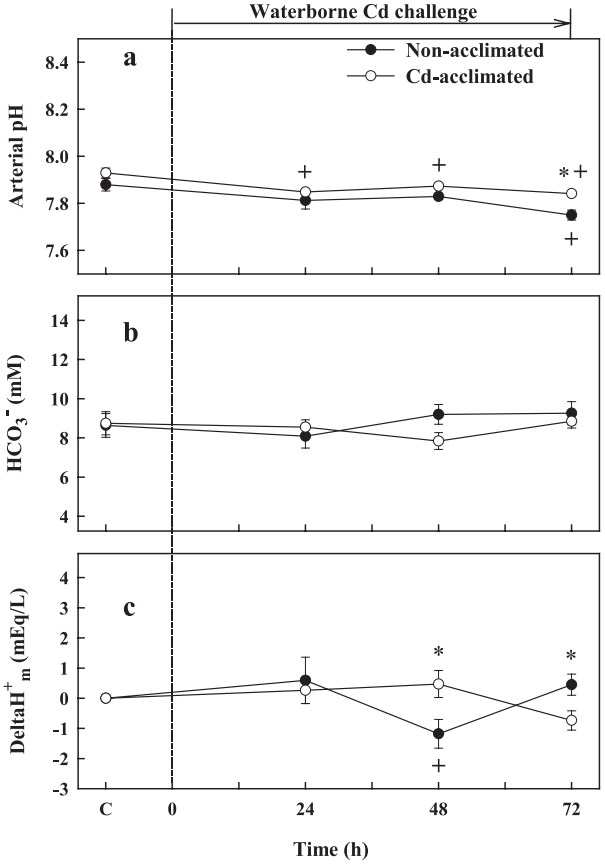

Fig. 2. (a) Arterial blood pH (pH_a), (b) arterial plasma HCO_3^-, and (c) net daily metabolic acid accumulation in the blood (ΔH_m^+) of rainbow trout before and after waterborne Cd challenge (10 μg/L). Format as per Fig. 1.

load (ΔH_m^+) was observed in fish after waterborne Cd exposure (Fig. 2c).

3.3. Hematology

Blood hematocrit (Hct, Fig. 3a) and hemoglobin (Hb, Fig. 3b) followed a similar trend and were initially 49% and 74% higher, respectively, in the Cd-acclimated fish relative to nonacclimated fish (Hct=19.2%; Hb=4.4 g/dL), suggesting that dietary Cd had an "increasing" effect on these hematological parameters. Both Hct and Hb fell gradually over time in the Cd-acclimated fish, but not in nonacclimated group where both parameters were conserved well. No significant differences attributable to dietary Cd existed after 24 h for Hct and after 48 h for Hb. Mean cellular hemoglobin concentrations (MCHC) were significantly higher in Cd-acclimated fish than in control fish before challenge, and remained constant in both groups over time (Fig. 3c). Initially, plasma protein concentration was slightly higher in the Cd-acclimated fish but the difference between the two treatment groups became significant only after the waterborne Cd challenge (Fig. 3d), suggesting some loss of water from the plasma compartment as an effect of waterborne Cd. Plasma protein concentrations were approximately 24% higher in the Cd-acclimated fish in comparison to the control group between 24 and 72 h.

3.4. Plasma ions and Cd

Prior to waterborne Cd challenge, no significant differences in plasma ions (Ca^{2+}, Mg^{2+}, K^+, Na^+, and Cl^-) between nonacclimated and Cd-acclimated fish were observed (Fig. 4). This suggests that the concentrations of these ions in the plasma were homeostatically conserved during chronic exposure to dietary Cd.

Plasma Ca^{2+} levels dropped over time after waterborne Cd challenge in both nonacclimated and Cd-acclimated fish (Fig. 4a). However, the decrease from the prechallenge level (~2.5 μmol/mL) was more pronounced in nonacclimated fish (44%) than in Cd-acclimated fish

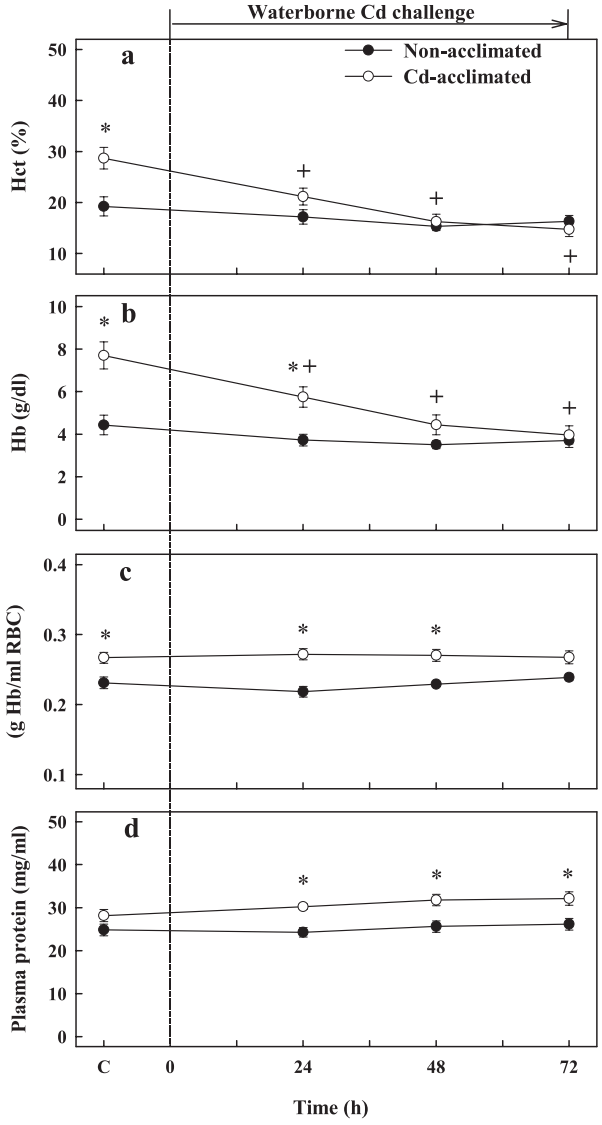

Fig. 3. Arterial hematological parameters in rainbow trout before and after waterborne Cd challenge (10 μg/L). (a) Hematocrit (Hct), (b) hemoglobin concentration (Hb), (c) mean cellular hemoglobin concentration (MCHC), (d) plasma protein concentration. Format as per Fig. 1.

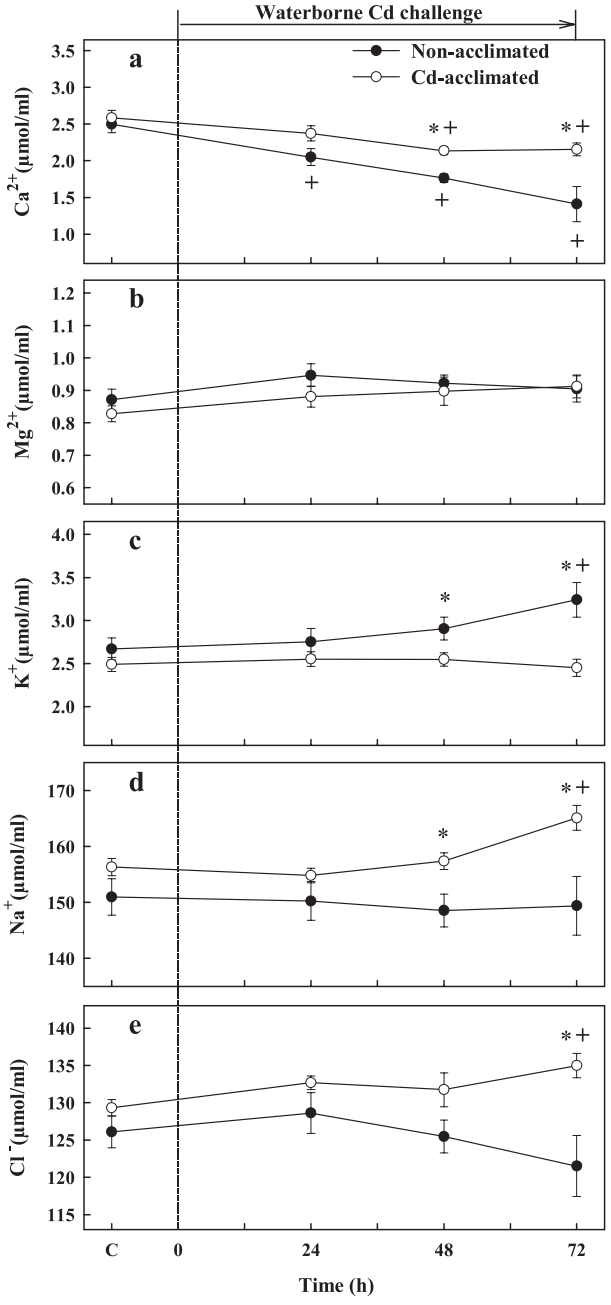

Fig. 4. Arterial plasma ion concentrations of Ca^{2+} (a), Mg^{2+} (b), K^+ (c), Na^+ (d), and Cl^- (e) in rainbow trout before and after waterborne Cd challenge (10 µg/L). Format as per Fig. 1.

(14%) by 72 h, suggesting that preacclimation to dietary Cd had a protective action against the negative effect of waterborne Cd on plasma Ca^{2+}. Unlike Ca^{2+}, plasma Mg^{2+} did not vary in either group over time (Fig. 4b). Plasma K^+ rose significantly in nonacclimated fish by 72 h; in Cd-acclimated fish, plasma K^+ was well conserved (Fig. 4c). In the case of plasma Na^+ (Fig. 4d) and Cl^- (Fig. 4e), the levels in the Cd-acclimated fish were elevated significantly by 48 h (Na^+) or 72 h (Cl^-) during waterborne Cd challenge. Neither ion varied significantly over time in the nonacclimated fish.

Dietary Cd exposure resulted in an almost 26-fold increase in plasma Cd level in Cd-acclimated fish relative to the background concentration in nonacclimated trout (0.89±0.14 ng/mL, Fig. 5). After Cd challenge, plasma Cd in Cd-acclimated fish was not elevated further, rather it tended to decrease (nonsignificantly) over time, maintaining significantly higher levels as compared with nonacclimated fish through 72 h. On the other hand, plasma Cd in nonacclimated fish became elevated slightly but significantly from 24 h onward after Cd challenge.

3.5. Stress indicators

The effects of Cd exposure on traditional plasma stress indicators (total ammonia, cortisol, glucose, and lactate) are presented in Fig. 6. Acclimation to dietary Cd (500 mg/kg for 45 days) resulted in a significant decrease of total ammonia (T_{amm}, 43%, Fig. 6a) and glucose (49%, Fig. 6c) in Cd-acclimated fish, but did not affect plasma cortisol (Fig. 6b) and lactate (Fig. 6d).

However, waterborne Cd challenge (10 µg/L) caused an elevation in all four measured stress parameters in nonacclimated fish but not in Cd-acclimated fish, suggesting that chronic exposure to dietary Cd protected fish against physiological stress caused by waterborne Cd. Plasma T_{amm} in nonacclimated fish was almost double the corresponding values for Cd-acclimated fish at 48 and 72 h (Fig. 6a). The most remarkable increase was found in the plasma cortisol concentration; at 72 h, it was more than 11 times the value for Cd-acclimated fish (13.9 ng/mL) and 12 times the prechallenge concentration (12.7 ng/mL, Fig. 6b). From 24 h onwards, plasma glucose was approximately 2 to 4 times greater in nonacclimated than in Cd-acclimated fish (~3 µmol/mL, Fig. 6c). Waterborne Cd also resulted in a significant decrease of plasma glucose concentration in Cd-acclimated fish over time. Similar to plasma cortisol (Fig. 6b), plasma lactate level in nonacclimated fish was the highest at 72 h with more than double the initial

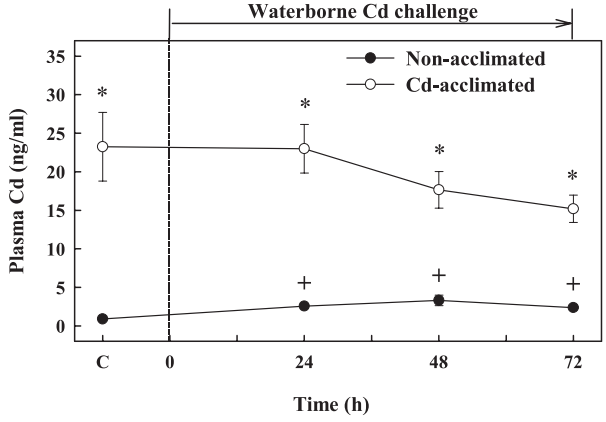

Fig. 5. Arterial plasma Cd concentration in rainbow trout before and after waterborne Cd challenge (10 µg/L). Format as per Fig. 1.

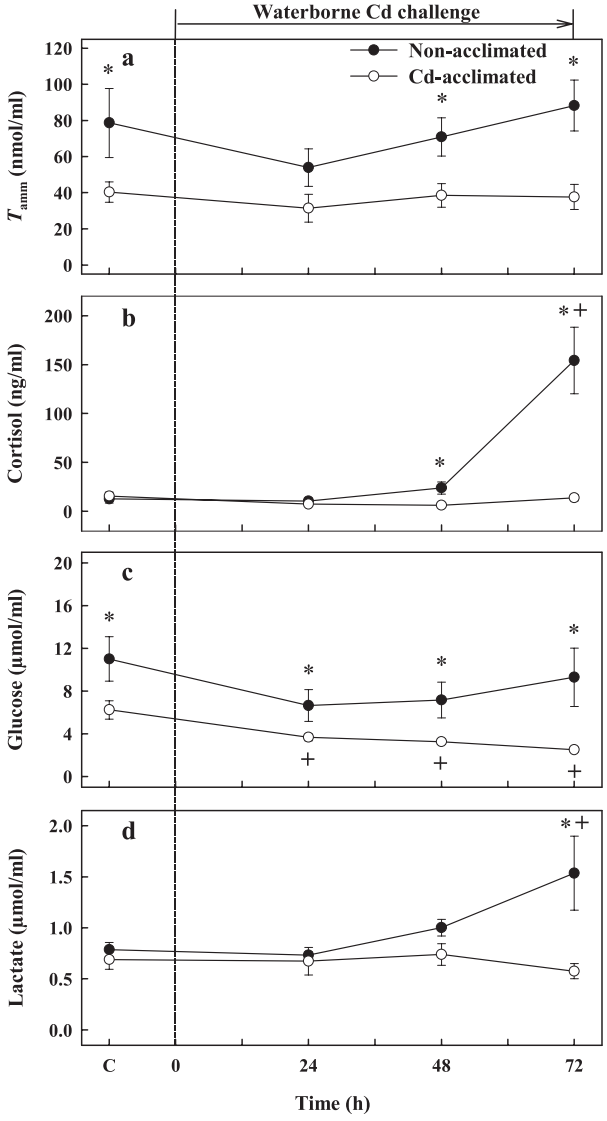

Fig. 6. Stress indices of arterial blood plasma in rainbow trout before and after waterborne Cd challenge (10 µg/L). (a) Total ammonia (T_{amm}), (b) cortisol, (c) glucose, (d) lactate. Format as per Fig. 1.

prechallenge value (0.7 µmol/mL) and the value for Cd-acclimated fish (0.6 µmol/mL, Fig. 6d). Plasma lactate concentration was not affected by waterborne Cd in Cd-acclimated fish.

4. Discussion

4.1. Effects of dietary Cd on respiratory and blood parameters

The physiological data prior to acute waterborne Cd challenge indicate that 45-day exposure to dietary Cd as high as 500 mg/kg had no major chronic effects on blood gases (Fig. 1), acid–base balance (Fig. 2), and plasma ions (Fig. 4) in trout. The most remarkable effects were the increase in the blood hematocrit (49%, Fig. 3a) and hemoglobin (74%, Fig. 3b), and the decrease in the plasma total ammonia (43%, Fig. 6a) and glucose (49%, Fig. 6a) of the Cd-acclimated fish relative to the control.

To our knowledge, there are no previous studies demonstrating the effects of dietary Cd or other metals on the respiratory and acid–base parameters of fish. However, during chronic sublethal exposure to waterborne Cd, a decrease in the O_2 diffusion capacity of the gills and an increase in ventilation rates in rainbow trout were observed (Majewski and Giles, 1981). The effects are likely caused by increased blood-to-water diffusion distance in the gills as a result of the cellular proliferation that accompanies the damage-repair process of acclimation to metals (Wood, 2001). Had similar branchial damage occurred due to dietary Cd, significantly lower P_{aO2} and pH_a and higher P_{aCO2} and ventilation rate in Cd-acclimated fish relative to control fish might have been expected.

Cd accumulated from the diet would presumably be taken up by the gills basolaterally via the circulatory system (Szebedinszky et al., 2001). The dietary Cd burden in the gills of the Cd-acclimated fish was expected to be at least 823±37 ng/g, the burden observed in our previous study in which rainbow trout were exposed to dietary Cd for 30 days under similar experimental conditions (Chowdhury et al., 2004b). In the present study, P_{aO2} (Fig. 1a), P_{aCO2} (Fig. 1b), and blood pH (Fig. 2a) were not significantly different in the Cd-acclimated fish relative to the control—i.e., there was no interference with arterial oxygenation and no respiratory acidosis. In addition, ventilation rate (Fig. 1c), plasma lactate concentration (Fig. 6d), and ΔH_m^+ (Fig. 2c), which would be expected to be increased if branchial diffusing capacity of O_2 were reduced (Pane et al., 2003), were not affected. Thus, any Cd-induced structural alteration in the gills attributable to chronic exposure to dietary Cd was below the threshold needed to induce notable disturbances of respiratory gas exchange or acid–base such as those caused by waterborne Cd, Zn, and Ni in trout (Majewski and Giles, 1981; Spry and Wood, 1984; Pane et al., 2003).

Increased blood hematocrit and hemoglobin can occur during environmental hypoxia (Wood and Johansen, 1972) and chronic or acute exposure of fish to waterborne metals, such as Cd (Majewski and Giles, 1981), zinc (Spry and Wood, 1984), copper (Dethloff et al., 1999), aluminum (Wood et al., 1988b), and nickel (Pane et al., 2003), in order to increase blood oxygen carrying capacity when branchial impairment of gas exchange occurs. On the contrary, anemia with reduced hematocrit and hemoglobin is also observed in higher vertebrates and fish in response to chronic Cd intoxication (Haux and Larsson, 1984; Gill and Epple, 1993; Liu et al., 1999). Inhibition of iron absorption and defective iron metabolism, shortened life span of erythrocytes, and/or impaired erythropoiesis caused by Cd are considered as possible reasons for anemia (see Gill and Epple, 1993; Liu et al., 1999). Although hypoxemia or respiratory impairment of gas exchange due to dietary Cd was not evident in the present study (see above), the reasons

for the increase of blood hematocrit (Fig. 3a) and hemoglobin (Fig. 3b) as well as elevation of MCHC (usually considered an indication of erythrocytic shrinkage or a change in the age structure of the erythrocyte population; Walsh et al., 1998) in the trout exposed to dietary Cd are not clear. Possibly, Cd may interact with an iron containing oxygen sensor (hemoprotein) in the cells which triggers the expression of the hormone erythropoietin, a precursor for the production of red blood cells and hemoglobin during oxygen deficiency. If Cd affects the oxygen-sensing efficiency of hemoprotein because of its well-known ability to compete with iron in biological systems (Bury and Grosell, 2003), an induction of hematocrit and hemoglobin is likely even in a condition when respiratory O_2 supply is not limiting.

The decrease of plasma ammonia and glucose in the dietary Cd-exposed fish suggests impaired homeostasis of these two important metabolites. A recent in vitro study suggests that Cd impairs basal or epinephrine-stimulated glucose release from eel hepatocytes (Fabbri et al., 2003).

Dietary Cd (500 mg/kg) resulted in remarkable build-up of plasma Cd level in the chronically exposed fish over 45 days (~ 24 ng/mL, Fig. 5). There was a 26-fold increase from the control level consistent with a similar increase of plasma Cd concentration over 30 days (28 times, 12.5 ng/mL) found in our previous study at the same dietary Cd dose (Chowdhury et al., 2004b). Elevated dietary Cd in plasma might contribute to the acclimation of fish via subtle physiological changes (see below).

Although dietary Cd did not substantially affect respiratory and blood parameters other than hematocrit, hemoglobin, glucose, and total ammonia, the present data suggest that chronic exposure to dietary Cd developed a protection in fish against waterborne Cd. For example, the reduction in plasma Ca^{2+} after exposure to 10 μg/L of waterborne Cd was very slight in dietary Cd-exposed fish over time and was much less than that in nonexposed fish (Fig. 4a). And as a stress response, plasma cortisol (Fig. 6b) rose in the nonexposed fish but not in the dietary Cd-exposed fish. Such protection is classic evidence of acclimation (McDonald and Wood, 1993), so clearly the phenomenon can "cross over" from one mode of exposure to another. This physiological evidence is in good agreement with the toxicological evidence of Szebedinszky et al. (2001) that chronic exposure to dietary Cd results in significantly increased tolerance (elevated 96 h LC50) to waterborne Cd in juvenile trout.

4.2. Responses to waterborne Cd challenge

After waterborne Cd challenge, P_{aCO2} was elevated in nonacclimated fish relative to the prechallenge level and became significantly greater than in Cd-acclimated fish at 48 h onwards (Fig. 1b). At the same time, blood pH also declined significantly in the nonacclimated fish relative to the initial level (see data point at 72 h, Fig. 1c). These effects on acid–base variables in the nonacclimated fish suggest that the fish were experiencing a mild, and possibly progressive, respiratory acidosis after waterborne Cd challenge. Respiratory acidosis with an increase in blood CO_2 tension and a decrease in blood pH during acute exposure of fish to waterborne cadmium, zinc, and nickel has been shown previously (Majewski and Giles, 1981; Spry and Wood, 1984; Pane et al., 2003). In the present study, the mild respiratory effect after waterborne Cd challenge was probably caused by gill damage and/or mucus secretion affecting gas exchange (Wood, 2001; Pane et al., 2003).

Plasma Ca^{2+} was reduced in both nonacclimated and Cd-acclimated fish after Cd challenge, although to a much greater extent in the former (Fig. 4a). This result was expected, since both acute and chronic waterborne Cd exposure resulted in plasma hypocalcemia in rainbow trout as well as other fish species (Giles, 1984; Pratap et al., 1989; Zohouri et al., 2001; Baldisserotto et al., 2004b). Similarly, waterborne Cd exposure reduced whole body influx of waterborne Ca^{2+} in rainbow trout (Reid and McDonald, 1988; Hollis et al., 2000; Baldisserotto et al., 2004b). The mechanistic explanation for plasma and/or whole body hypocalcemia is that Cd appears to be taken up at least in part via the Ca transport pathway at the gills and inhibits Ca^{2+} uptake in fish by competitively inhibiting high-affinity Ca^{2+}-ATPase located in branchial chloride cells (Verbost et al., 1987, 1989, Wood, 2001).

While waterborne Cd clearly affected plasma Ca^{2+} in both nonacclimated and Cd-acclimated fish, one of the important results in the current study is that pre-exposure to dietary Cd attenuated this effect, suggesting that chronic exposure to dietary Cd is protective against waterborne Cd toxicity, again in accord with the 96-h LC50 results of Szebedinszky et al. (2001). The postchallenge plasma Ca^{2+} level had fallen to almost half the prechallenge concentration (~2.5 μmol/mL) in nonacclimated fish at 72 h but the decrease was only 14% in the Cd-acclimated fish at the same time (Fig. 4a).

Relevant to the present findings, a protective effect of elevated dietary Ca^{2+} and Cd against inhibition of branchial Ca^{2+} uptake by waterborne Cd was found recently in juvenile rainbow trout (Zohouri et al., 2001; Baldisserotto et al., 2004a,b), suggesting that gastrointestinal tract and branchial pathways of Ca^{2+} and Cd uptake are interrelated. In the case of elevated dietary Ca^{2+}, the uptakes of both waterborne Ca^{2+} and its mimic Cd were downregulated in response to the increase in dietary Ca^{2+} uptake across the gastrointestinal tract, probably by the closure of apical Ca^{2+} channels in the gill chloride cells (Wood, 2001). The downregulation of waterborne Ca^{2+} uptake in trout pre-exposed to elevated dietary Cd was transient (Baldisserotto et al., 2004a) and probably occurred by a different mechanism: inhibition of basolateral Ca^{2+}-ATPase by dietborne Cd entering the chloride cells from bloodstream (Verbost et al., 1987, 1989). In trout exposed to elevated dietary Cd for an extended period of time (30 days), the

decreased uptake of waterborne Ca^{2+} was largely attenuated, suggesting a recovery or acclimation from this inhibition effect. Similar acclimation conceivably occurred in the adult trout exposed to elevated dietary Cd for 45 days in the present study. Alternatively or additionally, in the face of inhibitory effects on Ca^{2+} uptake across gut (dietborne) and gills (waterborne), dietary Cd may have induced a proliferation of branchial chloride cells, a protective or adaptive response that is central to the 'damage-repair' process of acclimation for the recovery from plasma electrolyte disturbance during chronic exposure to metal or acid (McDonald and Wood, 1993; Wood, 2001). Chloride cell hyperplasia was observed in fish during chronic exposure to waterborne and dietary Cd (Oronsaye and Brafield, 1984; Pratap and Wendelaar Bonga, 1993; Thophon et al., 2003) and was likely responsible for recovery of plasma Ca^{2+} during extended exposures (Giles, 1984; Pratap et al., 1989; Baldisserotto et al., 2004a).

Unlike plasma Ca^{2+}, plasma Mg^{2+} concentration was not affected by waterborne Cd challenge in either nonacclimated or Cd-acclimated fish (Fig. 4b). Transient hypermagnesemia has been recorded in fish during chronic exposure to waterborne or dietary Cd (Giles, 1984; Haux and Larsson, 1984; Pratap et al., 1989). However, Pratap et al. (1989) recorded hypermagnesemia only in low-Ca^{2+} water (0.2 mM); in high-Ca^{2+} water (0.8 mM), the effect was less pronounced or not seen. Thus, it appears that the effect of Cd on plasma Mg^{2+} is dependent on the ambient Ca^{2+} concentration and may not be seen if fish are exposed to Cd in hard water containing higher than 0.8–1.0 mM Ca^{2+} (present study).

Among monovalent plasma ions, K^+ was elevated in the nonacclimated fish after waterborne Cd exposure but in Cd-acclimated fish, it was well conserved (Fig. 4c), suggesting again that pre-exposure to dietary Cd developed protection against any disturbances in K^+ homeostasis. In contrast, hypokalemia in trout chronically exposed to waterborne Cd (Giles, 1984) or no change of plasma K^+ in tilapia chronically exposed to waterborne or dietary Cd (Pratap et al., 1989) has been reported in earlier studies. Increased plasma K^+ in the present study is probably attributable to stress and/or acidosis, and reflects an efflux of K^+ from the intracellular compartment of white muscle (Wood and McDonald, 1982; Wood et al., 1988a). Similar to the present findings, elevated plasma K^+ was commonly seen in stressed trout when challenged with acid and aluminum but not in the group previously exposed to the same for acclimation (Wood et al., 1988a, c).

Unlike plasma K^+, the other two monovalent ions Na^+ (Fig. 4d) and Cl^- (Fig. 4d) were elevated only in the Cd-acclimated fish after waterborne Cd challenge. This result contrasts with the reduced or unchanged plasma Na^+ and/or Cl^- reported in other fish chronically exposed to waterborne or dietary Cd (Giles, 1984; Pratap et al., 1989; Wood, 2001), although Reader and Morris (1988) observed increased Na^+ influx in brown trout exposed to elevated waterborne Cd. We suggest that the mechanism responsible for elevated levels of these plasma ions in the Cd-acclimated fish likely involves a greater uptake capacity with the proliferation of chloride cells during dietary acclimation (see above), a decrease in efflux rates due to mucus secretion during waterborne Cd challenge (Wood et al., 1988c, Sorensen, 1991; Wood, 2001), and/or fluid shift from plasma to tissue that may occur during metal stress (Wood et al., 1988c; Spry and Wood, 1984; Pane et al., 2003). Significantly greater levels of plasma protein in Cd-acclimated fish provide indirect evidence for the fluid loss from the plasma in Cd-exposed fish (Fig. 3d).

There was a remarkable elevation of plasma cortisol, a sensitive stress-indicating hormone secreted from interrenal tissue (Donaldson, 1981; Wendelaar Bonga, 1997), in the nonacclimated, naïve fish after waterborne Cd challenge (Fig. 6b). Cortisol mobilization is in accord with many previous studies examining the chronic or acute effects of waterborne Cd (Fu et al., 1990; Tort et al., 1996; Brodeur et al., 1998) as well as other metals, such as Zn, Al, Cu, and Ni (Spry and Wood, 1984; Wood et al., 1988c; Dethloff et al., 1999; Pane et al., 2003). Cortisol plays an important role in ion regulation, energy metabolism, and metal detoxification via metallothionein induction in fish (Fu et al., 1990; Wenderlaar Bonga, 1997). The cortisol-induced recruitment of chloride cells in gills and increase in ion transporting enzymes, particularly Na^+/K^+-ATPase, in gills, intestine, and kidneys are correlated with stimulated uptake of major ions (Ca^{2+}, Na^+ and Cl^-) by freshwater fish (Perry and Wood, 1985; Wenderlaar Bonga, 1997). Thus, the increase (11–12 times the prechallenge level and the value at 72 h for Cd-acclimated fish) of plasma cortisol in naïve fish after Cd challenge (Fig. 6b) fits well with the remarkable decrease (~44%) of plasma Ca^{2+} concentration at the same time (72 h vs. prechallenge, Fig. 4a), suggesting that hypocalcemia of this magnitude was the cue. This also explains why dietary Cd-acclimated fish with only a small drop in plasma Ca^{2+} (~14%, 72 h vs. prechallenge, Fig. 4a) did not exhibit an elevation of plasma cortisol from the basal level (Fig. 6b).

Since cortisol promotes carbohydrate and protein breakdown as a compensatory response to supply energy in stressed fish (Wenderlaar Bonga, 1997), increases in blood glucose and ammonia concomitant with cortisol surge have been documented previously (Fu et al., 1990; Pratap and Wendelaar Bonga, 1990; Wood, 2001). This agrees with the present results showing an increasing trend of plasma ammonia (Fig. 6a) and glucose (Fig. 6c) levels after Cd challenge. A significant increase in plasma lactate concentration (72 h, Fig. 6d), another stress indicator produced in the tissue during O_2 limitation, was observed in the nonacclimated fish at the same time after Cd challenge. This is probably attributable to a mild respiratory stress of hindered gas exchange caused by mucus secretion at gills after waterborne Cd exposure (see above).

In conclusion, chronic exposure to dietary Cd at the level employed here does not substantially affect respiratory and

blood parameters other than hematocrit, hemoglobin, glucose, and total ammonia, but produces acclimation by subtle changes which allow rainbow trout to cope with additional acute waterborne Cd challenge. The acclimation has a clear physiological basis, as dietary Cd-exposed fish exhibited a protection against the effects of waterborne Cd on acid–base status, plasma ions, and stress indices in the present study, and increased LC50 in a previous study (Szebedinszky et al., 2001). Although our Cd concentration in the diet is higher than those found in freshwater environments (see Szebedinszky et al., 2001), the study suggests that the phenomenon of acclimation may occur in natural fish population during chronic exposure to dietary Cd. Thus, acquired adaptive fitness of Cd-acclimated fish over naïve fish to cope with physiological stress may have ecological significance and suggests that not only the concentration of Cd in the ambient water but also the concentration of the diet is important in determining the extent of Cd toxicity to fish.

Acknowledgements

This study was supported by a Strategic Research Grant from the Natural Sciences and Engineering Research Council (NSERC) of Canada and by the International Copper Association (ICA), the Copper Development Association (CDA), the International Lead Zinc Research Organization (ILZRO), The Nickel Producers Environmental Research Association (NiPERA), Noranda-Falconbridge, and Teck-Cominco. C.M. Wood is supported by the Canada Research Chair Program. The authors wish to thank Dr. Peter Chapman as well as two anonymous reviewers for constructive comments on the manuscript.

References

Baldisserotto, B., Chowdhury, M.J., Wood, C.M., 2004a. Effects of dietary calcium and cadmium on cadmium accumulation, calcium and cadmium uptake from the water, and their interactions in rainbow trout. Aquat. Toxicol. (in press).

Baldisserotto, B., Kamunde, C., Matsuo, A., Wood, C.M., 2004b. A protective effect of dietary calcium against acute waterborne cadmium uptake in rainbow trout. Aquat. Toxicol. 67, 57–73.

Berntssen, M.H., Aspholm, O.O., Hylland, K., Wendelaar Bonga, S.E., Lundebye, A.K., 2001. Tissue metallothionein, apoptosis and cell proliferation responses in Atlantic salmon (*Salmo salar* L.) parr fed elevated dietary cadmium. Comp. Biochem. Physiol., C 128, 299–310.

Boutilier, R.G., Heming, T.A., Iwama, G.K., 1984. Physicochemical parameters for use in fish respiratory physiology. Fish Physiol. 10A, 403–430.

Brodeur, J.C., Daniel, C., Ricard, A.C., Hontela, A., 1998. In vitro response to ACTH of the interrenal tissue of rainbow trout (*Oncorhynchus mykiss*) exposed to cadmium. Aquat. Toxicol. 42, 103–113.

Bury, N., Grosell, M., 2003. Iron acquisition by teleost fish. Comp. Biochem. Physiol., C1 35, 97–105.

CCME (Canadian Council of Ministers of the Environment), 2002. Canadian Environmental Quality Guidelines (Update 2002).

Chowdhury, M.J., Baldisserotto, B., Wood, C.M., 2004a. Tissue-specific cadmium and metallothionein levels in rainbow trout chronically acclimated to waterborne or dietary cadmium. Arch. Environ. Contam. Toxicol. (in press).

Chowdhury, M.J., McDonald, D.G., Wood, C.M., 2004b. Gastrointestinal uptake and fate of cadmium in rainbow trout acclimated to sublethal dietary cadmium. Aquat. Toxicol. 69, 149–163.

Dallinger, R., Kautzky, H., 1985. The importance of contaminated food for the uptake of heavy metals by rainbow trout (*Salmo gairdneri*): a field study. Oecologia 67, 82–89.

Dang, Z.C., Berntseen, M.H.G., Lundebye, A.K., Flik, G., Wendelar Bonga, S.E., Lock, R.A.C., 2001. Metallothionein and cortisol receptor expression in gills of Atlantic salmon, *Salmo salar*, exposed to dietary cadmium. Aquat. Toxicol. 53, 91–101.

Dethloff, G.M., Schlenk, D., Khan, S., Bailey, H.C., 1999. The effects of copper on blood and biochemical parameters of rainbow trout (*Oncorhynchus mykiss*). Arch. Environ. Contam. Toxicol. 36, 415–423.

Donaldson, E.M., 1981. The pituitary–interrenal axis as an indicator of stress in fish. In: Pickering, A.D. (Ed.), Stress and Fish. Academic Press, London, pp. 11–47.

Fabbri, E., Caselli, F., Piano, A., Sartor, G., Capuzzo, A., 2003. Cd^{2+} and Hg^{2+} affect glucose release and cAMP-dependent transduction pathway in isolated eel hepatocytes. Aquat. Toxicol. 62, 55–65.

Farag, A.M., Boese, C.J., Woodward, D.F., Bergman, H., 1994. Physiological changes and tissue metal accumulation in rainbow trout exposed to foodborne and waterborne metals. Environ. Toxicol. Chem. 13, 2021–2029.

Fu, H., Steinebach, O.M., van den Hamer, C.J.A., Balm, P.H.M., Lock, R.A.C., 1990. Involvement of cortisol and metallothionein-like proteins in the physiological responses of tilapia (*Oreochromis mossambucus*) to sublethal cadmium stress. Aquat. Toxicol. 16, 257–270.

Giles, M.A., 1984. Electrolyte and water balance in plasma and urine of rainbow trout (*Salmo gairdneri*) during chronic exposure to cadmium. Environ. Toxicol. Chem. 8, 87–97.

Gill, T.S., Epple, A., 1993. Stress-related changes in the hematological profile of the American eel (*Anguilla rostrata*). Ecotoxicol. Environ. Saf. 25, 227–235.

Gill, T.S., Leitner, G., Porta, S., Epple, A., 1993. Response of plasma cortisol to environmental cadmium in the eel, *Anguilla rostrata* Lesueur. Comp. Biochem. Physiol. 104C, 489–495.

Harrison, S.E., Klaverkamp, J.F., 1989. Uptake, elimination and tissue distribution of dietary and aqueous cadmium by rainbow trout (*Salmo gairdneri* Richardson) and lake whitefish (*Coregonus clupeaformis* Mitchill). Environ. Toxicol. Chem. 8, 87–97.

Harrison, S.E., Curtis, P.J., 1992. Comparative accumulation efficiency of cadmium from natural food (*Hyalella azteca*) and artificial diet by rainbow trout (*Oncorhynchus mykiss*). Bull. Environ. Contam. Toxicol. 49, 757–764.

Haux, C., Larsson, A., 1984. Long-term sublethal physiological effects on rainbow trout, *Salmo gairdneri*, during exposure to cadmium and after subsequent recovery. Aquat. Toxicol. 5, 129–142.

Hollis, L., McGeer, J.C., McDonald, D.G., Wood, C.M., 1999. Cadmium accumulation, gill Cd binding, acclimation, and physiological effects during long term sublethal Cd exposure in rainbow trout. Aquat. Toxicol. 46, 101–119.

Hollis, L., McGeer, J.C., McDonald, D.G., Wood, C.M., 2000. Protective effects of calcium against chronic waterborne cadmium exposure to juvenile rainbow trout. Environ. Toxicol. Chem. 19, 2725–2734.

Kraal, M.H., Kraak, M.H.S., De Groot, C.J., Davids, C., 1995. Uptake and tissue distribution of dietary and aqueous cadmium by carp (*Cyprinus carpio*). Ecotoxicol. Environ. Saf. 31, 179–183.

Lacroix, A., Hontela, A., 2004. A comparative assessment of the adrenotoxic effects of cadmium in two teleost species, rainbow trout, *Oncorhynchus mykiss*, and yellow perch, *Perca flavescens*. Aquat. Toxicol. 67, 13–21.

Liu, J., Liu, Y., Habeebu, S.S., Klaassen, C.D., 1999. Metallothionein-nul mice are highly susceptible to the hematotoxic and immunotoxic

effects of chronic $CdCl_2$ exposure. Toxicol. Appl. Pharmacol. 159, 98–108.

Majewski, H.S., Giles, M.A., 1981. Cardiovascular–respiratory responses of rainbow trout (*Salmo gairdneri*) during chronic exposure to sublethal concentrations of cadmium. Water Res. 15, 1211–1217.

McDonald, D.G., Wood, C.M., 1993. Branchial mechanisms of acclimation to metals in freshwater fish. In: Rankin, J.C., Jensen, F.B. (Eds.), Fish Ecophysiology. Chapman and Hall, London, pp. 297–321.

McDonald, D.G., Hobe, H., Wood, C.M., 1980. The influence of calcium on the physiological responses of the rainbow trout, *Salmo gairdneri*, to low environmental pH. J. Exp. Biol. 88, 109–131.

McGeer, J.C., Szebedinszky, C., McDonald, D.G., Wood, C.M., 2000. Effects of chronic sublethal exposure to waterborne Cu, Cd, or Zn in rainbow trout: 1. Iono-regulatory disturbance and metabolic costs. Aquat. Toxicol. 50, 233–245.

Neeley, W.E., Phillipson, J., 1988. Automated enzymatic method for determining ammonia in plasma, with 14-day reagent stability. Clin. Chem. 34, 1868–1869.

Niyogi, S., Couture, P., Pyle, G., McDonald, D.G., Wood, C.M., 2004. Acute cadmium biotic ligand model characteristics of laboratory-reared and wild yellow perch (*Perca flavescens*) relative to rainbow trout (*Oncorhynchus mykiss*). Can. J. Fish Aquat. Sci. 61, 942–953.

Oronsaye, J.A.O., Brafield, A.E., 1984. The effect of dissolved cadmium on the chloride cells of the gills of the stickleback, *Gasterosteus aculeatus* L. J. Fish Biol. 25, 253–258.

Pane, E.F., Richards, J.G., Wood, C.M., 2003. Acute waterborne nickel toxicity in the rainbow trout (*Oncorhynchus mykiss*) occurs by a respiratory rather than ionoregulatory mechanism. Aquat. Toxicol. 63, 65–82.

Perry, S.F., Wood, C.M., 1985. Kinetics of branchial calcium uptake in the rainbow trout: effects of acclimation to various external calcium levels. J. Exp. Biol. 116, 411–433.

Pratap, H.B., Wendelaar Bonga, S.E., 1990. Effect of waterborne cadmium on plasma cortisol and glucose in the cichlid fish *Oreochromis mossambicus*. Comp. Biochem. Physiol., C 95, 313–317.

Pratap, H.B., Wendelaar Bonga, S.E., 1993. Effect of ambient and dietary cadmium on pavement cells, chloride cells, and Na^+/K^+-ATPase of the freshwater teleost *Oreochromis mossambicus* at normal and high calcium levels in the ambient water. Aquat. Toxicol. 26, 133–150.

Pratap, H.B., Fu, H., Lock, R.A.C., Wendelaar Bonga, S.E., 1989. Effect of waterborne and dietary cadmium on plasma ions of the teleost *Oreochromis mossambicus* in relation to water calcium levels. Arch. Environ. Contam. Toxicol. 18, 568–575.

Reader, J.P., Morris, R., 1988. Effects of aluminum and pH on calcium fluxes, and effects of cadmium and manganesium on calcium and sodium fluxes in brown trout (*Salmo trutta* L.). Comp. Biochem. Physiol., C 91, 449–457.

Reid, S.D., McDonald, D.G., 1988. Effects of cadmium, copper, and low pH on ion fluxes in the rainbow trout, *Salmo gairdneri*. Can. J. Fish. Aquat. Sci. 45, 244–253.

Shaffi, S.A., Manohar, Y.R., Nandan, M.J., 2001. Influence of protective agents on metal induced respiratory distress in *Labeo rohita* (Ham). Bull. Environ. Contam. Toxicol. 66, 611–616.

Soivio, A., Westmann, K., Nyholm, K., 1972. Improved methods of dorsal aorta catheterization: hematological effects followed for three weeks in rainbow trout. Finn. Fish Res. 1, 11–22.

Sorensen, E.M., 1991. Metal Poisoning in Fish. CRC Press, Florida.

Spry, D.J., Wood, C.M., 1984. Acid–base, plasma ion and blood gas changes in rainbow trout during short term toxic zinc exposure. J. Comp. Physiol. 154B, 149–158.

Stubblefield, W.A., Steadman, B.L., La Point, T.W., Bergman, H.L., 1999. Acclimation-induced changes in the toxicity of zinc and cadmium to rainbow trout. Environ. Toxicol. Chem. 18, 2875–2881.

Szebedinszky, C., McGeer, J.C., McDonald, D.G., Wood, C.M., 2001. Effects of chronic Cd exposure via the diet or water on internal organ-specific distribution and subsequent gill Cd uptake kinetics in juvenile rainbow trout (*Oncorhynchus mykiss*). Environ. Toxicol. Chem. 20, 597–607.

Thophon, S., Kruatrachue, M., Upatham, E.S., Pokethitiyook, P., Sahaphong, S., Jaritkhuan, S., 2003. Histopathological alterations of white seabass, *Lates calcarifer*, in acute and subchronic cadmium exposure. Environ. Pollut. 121, 307–320.

Tort, L., Kargacin, B., Torres, P., Giralt, M., Hidalgo, J., 1996. The effects of cadmium exposure and stress on plasma cortisol, metallothionein levels and oxidative status in rainbow trout (*Oncorhynchus mykiss*) liver. Comp. Biochem. Physiol., C 114, 29–34.

USEPA (U.S. Environmental Protection Agency), 2001. 2001 Update of Ambient Water Quality Criteria for Cadmium. EPA-822-R-01-001, Washington, DC.

Verbost, P.M., Flik, G., Lock, R.A.C., Wendelaar Bonga, S.E., 1987. Cadmium inhibition of Ca^{2+} uptake in rainbow trout gills. Am. J. Physiol. 253, R216–R221.

Verbost, P.M., Van Rooij, J., Flik, G., Lock, R.A.C., Wendelaar Bonga, S.E., 1989. The movement of cadmium through freshwater trout branchial epithelium and its interference with calcium transport. J. Exp. Biol. 145, 185–197.

Walsh, P.J., Wood, C.M., Moon, T.W., 1998. Red blood cell metabolism. In: Perry, S.F., Tufts, B.I. (Eds.), Fish Respiration. Fish Physiology, vol. 17. Academic Press, San Diego, U. S. A., pp. 41–73.

Wenderlaar Bonga, S.E., 1997. The stress response in fish. Physiol. Rev. 66, 591–625.

Wolf, K., 1963. Physiological salines for freshwater teleosts. Prog. Fish-Cult. 25, 135–140.

Wood, C.M., 2001. Toxic responses of the gill. In: Schlenk, D., Benson, W.H. (Eds.), Target Organ Toxicity in Marine and Freshwater Teleosts. Taylor and Francis, London, pp. 1–87.

Wood, C.M., McDonald, D.G., 1982. Physiological mechanisms of acid toxicity to fish. In: Johnson, R.E. (Ed.), Acid Rain/Fisheries. American Fisheries Society, Bethesda, MD, U. S. A., pp. 197–226.

Wood, C.M., McDonald, D.G., McMahon, B.R., 1982. The influence of experimental anemia on blood acid–base regulation in vivo and in vitro in the starry flounder (*Platichthys stellatus*) and the rainbow trout (*Salmo gairdneri*). J. Exp. Biol. 99, 221–237.

Wood, C.M., Playle, R.C., Simons, B.P., Goss, G.G., McDonald, D.G., 1988a. Blood gases, acid–base status, ions, and hematology in adult brook trout (*Salvelinus fontinalis*) under acid/aluminum exposure. Can. J. Fish. Aquat. Sci. 45, 1575–1586.

Wood, C.M., McDonald, D.G., Booth, C.E., Simons, B.P., Ingersoll, C.G., Bergman, H.L., 1988b. Physiological evidence of acclimation to acid/aluminum stress in adult brook trout (*Salvelinus fontinalis*): 1. Blood composition and net sodium fluxes. Can. J. Fish. Aquat. Sci. 45, 1587–1596.

Wood, C.M., Simons, B.P., Mount, D.R., Bergman, H.L., 1988c. Physiological evidence of acclimation to acid/aluminum stress in adult brook trout (*Salvelinus fontinalis*): 2. Blood parameters by cannulation. Can. J. Fish. Aquat. Sci. 45, 1597–1605.

Wood, C.M., Hogstrand, C., Galvez, F., Munger, R.S., 1996. The physiology of waterborne silver toxicity in freshwater rainbow trout (*Oncorhynchus mykiss*): 2. The effects of silver thiosulfate. Aquat. Toxicol. 35, 111–125.

Wood, S.C., Johansen, K., 1972. Adaptations to hypoxia by increased HbO_2 affinity and decreased red cell ATP concentration. Nature 237, 278–279.

Zall, D.M., Fisher, M.D., Garner, Q.M., 1956. Photometric determination of chlorides in water. Anal. Chem. 28, 1665–1678.

Zikic, R.V., Stajn, A.S., Pavlovic, S.Z., Ognjanovic, B.I., Saicic, Z.S., 2001. Activities of superoxide dismutase and catalase in erythrocytes and plasma transaminases of goldfish (*Carassius auratus gibelio* Bloch.) exposed to cadmium. Physiol. Res. 50, 105–111.

Zohouri, M.A., Pyle, G.G., Wood, C.M., 2001. Dietary Ca inhibits waterborne Cd uptake in Cd-exposed rainbow trout, *Oncorhynchus mykiss*. Comp. Biochem. Physiol., C 130, 347–356.