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Pre-exposure to Waterborne Nickel Downregulates Gastrointestinal Nickel Uptake in Rainbow Trout: Indirect Evidence for Nickel Essentiality

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Nickel (Ni) may be both a toxicant and a micronutrient, but its essentiality to aquatic animals is not established. Interactions between branchial and gastrointestinal routes of metal uptake are important for understanding metal regulation and essentiality in aquatic animals. Adult rainbow trout (*Oncorhynchus mykiss*) were pre-exposed to a sublethal concentration of waterborne Ni (7.43 μmol L^{-1}) or a control water (0.12 μmol L^{-1}) for 45 days, and subsequently, a gastrointestinal dose of radiolabeled Ni (1.08 μmol kg^{-1} wet wt) was infused into the stomach of both non-pre-exposed and Ni pre-exposed trout to test whether pre-exposure to waterborne Ni would affect gastrointestinal uptake. The fish pre-exposed to waterborne Ni exhibited a markedly greater level of total Ni in the blood plasma (~10-fold) but not in red blood cells (RBC). Pre-exposure downregulated the gastrointestinal uptake of radiolabeled Ni (new Ni) in the plasma and RBCs, providing evidence for the first time of homeostatic interaction between the two routes of Ni uptake. The plasma and RBC concentrations of new Ni in the non-pre-exposed and Ni pre-exposed groups were linear in the first 2 h and then approached a plateau. Only a small fraction of the infused dose (1.6–3.7%) was found in the internal organs of both groups at 24 h. Waterborne Ni, but not the infused Ni, greatly increased total Ni levels in the gills (6.1 fold), kidney (5.6 fold), scales (4.2 fold), and gut tissues (1.5–4.2 fold). It appears that gut, kidney and scales play important roles for Ni homeostasis by providing uptake, clearance and storage sites. Overall, our results suggest that Ni is subject to homeostatic regulation in the rainbow trout, a property that is characteristic of essential metals.

Introduction

Nickel (Ni) is a naturally occurring element and an anthropogenic contaminant for aquatic and terrestrial environments as it is used in many industrial products, including stainless steel, electroplating, pigments and ceramics (*1, 2*) Ni concentrations are usually below 10 μg L^{-1} in uncontaminated freshwaters, but may reach as high as several hundred to 1000 μg L^{-1} in Ni contaminated sites (*2*).

Several toxic effects of Ni including allergies, carcinogenesis, and cardiovascular and renal disorders have been identified in higher animals including humans (*3*). Based on limited studies, Ni causes respiratory disorder, Mg^{2+} antagonism and kidney lesions in fish and other aquatic animals (*4–6*). Freshwater fish take up waterborne and dietary Ni via the gills and gut (*5, 7*). Uptake mechanisms in fish are not known but probably involve iron (*8, 9*) and magnesium transport systems (*10*) as observed in mammals.

Environmental risk assessment of essential elements requires a different approach compared to nonessential metals, because both deficiency and excess require proper attention in setting environmental quality criteria (*11*). Therefore, it is important that the essentiality of an element is known so that regulatory guidelines are determined based on optimal concentrations for organisms. In this regard, Ni is not unequivocal for its essentiality to all biota (*12*).

Ni is known to be essential nutrient for microorganisms and aquatic plants due to its documented role as the cofactor for enzymes used in nitrogen fixation and hydrogen metabolism (*12*). The essentiality of Ni is now generally accepted for terrestrial animals based on numerous symptoms induced by Ni deficiency (*13*), but not undisputed, as a Ni-containing metalloenzyme has yet to be recovered from animal tissues (*13*). The consistent presence of Ni in animal tissues and maintenance of Ni homeostasis in the body by processes such as regulated uptake and excretion, storage of excess quantities in metabolically inactive sites (hair, fur, or feather), or binding to metallothioneins are also frequently presented as arguments for Ni essentiality in higher animals (*3, 13*).

The essentiality of Ni to aquatic animals is not established. However, inferences have been made in a few studies toward homeostatic regulation and thus possible essentiality of Ni in fish and other species based on the analysis of Ni bioaccumulation and tissue Ni distribution, and a comparison to undisputed essential metals like Cu and Zn (*7, 12*). To date, there has been no comprehensive study conducted exclusively to evaluate homeostatic mechanisms for Ni in aquatic animals, although several done recently in our laboratory have focused on renal clearance and transport of Ni in rainbow trout (*6, 14, 15*). These studies have demonstrated that the kidney can efficiently excrete and reabsorb Ni (*6*) and that renal Ni transport is a saturable process that is temperature and Mg^{2+} sensitive and can be altered during prolonged exposure to waterborne Ni (*14, 15*).

In the present study, adult rainbow trout were pre-exposed to waterborne "cold" Ni (7.43 μmol L^{-1}) for 45 days and subsequently a single dose of radiolabeled Ni (1.08 μmol kg^{-1} wet wt) was infused into the stomach of both non-pre-exposed and Ni pre-exposed trout to observe gastrointestinal uptake. Both the waterborne Ni concentration and gastric Ni dose were environmentally realistic (see below). Our primary goals were to evaluate tissue specific accumulation of Ni from waterborne exposure, and to test whether the increase of waterborne Ni in the body would affect gastrointestinal uptake. We hypothesized that such interaction between the two uptake routes is necessary for physiological regulation of Ni and would provide some insight into the essentiality of Ni for rainbow trout.

Experimental Section

Experimental Fish. Adult rainbow trout (~ 150 g) were obtained from a local hatchery (Humber Springs Trout Hatchery, Orangeville, Ontario, Canada) and held under laboratory conditions for at least two weeks before experimental use. Fish were held in 500 L tanks with a flow-through of moderately hard dechlorinated Hamilton City tapwater at approximately 2.0 L min^{-1}. As measured by graphite or flame

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atomic absorption spectrophotometer (AAS, Spectra 220, Varian, Australia), water composition was $Ca^{2+} \approx 1$ mM, $Mg^{2+} \approx 0.2$ mM, $Na^{+} \approx 0.6$ mM, and background Ni ≈ 4 μg L^{-1}. The dissolved organic carbon (DOC), measured by a Shimadzu TOC-5050A Total Carbon Analyzer (Mandel Scientific, Guelph, Ontario) was $\approx$2.5 mg L^{-1}. Water hardness (as $CaCO_3$) was 120 mg L^{-1} and titratable alkalinity to pH 4 was 1.9 mM. The temperature and pH of water were kept at 13 $\pm$ 1 °C and 8.0 $\pm$ 0.2, respectively, throughout the experiment. The fish were fed commercial, floating trout chow (Martin's Feed Mills, Ontario, Canada) at a ration of 1.5% of their body weight three times per week prior to the start of the experiment.

Pre-exposure to Waterborne Ni. For pre-exposure, a nominal Ni concentration of 6.82 μmol L^{-1} (400 μg L^{-1}) in the water was used for 45 days. Fish were held in two 500 L tanks ($n = 25$ per tank) receiving a flow of 1.5 L min^{-1}. In one tank, Ni was delivered as $NiSO_4 \cdot 6H_2O$ from a concentrated stock solution using a Mariotte bottle. The other tank was used as the control. Fish were fed trout chow at 1% of their body weight daily throughout the 45 day pre-exposure period. The composition of the food, as specified by the supplier (Martin's Feed Mills, Ontario, Canada) was: crude protein 40%, crude fat 11%, crude fiber 3.5%, Ca 1.0%, P 0.85%, Na 0.45%, and Ni 3.9 mg kg^{-1} dry weight. Water flow and dosing rates were monitored daily and adjusted if necessary. Water samples for dissolved Ni were taken every second day through a 0.45 μm filter, acidified with trace metal grade HNO_3 (Fisher Scientific), and analyzed to determine total dissolved Ni. The actual measured concentration of dissolved Ni was 7.43 $\pm$ 0.14 μmol L^{-1} (436 $\pm$ 8.3 μg L^{-1}, $n = 20$) in the exposure tank. The concentration in the control tank was 0.12 $\pm$ 0.02 μmol L^{-1} (6.8 $\pm$ 1.2 μg L^{-1}, $n = 20$).

The concentration (400 μg L^{-1}) used for the prolonged exposure is sublethal and is only 2.6% of 96 h LC50 for rainbow trout (*5*). This concentration is at the upper range of freshwater quality criteria values (25–470 μg L^{-1}) for aquatic life in the United States and Canada (*1, 16*), but found in Canadian lakes (water, $\sim$111 – 338 μg L^{-1}; sediment, > 1,000 μg g^{-1} dry wt) impacted by mining and industrial activity (*17, 18*).

Cannulation. The Ni exposed (235 $\pm$ 9.6 g) and control (232 $\pm$ 8.4 g) fish were anesthetized with 0.075 g L^{-1} of MS222 (neutralized to pH 8.0 with $\sim$1 mmol L^{-1} NaOH) and surgically fitted with an arterial catheter (*19*) and a stomach catheter (*20*). The former was inserted into the dorsal aorta for sampling blood and the other down the esophagus for dosing of radiolabeled Ni solution into the stomach. During surgery, the anesthetic solution irrigating the gills of Ni-pre-exposed fish was spiked with $NiSO_4 \cdot 6H2O$ to yield a Ni concentration comparable to that to which these fish had been exposed; ionic composition was also the same. After surgery, fish were transferred to individual darkened plexiglass chambers (3 L) served with a water flow of 250 mL min^{-1} and continuous aeration, and allowed to recover for 48 h before gastrointestinal infusion of Ni. During the recovery period, Ni exposure to this group of fish was still maintained by delivering Ni solution to their holding chambers from a Mariotte bottle as described above.

Experimental Procedures. After recovery, an initial blood sample (300 μl) was obtained via the arterial catheter from both the control and Ni pre-exposed fish and replaced with Cortland saline (*21*). Immediately after this sampling, waterborne Ni exposure was terminated and fish were given a single dose (1.08 μmol kg^{-1} or 63.4 μg kg^{-1}) of radioactive Ni (^{63}Ni) solution into the stomach via the stomach catheter. The solution was prepared by adding ^{63}Ni stock ($^{63}NiCl_2$; specific activity: 363.2 kBq μg^{-1}; PerkinElmer, Boston, MA) to 0.9% NaCl solution containing an appropriate concentration of stable Ni so that the final radioactivity in the solution was 1727 kBq ml^{-1} and total Ni concentration was 38.1 μg mL^{-1} (0.65 μmol ml^{-1}). Based on the known Ni concentrations in the benthic invertebrates of Ni impacted lakes ($\sim$50 $\mu g\, g^{-1}$ dry wt or $\sim$10 μg g^{-1} wet wt; (*18*)), and assuming a daily ration in the wild equivalent to 2% wet weight relative to fish body weight (i.e., 20 g per kg), the gastrointestinal dose used in this study (63.4 μg kg^{-1} wet wt) is approximately 30% of the daily dietary Ni dose one would expect for fish living in the lakes. Conversely, it is about 160% of the background daily dose the trout in this study received from their commercial trout diet (analysis below) during the pre-exposure period.

After infusion of radiolabeled Ni into the stomach, blood samples were taken via the catheter following the method used previously for Cd (*22*). Approximately 300 μl of blood was removed via the catheter at 10, 20, 40, 60, 120, 240, 360, 480, 720, 1440 min after gastrointestinal infusion and replaced with an equal volume of Cortland saline (*21*). Blood samples were centrifuged at 14,000 *g* for 3 min to separate plasma from blood cells. Both the blood plasma and cells were digested in 1N HNO_3 (trace metal grade, Fisher Scientific) at 60 °C for 48 h and analyzed for radioactivity and total Ni concentration.

After the final blood sampling (1440 min or 24 h), the fish were euthanized immediately by an overdose of MS-222 (0.6 g L^{-1}) and dissected to obtain tissue samples. The liver, gall bladder including bile, gill filaments, and kidney were obtained as whole organs. For gut tissues, the whole gastrointestinal tract was removed from the body cavity and cut into four pieces: whole stomach, anterior intestine including pyloric cecae (hereafter referred to as cecae), midintestine, and posterior intestine. The gut tissues were thoroughly rinsed with distilled water to clear mucus, food fragments, and nonabsorbed Ni from the lumen, and analyzed individually. In order to determine background concentrations of "cold" Ni in the tissues before dosing, six control and six Ni-pre-exposed fish were sampled directly from the holding tanks, and tissue samples were obtained as above. All tissue samples were digested in 1 N HNO_3 at 60 °C for 48 h for the determination of beta radioactivity and total Ni concentrations.

After removal of the above tissue samples, the fish carcass was homogenized in a blender with 500 mL of 1N HNO_3. Three aliquots of approximately 15 mL of the suspension for each fish were collected in plastic vials and digested by placing the vials in an oven at 60 °C for 48 h to determine radioactivity and total Ni in the carcass.

Measurements and Calculations. To determine ^{63}Ni radioactivity, an aliquot (200 – 700 μl) of digested samples was added to 10 mL of Ultima Gold AB scintillation cocktail (PerkinElmer) and counted in a liquid scintillation counter (LKB Wallac, 1217 Rackbeta, Helsinki, Finland). The counts were corrected for background, radiodecay, and quenching, and converted to absolute values (ng Ni per unit wet weight or volume) to obtain newly accumulated Ni

$$\text{New Ni} = R/(M \cdot SA)$$

where R is the ^{63}Ni radioactivity in the sample after correction (cpm), M is the tissue wet mass (g) or plasma volume (mL), and SA is the measured specific activity of radioactive Ni in the infusion solution (cpm ng^{-1} total Ni).

To determine total Ni in the exposure water, plasma, RBCs, and tissue samples, we used a graphite furnace AAS (SpectrAA 220, Varian, Australia). Absorbance of the appropriately diluted unknown samples (in duplicate) was related to the absorbance of a series of known standards made from a certified stock solution of Ni (Fisher Scientific) to obtain Ni concentrations expressed on the basis of per-gram wet tissue and RBC, per-milliliter plasma, or per-liter water. A reference aqueous material for trace metals (TM-15, Environment Canada, Burlington, ON, Canada) was analyzed along with

Ni samples for validating the analytical results. The recovery of Ni in TM-15 was 98–102%.

Specific growth rates of fish (% per day) during the 45-day pre-exposure period were determined from the change over time of the natural log (*ln*) of fish weights (*23*). To calculate the relative distribution of the infused radiolabeled Ni, we converted the total burdens of new Ni (nmol) in the plasma, RBCs, sampled tissues, and carcass into the percentage of infused dose (*23*). To calculate the total burden in blood compartments, we assumed the relative mass of blood in rainbow trout to be 6.0% (*24*), and the RBCs were assumed to represent 30% of the mass of whole blood, the rest being plasma (*25*). The fraction (%) of radiolabeled Ni dose internalized beyond the gut wall was calculated by adding the percentages of new Ni found in nongut internal tissues, carcass, plasma, and RBCs. The percentage of the Ni dose that was lost or excreted from the fish was calculated by subtracting the percentage of internalized Ni plus that found in gut tissue from the infused dose (100%).

Statistical Analysis. Data are presented as mean ± SEM (*n*) where *n* is the number of fish. To test significant differences between plasma and RBC concentration-time profiles in the control and Ni-pre-exposed groups,we used a two-factor ANOVA, with pre-exposure and time as the main factors. Student's unpaired *t*-test or Newman−Keuls test was applied to compare growth rates and tissue levels of total and newly accumulated Ni in treatment groups. Percentages and proportions were arcsine transformed before statistical analysis. All statistical tests were performed using the computer software InStat (GraphPad, San Diego, CA, U.S.A). The significance level of $p \leq 0.05$ was used throughout.

Results and Discussion

No mortality occurred during pre-exposure of trout to waterborne Ni at the sublethal level of 7.43 μmol L^{-1} for 45 days. Specific growth rates of the nonexposed (0.80 ± 0.1% per day) and Ni pre-exposed fish (0.73 ± 0.09% per day) were not significantly different. Similar results with no significant effects on survival and/or growth were observed when rainbow trout at embryonic, fingerling or adult stages were chronically exposed to waterborne Ni up to 7.34 μmol L^{-1} for a prolonged period (*6, 26, 27*).

The concentration of total Ni in the blood plasma of the nonexposed naïve fish (never exposed to any experimental elevations of Ni) was 3.27 ± 0.25 μmol L^{-1} (Figure 1a), approximately 27× higher than the background Ni level (0.12 μmol L^{-1}) in control water. The plasma concentration (32.4 ± 0.25 μmol L^{-1}) in the fish chronically exposed to waterborne Ni (7.43 μmol L^{-1}) for 45 days was approximately 10-fold greater than that in the naïve fish, but the bioconcentration factor relative to the water was now only about 5×. Similar loading of plasma Ni in rainbow trout both from acute and chronic exposures to waterborne Ni at the present or higher levels was also observed previously (*5, 27*), suggesting that cellular uptake and/or renal clearance of plasma Ni were slower than the Ni uptake in the plasma from the exposure water (*6, 27*). After a chronic study for 85 d, Ni concentrations in plasma of rainbow trout exposed to 2.0 to 7.3 μmol L^{-1} appeared to plateau at 80−100 μmol L^{-1} (*26*), which was coupled with the inference that active regulation of Ni had occurred in these fish during prolonged exposure.

Upon a gastrointestinal dose of 1.08 μmol kg^{-1} radiolabeled Ni, the total plasma Ni concentrations did not significantly change over 24 h in either the nonexposed or Ni pre-exposed fish and ANOVA indicates that the levels remained significantly greater in the latter overall (Figure 1a). No change in total Ni over time might be explained as the "masking effect" of the high concentration of endogenous Ni on relatively smaller concentrations of new Ni (Figure 1b), as observed for Zn in rainbow trout (*22*).

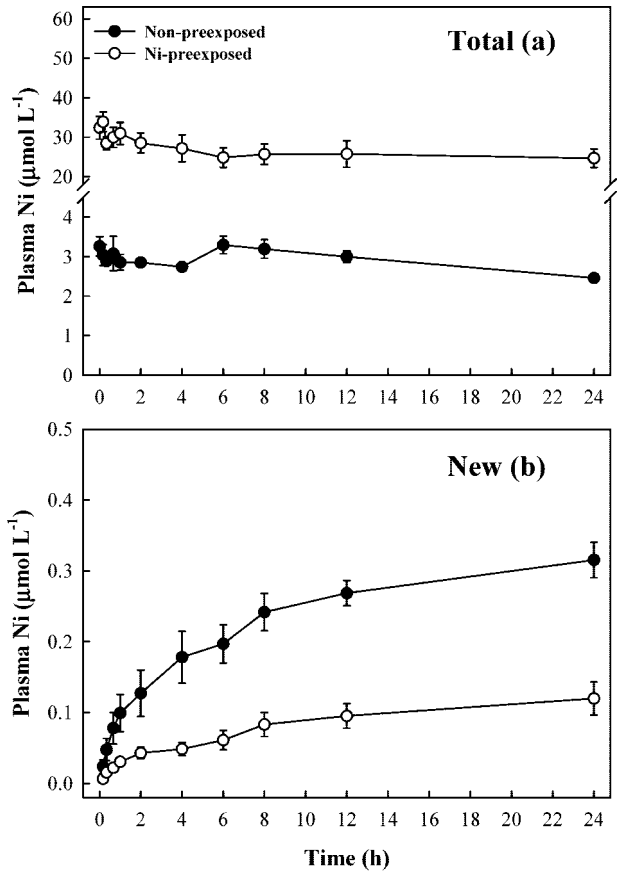

FIGURE 1. Plasma concentration−time profile of total Ni (a) and newly accumulated Ni (b) in non-pre-exposed and Ni pre-exposed rainbow trout after a gastrointestinal dose of radiolabeled Ni (1.08 μmol kg^{-1} wet wt) infused into the stomach. The fish were pre-exposed to waterborne stable Ni (6.82 μmol L^{-1}) for 45 days. Symbols represent experimental values (mean ± SE, n = 6–7). ANOVA revealed significant differences between non-pre-exposed and Ni pre-exposed fish both for total Ni ($p < 0.001$; panel a) and newly accumulated Ni ($p < 0.05$; panel b).

Plasma new Ni (radiolabeled Ni) levels were significantly greater in the nonexposed fish than in Ni pre-exposed fish (Figure 1b). The mean concentrations of new Ni in Ni pre-exposed fish were 27 – 38% of the concentrations in nonexposed fish over 24 h after gastrointestinal Ni dose (Figure 1b). The results suggest that pre-exposure to waterborne Ni downregulated the gastrointestinal uptake of radiolabeled Ni, providing the first evidence of homeostatic interaction between the two routes (gills and gut) of Ni uptake in fish. Similar interactions demonstrating a homeostatic regulation of essential metals (e.g., Cu and Zn) or their mimicking ions (Na^+, Ca^{2+}) following pre-exposure to dietary sources have been documented in fish. For example, dietary Cu or Na^+ pre-exposure downregulates branchial uptake of waterborne Cu and Na^+ (*28–30*), whereas dietary Cu deprivation upregulates branchial uptake in rainbow trout (*29*). Pre-exposure to elevated Ca^{2+} in the diet reduces branchial Ca^{2+} and Zn uptake (*31*). Contrary to Ni (present study), previous exposure to waterborne Cu resulted in no effect on the dietary uptake rate of Cu (*32*). Despite this difference, it appears that branchial (waterborne) and gastrointestinal (dietary) pathways of Ni uptake are interrelated, as observed for other essential metals and ions (see above), and that the gut plays a key role in Ni homeostasis in rainbow trout. The presence of protective mechanisms that control Ni uptake across the gut has also been suggested for lake whitefish fed dietary Ni (17 μmol g^{-1} diet) for 104 days, because Ni

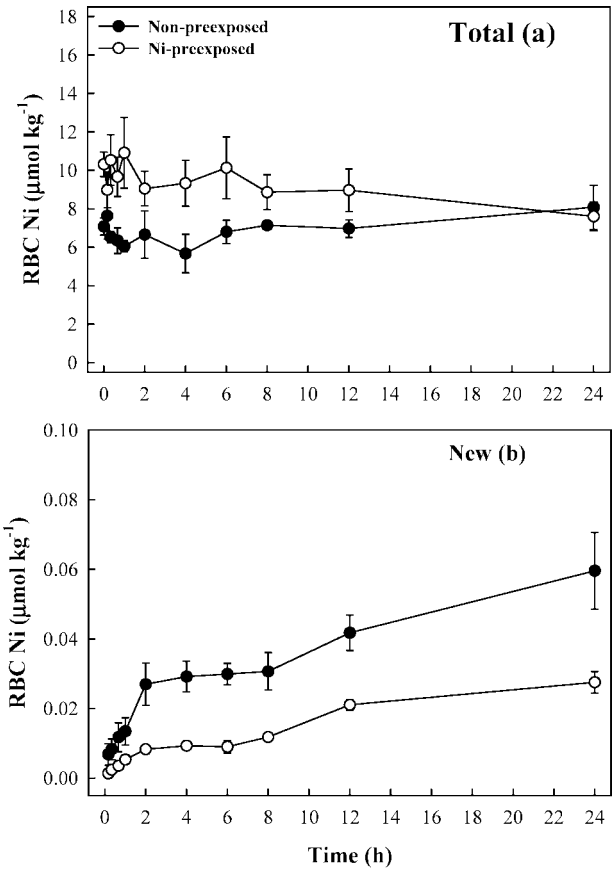

FIGURE 2. Red blood cell (RBC) concentration−time profile of total Ni (a) and newly accumulated Ni (b) in non-pre-exposed and Ni pre-exposed rainbow trout after a gastrointestinal dose of radiolabeled Ni (1.08 μmol kg^{-1} wet wt) infused into the stomach. The fish were pre-exposed to waterborne stable Ni (6.82 μmol L^{-1}) for 45 days. Symbols represent experimental values (mean ± SE, n = 6–7). ANOVA revealed significant differences between non-pre-exposed and Ni pre-exposed fish for new Ni (p < 0.05; Figure 2b).

concentrations in the gut tissues were the highest on day10, but decreased afterward (*7*). For Cu, the gill appears to perform the major homeostatic role via adjustment of branchial Cu transport proteins, whereas the gut serves for bulk acquisition (*32*).

The plasma concentrations of new Ni in both groups increased linearly in the first 2 h and then approached a plateau (Figure 1b), suggesting that a saturable process might be involved in Ni transport across gut epithelial cells. Nothing is known about the mechanisms of Ni uptake in fish, but in mammals, the presence of a saturable mechanism in the gut has been suggested (*33*). More recently, the proton-coupled divalent metal transporter DMT1 (also known as DCT1 or Nramp2) has been implicated for cellular Ni transport in mammals (*8, 34*). Pre-exposure to waterborne Ni may downregulate DMT1 expression, should this transporter be involved in the uptake of Ni across the gut of the rainbow trout. Further research is necessary to understand the underlying molecular mechanisms of Ni transport across the fish gut.

RBC Ni levels (Figure 2a) behaved very differently from plasma Ni levels (Figure 1a). Prolonged exposure of fish to elevated waterborne Ni for 45 days resulted in only a small increase (~1.5 fold) of RBC total Ni concentration to 10.31 ± 0.63 μmol kg^{-1} RBC relative to the background in the naïve fish (7.07 ± 0.44 μmol kg^{-1} RBC, Figure 2a). Thus RBC Ni concentration was kept almost constant, being approximately 2-fold above plasma levels of 3.27 ± 0.25 μmol L^{-1} in control fish, and well below plasma levels of 32.4 ± 0.25 μmol L^{-1} in chronically exposed fish. Gastrointestinal dosing of radiolabeled Ni also did not affect total Ni in the RBCs, as the concentrations were not significantly different between the treatment groups and over time (Figure 2a). The high background level of RBC Ni in the naïve fish relative to a nonessential metal (e.g., Cd, see below), but small increase during prolonged exposure suggests that Ni might be essential for RBC function, and that the RBC concentration of Ni is homeostatically regulated. Consistent with these results, high background levels of RBC Zn (~150 μmol kg^{-1}) but strong regulation with negligible erythrocytic accumulation of Zn were observed when rainbow trout were chronically exposed to waterborne Zn under similar experimental conditions (*22*). However, Cd which is not known to be essential, showed results contrary to those for Ni and Zn. Low background levels (~ 0.05 μmol kg^{-1}) but high loading levels (2–19 fold) in RBCs were observed when rainbow trout were chronically exposed to waterborne or dietary Cd for 30 days (*22, 23*).

The RBC new Ni−time profiles (Figure 2b) and the plasma new Ni−time profiles (Figure 1b) were similar: the RBC concentrations of new Ni were significantly smaller (19–50%) in Ni-pre-exposed fish relative to those in non-pre-exposed fish and the levels tended to plateau in both groups over time (Figure 2b). The similar concentration-time profiles and smaller concentrations of new Ni in both blood compartments (plasma and RBC) of Ni pre-exposed fish reinforce our conclusion that gastrointestinal uptake of Ni is systemically regulated in response to waterborne or dietary Ni. Overall, the new Ni concentrations in the plasma (Figure 1b) were greater (3−8 fold) than the new Ni concentrations in the RBCs (Figure 2b) in both treatment groups, which translates into the fact that the new Ni in the plasma compartment accounted for approximately 78–89% of the blood new Ni taken up from gastrointestinal dose.

After waterborne Ni pre-exposure, the highest concentrations of tissue total Ni were observed in the kidney (58.4 ± 2.2) > scales (53.9 ± 3.5) > blood (42.7 ± 4.5) > gills (40.4 ± 4.3) > anterior (cecae) and posterior intestine (~30.0 ± 6.0 μmol kg^{-1}), whereas concentrations in bile, midintestine, liver, and stomach were relatively low (9.6 – 12 μmol kg^{-1}; Figure 3a). However, the significant increases (accumulation) relative to the nonexposed naïve fish were observed in the order: gills (6.1 fold) > kidney (5.6 fold) > posterior-intestine and scales (4.2 fold) > blood and anterior intestine (3.5 fold) > liver (2.1 fold) > stomach (1.8 fold) > midintestine (1.5 fold). Our data on tissue concentrations and relative accumulations of Ni are in agreement with the patterns of Ni accumulation measured previously in rainbow trout and other fish exposed to waterborne Ni (*5, 35, 36*) or dietary Ni (*7*) for a prolonged period. In all cases, a general pattern is that Ni is preferentially accumulated in the kidney, gills, intestine, and blood.

The kidney has been identified as the most common target for Ni in fish (*7, 12, 35, 36*) and mammals (*13*) with implications that renal clearance is a major process of Ni regulation. In rainbow trout, the kidney not only excretes but also reabsorbs Ni during urine processing (*6*). Both functions are probably important for the regulation of Ni which is likely essential but potentially toxic at high levels. Strong accumulation of Ni has also been observed in the scales of rainbow trout (present study, Figure 3a) and lake whitefish (*7*). In addition to kidney, scales appear to be an important organ of Ni regulation in fish by serving as a storage or excretion site (by sloughing).

The gastrointestinal Ni dose did not increase total Ni concentrations in any of the tissues except midintestine. Indeed, significantly smaller concentrations were observed in a few tissues in both non-pre-exposed (bile, stomach, and midintestine) and Ni pre-exposed fish (gills and scales; Figure

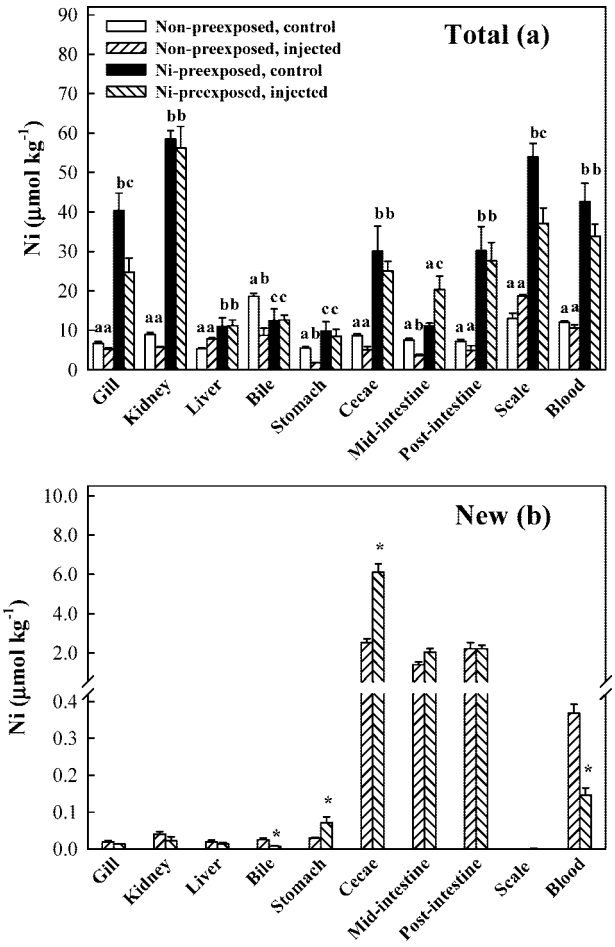

FIGURE 3. (a) Concentrations of total Ni in the tissues of noninfused, non-pre-exposed control fish, in noninfused, Ni pre-exposed control fish, and in both non-pre-exposed and Ni pre-exposed rainbow trout 24 h after a gastrointestinal dose of radiolabeled Ni (1.08 μmol kg^{-1} wet wt) infused into the stomach. (b) Concentrations of newly accumulated radiolabeled Ni in the same tissue samples. The fish were pre-exposed to waterborne stable Ni (6.82 μmol L^{-1}) for 45 days. Results are presented as mean ± SE (n = 6–7). For total Ni (a), bars with different letters are significantly different (p < 0.05) for the same tissue. For new Ni (b), * represents a significant difference between non-pre-exposed and Ni pre-exposed fish for the same tissue (p < 0.05).

3a). Greater concentrations of new Ni were found only in gut tissues, of which stomach and cecae showed significant differences between the two groups (Figure 3b). The analysis of new Ni distribution in fish tissues at 24 h indicates that only a small fraction of the radiolabeled Ni dose (1.08 μmol kg^{-1} fish) was in the internal organs (excluding gut) of both non-pre-exposed (3.7 ± 0.4%) and Ni pre-exposed fish (1.6 ± 0.4%). Approximately 2.3 ± 0.2% and 5.9 ± 0.7% of the dose were found in gut tissues, respectively, values which were significantly different. Thus, the rest of the dose (~92–94%, calculated but not measured) was still in the lumen and/or eliminated via the urine and feces from the fish by 24 h. Consistent with our results, in mammals, a majority of ingested Ni is eliminated in feces, with only 1–10% of Ni being absorbed through the intestine and subsequently eliminated in urine (*3*). Very low internalization of radiolabeled Ni explains why new Ni concentrations in internal organs (<0.05 μmol kg^{-1}, Figure 3b) and carcass (<0.04 μmol kg^{-1}, data not shown) were also very low, and consequently did not reflect in the tissue total Ni concentrations (Figure 3a). Greater concentrations of new Ni in gut tissues is a reflection of Ni exposure route, whereas the greater proportion in the gut of the Ni pre-exposed trout (2.3% versus 5.9%) suggests that waterborne Ni exposure increased Ni binding capacity in the gut. Possibly, induction of the synthesis of the metal binding protein metallothionein (*37*) may have occurred in response to the chronic waterborne Ni pre-exposure, as observed in lake whitefish fed a high Ni diet (*4*).

Our results reveal that pre-exposure to waterborne Ni downregulates the gastrointestinal uptake, suggesting that branchial (waterborne) and gastrointestinal (dietary) pathways of Ni uptake are interrelated, as observed for other essential metals and ions. RBC Ni concentrations are clearly regulated in the face of large variations in plasma Ni concentrations. It appears that the gut plays a key role in Ni homeostasis in rainbow trout and a rapid process is involved in Ni transport across gut epithelial cells. Previous findings on renal Ni clearance and transport mechanisms (*6, 14, 15*), together with present data on tissue Ni burdens suggest that the kidney and scales are important organs of Ni regulation or detoxification in fish by serving as clearance or storage sites. Overall, our results suggest that Ni is subject to homeostatic regulation in the rainbow trout, a property that is characteristic of essential metals (*12*).

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