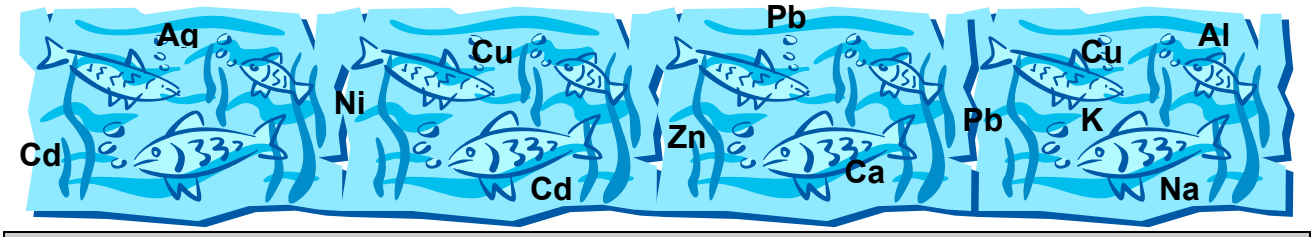
NSERC – Industry Project on Metal Bioavailability
Research Newsletter



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News

Welcome Aboard

Joel Klinck joined the Wood lab in January of 2006 as a M.Sc. student. Joel graduated from Wilfrid Laurier University with a B.Sc. Hons. At Wilfrid Laurier Joel examined the protective effect of DOC on Hg toxicity at the fish gills supervised by Dr. Rick Playle. Continuing on this legacy, at McMaster, Joel will study the transport of food borne metals with the long term goal of incorporating these effects into the BLM and extend the BLM to multiple metal mixtures. Mina Girgis NSERC summer student assisted PDF Jasim Chowdhury on his studies of extending the BLM to predict chronic Cu toxicity in the rainbow trout. Mina will continue during the term as a 4th year Hons. Student .His thesis will study the effect of Cu on the biochemical properties and activity of two important transporting proteins the Na-K ATPase and the Na-H^{+} ATPase. Our former 4th year Honours student Erin Leonard has now started an M.SC. at McMaster, studying the mechanisms of Ca^{2+} and Cd^{2+} transport in chironomids under the primary supervision of Dr. Mike O'Donnell.

Dr. Jasim Chowdhury is back at the helm as senior postdoc on the NSERC-CRD grant. He returns after a completing a term with the U.S. EPA evaluating endocrine disruption in *Xenopus laevis*. Currently, Jasim is primarily involved in developing the BLM to predict chronic Cu toxicity in rainbow trout. He takes over from Dr. Natasha Franklin who returned to her home in Sydney Australia and is now PDF at CSIRO working on Nanovector Technology. During her tenure at McMaster Natasha was instrumental in developing the acute BLM for Cu toxicity in a tropical organism the zebrafish to meet one of the primary objectives of the NSERC-CRD grant of extending the BLM to a wider range of target organisms. This summer another of our PDF's Dr. Patty Gillis was successful in obtaining funds from CDA to evaluate Cu toxicity in glochidia larvae of freshwater mussels. Patty is currently working on this project independently at the University of Guelph. At McMaster Patty was closely involved in setting up the invertebrate related BLM studies as part of the NSERC-CRD grant. Dr. Tania Ng, has joined the Metals Bioavailability Group as PDF in August 06 as Patty's replacement. Tania brings with her a variety of expertise on cellular fractionation and mathematical modelling related to metal bioavailability in invertebrates which she has started to employ on trophic transfer of Cd from blackworms to trout. Dr. Chris Glover a former PDF under CMW but currently at the National Institute of Nutrition and Seafood Research, Bergen, Norway, was on a sabbatical to McMaster in the summer and studied *in vitro* Cu bioavailability across gut epithelia in trout using brush-border membrane vesicles technique. Chris starts a job as an aquatic research manager at Landcare New Zealand in the fall.

Travel

At the Bamfield Marine Sciences Centre on Vancouver Island, B.C. this summer, a research team comprising Chris Wood, Carol Bucking, John Fitzpatrick and Sunita Nadella

continued the studies initiated last year on *Mytilus* with the aim of extending the BLM to the marine environment. Range finding 48 h toxicity tests were performed for Cu, Zn, Cd, Ni and Pb. Early life stages of these bivalves appeared to be extremely sensitive to Cu and Pb and moderately to Zn. The effect of metal toxicity was also evaluated on sperm and eggs separately. The former was much more sensitive than the latter, though neither was as sensitive as the early embryo.

Conference presentations

- *The following papers were presented by the Metals Bioavailability Group at the Annual Fish Physiology and Biochemistry Workshop, Elmhirst's Resort, Keene, Ontario, February 3-5, 2006*

- **Kam J., Franklin N., Wood C.M. (2006).** Diet selection in rainbow trout under chronic dietary metal exposure. 15th Annual Fish Physiology and Biochemistry Workshop, Elmhirst's Resort, Keene, Ontario, February 3-5, 2006

- **Leonard E., Bucking C., Wood C.M. (2006).** Characterization of Ni uptake in rainbow trout. 15th Annual Fish Physiology and Biochemistry Workshop, Elmhirst's Resort, Keene, Ontario, February 3-5, 2006

- **Bechard K., Gillis P., Wood C.M. (2006**). Using chironomids to study trophic transfer. 15th Annual Fish Physiology and Biochemistry Workshop, Elmhirst's Resort, Keene, Ontario, February 3-5, 2006.

The following paper was presented by the Metals Bioavailability Group at the annual meeting of the Canadian Society of Zoologists, University of Alberta, Edmonton, AB. 2nd -7th May 2006.

- **Wood C.M. (2006).** Rick Playle – An Appreciation. 45th annual meeting of the Canadian Society of Zoologists, University of Alberta, Edmonton, AB. 2nd -7th May 2006. (Invited Platform presentation).

The following papers were presented by the Metals Bioavailability Group at the VIIth International Congress on the Biology of Fish, 18-22 July, St. John's, Newfoundland

- **Craig, P.M., Wood, C.M., McClelland, G.B. (2006).** Softwater acclimation and ion regulation as a precursor to acute and chronic metal exposure in zebrafish (*Brachydanio rerio*). VIIth International Congress on the Biology of Fish, 18-22 July, St. John's, Newfoundland

- **De Boeck, G., Hattink, J., Franklin, N., Bucking, C., Wood, S., Walsh, P., Wood, C.M. (2006).** Disruption of ionoregulation in spiny dogfish under metal exposure. VIIth International Congress on the Biology of Fish, 18-22 July, St. John's, Newfoundland

- **Wood, C.M. (2006).** How hard is that diet? Implications for metal accumulation and toxicity. VIIth International Congress on the Biology of Fish, 18-22 July, St. John's Newfoundland.

The following paper will be presented by the Metals Bioavailability Group at the Annual Meeting of the American Physiological Society in Virginia Beach, Virginia, Oct. 8-11th 2006.

- **Craig, P.M., Wood, C.M., McClelland, G.B. (2006).** In vivo effects of copper on oxidative stress pathways in the tropical zebrafish. Annual Meeting of Amer. Physiol. Soc. Oct. 2006

The following papers will be presented by the Metals Bioavailability Group at the 26th Annual Meeting of the Society of Environmental Toxicology and Chemistry (SETAC) in Montreal Nov.2006

- **Craig, P.M., Wood, C.M., McClelland, G.B. (2006).**Toxicogenomics and the BLM: Zebrafish as a tropical model for chronic copper exposure. Society for Environmental Toxicology and Chemistry Annual Meeting, Montreal, Nov 2006.

- **Chowdhury, M.J., Girgis, M.N., Wood, C.M. (2006).** Towards a chronic BLM for copper toxicity to rainbow trout: defining chronic endpoints and binding constants. Society for Environmental Toxicology and Chemistry Annual Meeting, Montreal, Nov 2006.

- **Klinck, J.S., Nadella, S.R., Ojo, A., Wood, C.M. (2006).**Dietary copper and cadmium uptake in rainbow trout (*Oncorhyncus mykiss*): the role of calcium mediated transport. Society for Environmental Toxicology and Chemistry Annual Meeting, Montreal, Nov 2006.

- **Gillis, P., Wood, C.M.(2006)** The effect of waterborne cadmium exposure on sodium and calcium regulation in *Chironomus riparius* larvae. Society for Environmental Toxicology and Chemistry Annual Meeting, Montreal, Nov 2006.

- **Wood, C.M., Niyogi, S., Kamunde, C., Baldisserotto, B., Franklin, N., Chowdhury, M.J., Pyle, G. (2006).**The influence of dietary chemistry on responses of fish to metals: towards a dietary BLM. Society for Environmental Toxicology and Chemistry Annual Meeting, Montreal, Nov 2006.

- **Kozlova, T., J. McGeer, CM Wood. (2006)** Development of an acute Biotic Ligand Model for Ni toxicity to *Daphnia pulex* in soft water: effects of Ca, Mg, Na, K, Cl, pH and dissolved organic matter. Society for Environmental Toxicology and Chemistry Annual Meeting, Montreal, Nov 2006.
- **Green, W., Mirza, R., Wood, C.M. and Pyle, G. (2006)** Binding characteristics of Copper on the olfactory epithelium of the fathead minnow (*Pimephales promelas*): A Biotic Ligand Model approach. Society for Environmental Toxicology and Chemistry Annual Meeting, Montreal, Nov 2006.

Research Highlights

This issue will highlight research conducted by Sunita Nadella who recently completed her M.Sc. at McMaster under the supervision of Chris Wood. An extended version of this paper has been recently submitted for publication.

Mechanisms of dietary Cu uptake in freshwater rainbow trout: evidence for Na-assisted Cu transport and a specific metal carrier in the intestine.

Nadella , S.R., Grosell, M. and C.M. Wood

Introduction

Recent studies investigating interactions between branchial and gastrointestinal uptake of Cu (Kamunde *et al.* 2001, 2002 a, b) provide evidence that under normal levels of Cu in the water and food, the rainbow trout sources over 80% of its Cu from food. However, while the mechanisms of waterborne Cu uptake have been the focus of most current research, mechanisms of dietary Cu uptake in fish have received little attention.

Two possible candidate proteins responsible for the absorption of dietary Cu in mammals have emerged: DMT1 was shown to be capable of transporting a range of metals including Fe, Zn, Cu, and Mn (Gunshin *et al.* 1997). Ctr1 has also been shown to have Cu transport activity in transfected cell lines, displaying a substrate preference for Cu^{1+} (Lee *et al.* 2002 a). However, mammalian Ctr1 mRNA expression is not regulated following dietary Cu restriction (Lee *et al.* 2000). Alternatively Sharp (2003) has reported that high Cu levels could modify the expression of DMT1 in Caco-2 cells and suggest that DMT1 and not Ctr1 acts as the major intestinal Cu transporter. Arredondo *et al.* (2003) have recently demonstrated an association between Cu and Fe transport and suggest that, DMT1 preferentially transports monovalent Cu^{1+} relative to Cu^{2+} in cultured Caco -2 cells.

In addition there is older evidence for a Na^+-related pathway of Cu absorption in mammals. Wapnir and Stiel (1987) demonstrated that Na removal inhibited Cu absorption in the perfused rat intestine. Further investigation revealed an inhibition of Cu uptake in the presence of amiloride, an inhibitor of Na^+ channels and some Na^+-linked transporters (Wapnir 1991), providing evidence for Cu entry via a Na^+-linked mechanism.

In the African walking catfish, basolateral transport appears to involve a Cu ATPase and/or a Cu/anion symport (Handy et al. 2000, 2002). Removal of Na^+ from the mucosal solution tended to slow rather than accelerate Cu transport, suggesting that Na^+ channels are not involved, but pointing to the potential presence of a Na^+-linked mechanism similar to that postulated by Wapnir (1991) in the rat. Very recently, Burke and Handy (2005) have developed an isolated trout enterocyte preparation in which uptake is thought to be dominated by apical transport rates. In these dispersed cells, there was no evidence that lowering the bathing Na^+ concentration altered the rate of Cu accumulation at low Cu concentrations, but at very high Cu levels in the bathing solution (800 μM), Na^+ removal did accelerate Cu uptake.

Arising from this somewhat confusing background, our objective was to characterize Cu uptake in the isolated but intact intestine of the rainbow trout. A preceding study (Nadella *et al.* 2006 b) has established the *in vitro* trout gut sac made from either the mid- or posterior intestine as a robust preparation which exhibits saturable, apparently carrier-mediated Cu-uptake over the normal concentration range of Cu in the chyme, and which maintains stable transport rates for up to 4 h. The anterior intestine is less suitable for this approach because it contains the

delicate pyloric caecae. In particular, we focused on the mid and posterior intestinal segments and employed a mucosal concentration (50 µM) typical of that measured in the chyme *in* vivo (Nadella *et al.* 2006 a). Possible Na^+-sensitive Cu uptake was assessed by manipulation of Na levels in the gut lumen and use of phenamil and Ag that are known inhibitors of Na^+ transport. P_{CO2} elevation at constant pH was used to investigate the possible contribution of proton supply. The potential of DMT1 and Ctr1 to mediate Cu transport was investigated through examination of pH sensitivity, known to stimulate both transport proteins and the possibility of inhibition of Cu uptake in the presence of 10-fold excess of other divalent metals. Transepithelial potential (TEP) was also measured in these experiments to determine whether any of the observed effects on Cu transport were mediated indirectly by changes in the voltage gradient.

Results

Results were qualitatively similar in mid and posterior intestinal segments, different only in minor quantitative detail.

Mucosal NaCl concentrations manipulated over the range 3-280 mM revealed a clear stimulatory effect on Cu transport (Fig. 1A). Serosal transepithelial potential (TEP) measured against a mucosal reference set at 0 mV was -6 mV at 3 mM Na^+ in the mid intestine (Fig. 1 B). Increasing Na^+ concentration resulted in a progressively less negative TEP reaching +1.2 mV at 280 mM Na^+.

Cu transport occurred simultaneously with considerable fluid transport. Net water flux was elevated 2-5 fold with increasing mucosal Na^+ concentration in both mid and posterior intestine. However, reversing fluid transport by manipulation of the osmotic gradient had no effect on Cu transport rate in either segment indicating that solvent drag was not involved. Similarly increasing osmolality with mannitol had no effect. Replacing all Cl^- with SO_4^{2-} confirmed the Na^+-dependent pattern of Cu transport seen with NaCl manipulation i.e. the effect was independent of the anion suggesting Cu transport to be sensitive to Na^+ and insensitive to Cl^-.

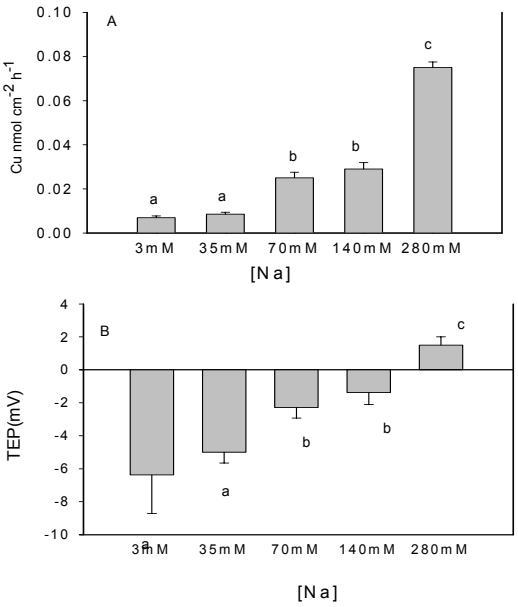

Fig. 1 Effect of Na on A. Cu transport and B. Transepithelial Potential in the mid intestine

Mid and posterior intestinal segments exposed to 100 µM phenamil exhibited an approximate 35% and 20% decrease in Cu and Na transport respectively (Fig 2) compared to drug-free, DMSO solvent controls.

Inducing hypercapnia (at constant pH = 7.4) caused a general trend of elevated Cu transport (3-fold increase).Na^+ transport was also elevated in both segments by about 50%, which was significant at 1% CO_2, with no further rise at 3% CO_2

Cu uptake declined by approximately 50% in the mid intestine when the pH of mucosal saline was raised to 8.0.

The presence of a 10-fold excess of Zn (500 µM $ZnSO_4$) in the mucosal saline significantly decreased transepithelial transport of Cu upto 75%. Similarly, Cu uptake rate significantly declined by 80% in the mid intestine when exposed to 500 µM Fe (as $Fe(NO_3)_3$) while Na^+ transport was not affected. (Fig.3). Curiously, 500 µM Ag^+ (as $AgNO_3$) stimulated both Cu and Na^+ uptake.

Discussion

Based on observations of a greater transport of Cu in the presence of Na^+, our data provide evidence for an apparent Na^+-assisted pathway of intestinal Cu uptake in rainbow trout. Alternately, sensitivity of Cu uptake to pH and competition with other divalent cations suggests a specific metal transporter with features similar to a recently characterized Cu transporter from Ctr1-deficient embryonic cells in mammals (Lee *et al.* 2002). Consequently we discuss if these two mechanisms are linked, or whether separate mechanisms of Cu transport exist in the trout gut, one which is Na^+-assisted and the other a metal-specific pathway independent of the Na^+-gradient.

Na^+-dependent Cu uptake

A notable finding of this study was the observation of a Na^+ concentration-dependent increase in Cu transport (Fig. 1 A) which provides compelling evidence that Na^+ may be involved in the uptake of Cu in trout intestine. Na^+-sensitive Cu transport has been earlier described in rainbow trout gills (Grosell and Wood 2002). However the mechanism at the gills where branchial Cu uptake was reduced with increasing ambient Na^+ concentration is diametrically different from our observation of stimulated Cu uptake across the intestinal epithelium.

Under normal physiological Na^+ levels and symmetrical conditions, the measured TEP was about -1.5 mV, which would slightly assist Cu uptake. Serosal transepithelial potential (TEP) was progressively less negative with increasing Na^+ levels (Fig. 1 B), suggesting that stimulation of Cu uptake was not related to changes in TEP as the trend towards a serosa-positive electrical gradient with increasing luminal Na^+ would have provided progressively less assistance for the transport of positively charged Cu ions.

Increasing the osmolality of the luminal fluid to mimic the highest Na^+ concentration had no significant effect on Cu transport thereby eliminating osmotic pressure as an explanation. Furthermore, the contribution of solvent drag and the nature of the accompanying anion was clearly not a contributing factor. Thus the interaction of Na^+ with Cu transport appears to be direct rather than indirect. In accord with the present results, Na^+-dependent stimulation of Cu uptake has also been demonstrated in the jejunum and ileum of rat (Wapnir and Stiel, 1987), though its exact mechanism remains unclear. In trout the intestinal Na^+-Cu interaction appears to be important *in vivo* as well as *in vitro*. Very recently Kjoss *et al.* (2005) found that juvenile rainbow trout that received elevated dietary Na^+ along with high dietary Cu showed the greatest Cu retention, a result which was considered to reflect a positive interaction between Cu and Na^+ transport in the gastrointestinal tract. In accord with the above observations, Na^+ removal from the lumen of the intestine in African Walking catfish tended to slow Cu transport (Handy et al. 2000, 2002). In contrast increased Cu accumulation in isolated intestinal cells from rainbow trout was observed when Na^+ was lowered from 140 mM to 11 mM (Burke and Handy, 2005). However, this response was observed only at a high Cu concentration of 800 μM where there may be a different route of Cu transport. At lower concentrations, down to the 50μM Cu used in the present study, Burke and Handy (2005) observed no interaction of Na^+ on Cu transport.

The partial inhibition of both Cu and Na^+ uptake with 100 μM phenamil, an amiloride analogue (Fig. 2) implicates an apical Na^+ channel or Na^+/H^+ exchanger in the process.

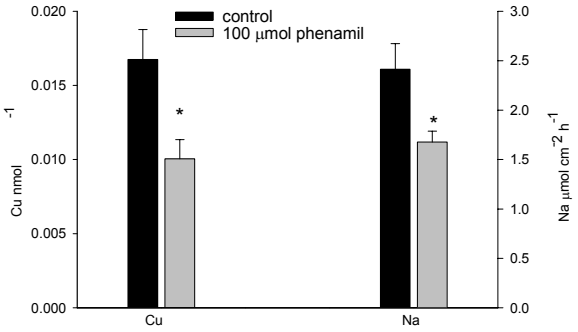

Fig. 2 Effect of phenamil on Cu and Na transport in the mid intestine.

Similar evidence linking the transport of Cu to a Na^+-coupled mechanism is available from the rat

intestine on the basis of a considerable decrease in Cu absorption with 1 mM amiloride (Wapnir, 1991).

The results from the present study with trout intestinal sacs correspond with a reported inhibition of Cu uptake in rainbow trout gills exposed to 100 μM phenamil (Grosell and Wood, 2002). However, Grosell and Wood (2002) reported a discrepancy between the inhibitory effect of phenamil on Na^+ and Cu uptake, phenamil inhibiting Na^+ uptake more effectively than Cu uptake. This is not the case in the trout intestine where phenamil inhibited Cu uptake to an equal or greater extent. When taken together with our finding of stimulated Cu uptake with increasing Na^+ levels in the trout intestine (in contrast to inhibition of Cu uptake by increasing Na^+ levels at the trout gills), this clearly indicates that Na^+-dependent Cu uptake in the trout intestine occurs via a mechanism different from that found in the gills.

Additional evidence linking Na^+ and Cu transport in the trout intestine was the significant increases in both Na^+ and Cu transport observed under hypercapnia, a treatment intended to induce intracellular acidosis. Previous studies in isolated frog skin (Harvey, 1992) indicate that H^+ excretion is dependent on availability of CO_2 and the transport of Na^+. The former provides the source of protons from the catalyzed hydration of CO_2 and the latter provides the electrically balancing positive charge via entry through apical Na^+ channels which are electrically coupled to the proton pumps, or via a Na^+/H^+ antiport (NHE). Substantial evidence exists for the presence of amiloride-sensitive NHE isoforms associated with a Na^+/H^+ antiport on both the apical and basolateral membranes of the mammalian gastrointestinal tract (Hoogerwerf *et al.* 1996). It is possible that such a mechanism exists in the trout gut as the normally high external $[Na^+]$ in the gut lumen, arising from a largely carnivorous diet, would thermodynamically favor Na^+/H^+ exchange and explain the increased uptake of Na^+ under induced hypercapnia. By this scenario, high P_{CO2} would drive Na^+ uptake by the NHE (or H^+ ATPase/Na^+ channel) and at the same time acidify the boundary layer on the mucosal surface. It remains to be determined whether Cu is transported at a higher rate along with Na^+ by a common mechanism, or because the elevated H^+ extrusion creates a suitable microenvironment facilitating Cu transport.

Na-independent Cu uptake

Two of the possible candidate proteins responsible for the absorption of dietary Cu in mammals are recognized to be pH-sensitive. Ctr1 is stimulated by low extracellular pH (Lee *et al.* 2000) and the transport of metal ions by all the members of the NRAMP family, which includes DMT1, is driven by protons (Gunshin *et al.* 1997). No effect on Cu transport was seen when pH was lowered to 6.0, but a marked decrease in Cu uptake was observed when pH was raised to 8.0. These effects were specific to Cu and not Na^+ transport suggesting the presence of a non Na^+-dependent pathway for Cu uptake that demonstrates characteristics of DMT1 or Ctr1 mediated transport. However, the pH effect upon intestinal Cu uptake in rainbow trout could also be explained by altered Cu speciation, related to the decline in % total concentration of Cu^{2+} as it is replaced mainly by $CuCO_3$ at high pH. This result might suggest that Cu transport is dependent on the presence of Cu^{2+} as the primary substrate and thereby allude to a role for DMT1 in Cu transport according to the traditional view that DMT1 preferentially transports divalent metals (Gunshin *et al.* 1997). However, the experiments of Gunshin *et al.* (1997) were performed in the presence of 100 μM ascorbic acid, a reducing agent, so it is possible that DMT1 transports Cu^{1+}. Indeed Arredondo *et al.* (2003) recently provided evidence that DMT1 may preferentially transport Cu^{1+} over divalent Cu^{2+} in cultured human intestinal Caco-2 cells. Further complicating interpretation is the fact that treatment with the reducing agent ascorbate (100 μM-2.5 mM) had no effect on Cu uptake on our trout gut system (Nadella *et al.* 2006 b) indicating either that the valence of Cu present is not critical or that sufficient quantities of endogenous reductase are present on the intestinal epithelium of trout. Given that DMT1 accepts Cu^{1+} as substrate, an endogenous reductase could also facilitate Cu uptake via this H^+-metal symport.

At the gene level, DMT1 has been identified in fish (Donovan *et al.* 2002) and, it has been implicated in intestinal Fe uptake in flounder (Bury *et al.* 2001) and the branchial uptake of Fe in the zebrafish (Bury and Grosell, 2003)

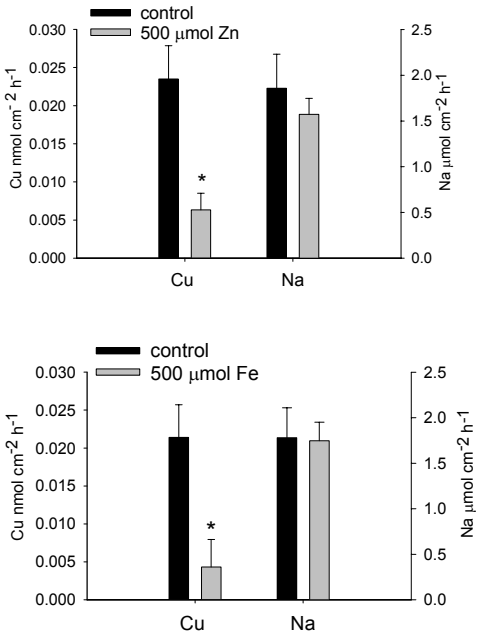

Fig. 3 Effect of Zn and Fe on Cu and Na transport in the mid intestine

The present study revealed significant inhibition of Cu uptake in the presence of 10-fold greater Fe (500 μM $Fe(NO_3)_3$) or Zn (500 μM $ZnSO_4$) in the intestine of the rainbow trout (Fig.3). Notably Glover and Hogstrand (2003) also reported significant inhibition of intestinal Zn absorption by equimolar Cu levels in rainbow trout *in vivo*. In the present study Na^+ uptake rate remained unchanged in the presence of either metal cation, indicating a specific effect on Cu transport. DMT1 accounts for atleast 50% of Cu transport in human intestinal Caco-2 cells (Arredondo *et al.* 2003). Very recently Knöpfel *et al.* (2005) observed that DMT1 was involved in Cu uptake in the rat small intestine BBM vesicles.

In mammalian systems, there is also extensive evidence of inhibition of Cu absorption when dietary or intra-luminal Zn: Cu ratios are elevated. (Oestreicher and Cousins, 1984).Taken together with these mammalian observations, the present evidence of Cu-Fe and Cu-Zn interaction in the trout intestine and the identification of DMT1 in the zebrafish argue that Cu uptake in the trout intestine occurs at least in part by DMT1.

A Proposed Model for Intestinal Copper Transport in the Rainbow Trout

Overall our data clearly implicate a Na^+-dependent mechanism in mediating Cu transport across the trout intestine, but there is a dichotomy in the results. On the one hand, phenamil partially inhibits both Na^+ and Cu transport and hypercapnia stimulates both Na and Cu transport, results which are best explained by both Cu and Na^+ entering through an apical Na^+-channel as in the trout gill (Grosell and Wood, 2002). On the other hand, elevated luminal Na^+ stimulates rather than competitively inhibits Cu transport and the presence of Ag^+ stimulates both Na^+ and Cu transport, results which are best explained by a Na^+-Cu co-transport system. However we propose that these apparently dichotomous trends can be explained by a common mechanism. Based on evidence that treatments which accelerate Na^+ transport also accelerate Cu uptake, while treatments which depress Na^+ transport inhibit Cu uptake, we propose a novel Na^+-assisted mechanism of Cu uptake. By this scenario, the Na^+ gradient stimulates Na^+/H^+ exchange via an NHE type transporter or a Na^+ channel/H^+ATPase system at the brush border. The resulting increase in H^+ concentration in the brush border microenvironment would thereby create a suitable H^+ gradient for the effective transport of Cu via either DMT1 and/or Ctr1. Increased H^+ extrusion associated with high P_{CO2} would also have this effect. This proposed mechanism serves to link the observed stimulation of Cu transport with increasing Na^+ transport to the inhibition of Cu transport in the presence of phenamil. Since neither DMT1 nor Ctr1 are ATP-dependent, this step would be responsible for the low Q_{10} values associated with apical uptake (Nadella *et al.* 2006 b), while the higher Q_{10} values for overall transport would be explained by Cu extrusion via a Menkes type Cu ATPase at the basolateral membrane. Based on pH sensitivity and inhibition of Cu uptake with

Fe and Zn, DMT1 appears as the most likely candidate involved in apical Cu transport in the trout intestine, although a role for Ctr1 cannot be excluded. Interestingly another Cu transporter has been identified in Ctr1 deficient mouse embryonic cells (Lee *et al.* 2002 b), that transports Cu in the absence of Ctr1 with Km ~ 10 µM, in a saturable, time-, temperature- and pH- dependent manner. The transport is competed by Zn, but function is not affected by Ag^{+} or ascorbate. It is possible that such a transporter exists in the trout intestine as the present study, together with that of Nadella et al (2006 b) have reported almost identical Cu transport characteristics.

Note: This research was supported by funding from the Human Health Program of ICA to Chris Wood and Martin Grosell.

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