# Amino acid modulation of *in vivo* intestinal zinc absorption in freshwater rainbow trout

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## Summary

The composition of the intestinal lumen is likely to have considerable influence upon the absorption, and consequently the nutrition and/or toxicity, of ingested zinc in aquatic environments, where zinc is both a nutrient and a toxicant of importance. The effects of amino acids upon intestinal zinc uptake in freshwater rainbow trout (*Oncorhynchus mykiss*) were studied using an *in vivo* perfusion technique. The presence of histidine, cysteine and taurine had distinct modifying actions upon quantitative and qualitative zinc absorption, compared to perfusion of zinc alone. Alterations in zinc transport were not correlated with changes in levels of free zinc ion. The chemical nature of the zinc–amino acid chelate, rather than the chelation itself, appeared to have the most important influence upon zinc absorption. L-histidine, despite a strong zinc-chelating effect, maintained quantitative zinc uptake at control (zinc alone) levels. This effect correlated with the formation of $Zn(His)_2$ species. D-histidine at a luminal concentration of $100\,mmol\,l^{-1}$ significantly enhanced subepithelial zinc accumulation, but reduced the fraction of zinc that was retained and absorbed by the fish. The possibility of a $Zn(His)_2$-mediated pathway for intestinal uptake is discussed. L-cysteine specifically stimulated the accumulation of zinc post-intestinally, an effect attributed to enhanced zinc accumulation in the blood. Taurine increased subepithelial zinc accumulation, but decreased the passage of zinc to post-intestinal compartments. Amino acids are proposed to have important roles in modifying intestinal zinc uptake with potential implications for environmental toxicity as well as aquaculture.

Key words: zinc, uptake, intestine, dietary metal, zinc-binding ligand, transport, histidine, cysteine, taurine, amino acid, fish, rainbow trout, *Oncorhynchus mykiss*.

## Introduction

In aquatic ecosystems zinc is both an essential micronutrient and a toxicant of considerable significance. Biological roles of zinc are mediated by its structural and/or functional importance in more than 300 enzymes and other proteins (Vallee and Falchuk, 1993). Consequently, zinc deficiency in fish leads to physiological perturbation of growth, reproduction, vision and immunity (Watanabe et al., 1997). Conversely, excessive environmental zinc can have severe impacts upon the survival of aquatic organisms (Eisler, 1993). Two major routes for zinc uptake exist in fish (Hogstrand and Wood, 1996). Waterborne zinc is absorbed mainly *via* the gill, while the gut assimilates particulate zinc from diet and imbibed water. Given the speciation of zinc in natural waters (Rozan et al., 2000) and reliance upon food sources that may bioaccumulate the metal (Dallinger et al., 1987), the gut is likely to be the major source for zinc assimilation. The diet, however, is a complex chemical mixture and its composition will have an important influence upon zinc uptake and absorption, and consequently zinc nutrition and toxicity.

The accompanying paper (Glover and Hogstrand, 2002) characterises the *in vivo* uptake of zinc in freshwater rainbow trout using an intestinal perfusion system. This method allowed kinetic characterisation of zinc uptake and highlighted important differences between intestinal uptake in freshwater fish and studies in mammals and marine teleosts. This technique has been used in this study to investigate the roles of amino acids in modifying the uptake and absorption of zinc. In fish the introduction of an amino acid–zinc chelate to the diet has been reported to increase zinc concentrations in the body of rainbow trout, compared to inorganic zinc treatments (Hardy et al., 1987; Paripatananont and Lovell, 1995). This has potential benefits for the aquaculture industry where increased Zn(II) absorption is associated with improved fish growth and health (Watanabe et al., 1997).

Facilitatory effects of amino acids on zinc absorption have been documented in a number of mammalian and invertebrate tissues (e.g. Wapnir et al., 1983; Ackland and McArdle, 1990; Bobilya et al., 1993; Buxani-Rice et al., 1994; Horn et al., 1995; Vercauteren and Blust, 1996). Histidine and cysteine have high binding affinities for zinc, with dissociation

constant ($K_d$) values of 12.1 and 18.2, respectively, for Zn(II)-(amino acid)$_2$ species (Martell and Smith, 1974). Amino acids may increase bioavailability by removing chelated zinc from dietary zinc-binding constituents such as phytic acid. Evidence of such an action has been presented in studies of zinc absorption in fish where the beneficial effects of adding zinc as an amino acid chelate were greater in diets that contained high levels of phytic acid (Paripatananont and Lovell, 1995). This increase in bioavailability may be achieved in one of two ways. Amino acids may act to shuttle zinc from dietary components with low zinc binding affinity to uptake surfaces with higher affinity (Wapnir et al., 1985; Ackland and McArdle, 1990; Bobilya et al., 1993). Alternatively, the formation of an amino acid–zinc chelate may create a substrate for transport across the epithelial surface (Wapnir et al., 1983; Ashmead et al., 1985).

Experimental evidence regarding the exact mechanism of the enhancement effect of amino acids upon zinc uptake is scant. Physiological investigations have yet to yield a candidate transporter. Although the basic amino acid carrier $y^+$ was a candidate for mediating the uptake process in human erythrocytes, subsequent experiments suggest this is not the case (Horn et al., 1995). In lobster hepatopancreas epithelia, it was proposed that zinc may combine with L-proline to form a more readily transportable complex, in addition to acting at an allosteric binding site to stimulate the absorption of L-proline (Monteilh-Zoller et al., 1999).

The role of amino acids in altering the uptake, fate and metabolism of intestinally introduced zinc was the focus of the present study. Histidine and cysteine were chosen as amino acids that are known to have biological relevance as zinc chelators. Concentrations were tested that altered free zinc ion activity and the nature of amino acid–zinc chelates. Taurine was included as an amino acid of biological importance, but without known chelation effects. The results demonstrated the complex nature of *in vivo* intestinal zinc uptake, including an L-histidine-mediated uptake pathway, and specific actions of D-histidine and L-cysteine upon qualitative zinc absorption. The results suggested that dietary constituents could play a vital role in modulating intestinal zinc uptake, with potential implications for aquaculture and environmental toxicity.

## Materials and methods

### Animals

Rainbow trout (*Oncorhynchus mykiss* Walbaum; 80–366 g, mean ± S.D. 149±6 g, N=103) were obtained from Wolf Creek Dam National Fish Hatchery, Kentucky, USA and transported to holding facilities at the University of Kentucky. Fish were maintained in 400 l fibreglass tanks with flowing, aerated and dechlorinated Lexington city tapwater. The water temperature was maintained in the range of 11–15 °C, varying with season. Food was withheld from fish for at least 5 days before experimentation.

### Experimental procedure

The *in vivo* intestinal cannulation procedure was identical to that described by Glover and Hogstrand (2002). Experimental perfusion solutions consisted of $^{65}$Zn(II) (as ZnCl$_2$: approx. 4 kBq ml$^{-1}$, New England Nuclear) in a 77 mmol l$^{-1}$ NaCl saline, with Zn(II) added as ZnSO$_4$·7H$_2$O to a final concentration of 50 μmol l$^{-1}$. Amino acids (L- or D-histidine, L- or D-cysteine, taurine) were included individually in perfusates with Zn(II) at the concentrations stated (2 or 100 mmol l$^{-1}$). These two concentrations resulted in dramatically different Zn(II)–amino acid chelate speciation (Table 1), and allowed the testing of hypotheses regarding potentially important uptake moieties. Solutions were made fresh from stock on the day of experiment. The pH of perfused solutions was neither adjusted, nor buffered and ranged from 6.0–6.4. Experimental perfusions were of 3 h duration.

Upon completion of experimental perfusion, tissue samples (intestinal epithelium and subepithelium, blood, carcass) were collected, treated and analysed for $^{65}$Zn(II) activity in the manner described previously (Glover and Hogstrand, 2002). Throughout the text reference is made to a number of tissue compartments. These are defined as follows. Tissue scraped from the mucosal surface of the intestine is referred to as the 'epithelial' compartment. This will include both mucus, and mucosal intestinal cells. 'Subepithelium' denotes that intestinal tissue remaining following scraping. The carcass remaining following intestinal excision is the 'body' compartment. 'Post-intestinal' parameters are those calculated using pooled blood and body data. 'Retained Zn(II) fraction' is the proportion of perfused Zn(II) that is sequestered by the animal, and includes

Table 1. *Zn(II) speciation in the experimental treatments investigated*

|  | Zn$^{2+}$ | ZnCl$^+$ | Zn(Cys) | Zn(Cys)$_2$$^{2-}$ | Zn(His)$^+$ | Zn(His)$_2$ |
|---|---|---|---|---|---|---|
| Control [Zn(II) alone] | 87 | 11 | – | – | – | – |
| Zn(II)+2 mmol l$^{-1}$ Cysteine | 6 | 0 | 86 | 8 | – | – |
| Zn(II)+100 mmol l$^{-1}$ Cysteine | 0 | 0 | 17 | 83 | – | – |
| Zn(II)+2 mmol l$^{-1}$ Histidine | 4 | 0 | – | – | 65 | 31 |
| Zn(II)+100 mmol l$^{-1}$ Histidine | 0 | 0 | – | – | 4 | 96 |

Values are the percentage of Zn(II) present as a given species, and were calculated *via* the geochemical equilibrium modelling system MINEQL+ (Version 4.01; Environmental Research Software).

Zn(II) was added as ZnSO$_4$·7H$_2$O (ACS Grade, Sigma Chemical Co., St Louis, MO, USA), and speciation calculated at experimental temperatures of approximately 15 °C.

that Zn(II) which is trapped by the 'epithelium'. Because this compartment may be regularly sloughed, Zn(II) accumulated here may not be available for uptake. Hence the 'absorbed Zn(II) fraction' is the proportion of perfused Zn(II) that accumulates in post-epithelial compartments, and can threfore be considered Zn(II) which is truly absorbed.

For all treatments a $^{65}$Zn(II) budget was constructed. For some experiments it was noted that not all $^{65}$Zn(II) could be accounted for. This was likely a consequence of Zn(II) binding to experimental apparatus. To correct for this, parameters were adjusted based on the percentage of recovered radioactivity (Hardy et al., 1987). For calculation of epithelial, subepithelial and post-intestinal accumulation rates, data were standardised to account for differences in the retained Zn(II) fraction that otherwise may have distorted patterns of accumulation. Retained and absorbed Zn(II) fractions are quantitative measures of the amount of Zn(II) entering the animal. Standardised accumulation parameters therefore represent the qualitative change in Zn(II) distribution caused by the amino acid treatments. All calculations were based on equations described in the accompanying paper (Glover and Hogstrand, 2002).

Data are expressed as means ± S.E.M. Significant effects ($P<0.05$) of treatments were tested using analysis of variance (ANOVA), unless otherwise stated.

## Results

The geochemical equilibrium modelling program MINEQL+ (Version 4.01; Environmental Research Software) was used to determine Zn(II) speciation within the perfusates used (Table 1). While 87 % of Zn(II) in control experiments was present as free $Zn^{2+}$ ion, 94–100 % of Zn(II) in histidine and cysteine experiments was chelated. At 2 mmol l$^{-1}$ amino acid concentrations the majority of Zn(II) was bound in mono-amino acid complexes, while at 100 mmol l$^{-1}$ most Zn(II) was present as bis-amino acid species. Zn(II) speciation in taurine experiments was not calculated owing to insufficient information about the chemical equilibria of taurine with perfusate constituents. The dissociation constant of taurine–Zn(II) complexes ($K_d$=4.6) (Sakurai and Takeshima, 1983), is much lower than those of cysteine ($K_d$=18.2) and histidine ($K_d$=12.1) with Zn(II). It was likely, however, that at the high amino acid concentrations used, a significant proportion of Zn(II) was bound to taurine. While the amino acid concentrations used were supraphysiological, the altered Zn(II) speciation induced allowed differentiation between the effects of Zn(II) chelation and the nature of the chelated species. These concentrations also permitted comparison with studies in the mammalian literature (e.g. Wapnir et al., 1983; Aiken et al., 1992b).

Plots of the appearance of $^{65}$Zn(II) from the efferent cannulae (Fig. 1) showed that the slopes of the linear portion of recovered $^{65}$Zn(II) differed in the presence of L-histidine and L-cysteine at 100 mmol l$^{-1}$. These treatments also reached steady state more rapidly than the control. Slopes obtained in taurine experiments and for 2 mmol l$^{-1}$ cysteine and histidine
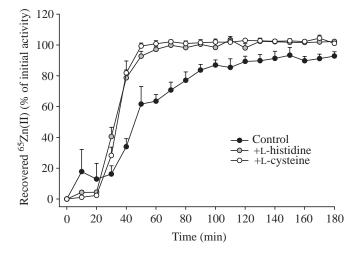


Fig. 1. Profile of $^{65}$Zn(II) activity obtained from the efferent cannula following perfusion of the intestine with 50 µmol l$^{-1}$ Zn(II) alone (control), or in solution with 100 mmol l$^{-1}$ L-cysteine or L-histidine. Values are means ± S.E.M. ($N$=4–6 for amino acid treatments, $N$=10 for control). Slope values (3.98 for L-cysteine, 3.71 for L-histidine, and 2.71 for Zn(II) alone) were calculated over the linear phase of outflow (approx. 20–50 min).
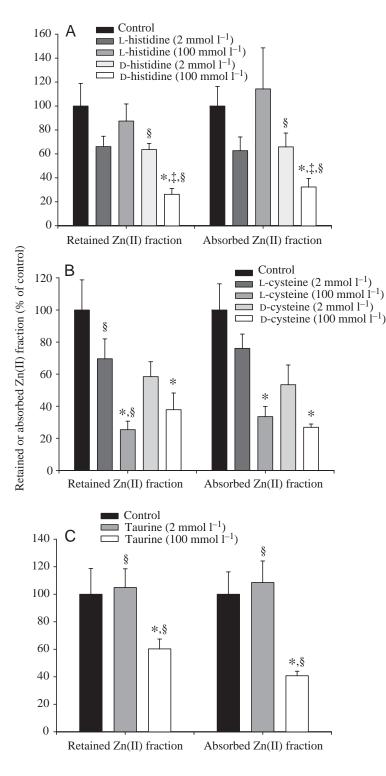
treatment groups were unchanged from the controls [Zn(II) alone]. This effect was stereospecific, as neither D-histidine nor D-cysteine exhibited a significantly altered slope of recovered $^{65}$Zn(II) (data not shown).

Retained and absorbed Zn(II) fractions [measures of quantitative Zn(II) absorption] were closely correlated. Alterations in the retained fraction resulted in concomitant changes in the absorbed fraction, with no significant difference in the magnitude of the change between the two measures (Fig. 2A–C). In control treatments, where Zn(II) was perfused alone, 27 % of the total perfused Zn(II) was retained by the fish, while 11 % of the total perfused Zn(II) was considered absorbed. In Fig. 2A–C these figures were normalised to 100 % to allow direct comparison between treatment effects on retained and absorbed Zn(II) fraction.

Of all the amino acid solutions tested, none significantly increased the retained or absorbed fraction of perfused Zn(II) above that observed with Zn(II) alone. The inclusion of 100 mmol l$^{-1}$ amino acids in Zn(II) perfusates, however, generally resulted in decreased retained and absorbed Zn(II) fractions (Fig. 2A–C). The exception was L-histidine, which produced no alteration in these parameters. This effect was statistically different from the response to D-histidine at the same concentration. There were no other stereospecific effects noted. Amino acid treatments at 2 mmol l$^{-1}$ caused a consistent, yet insignificant, decrease in retained and absorbed Zn(II) fractions.

Amino acids had little impact upon epithelial (mucus and epithelial cells) Zn(II) accumulation (Fig. 3A–C). The only effect of note was a significant stimulation of epithelial accumulation with 100 mmol l$^{-1}$ D-histidine when compared with the corresponding 2 mmol l$^{-1}$ treatment.

Zn(II) accumulation in the subepithelial compartment (intestinal tissue remaining following scraping) was significantly and markedly increased in the presence of 100 mmol l$^{-1}$ D-histidine (Fig. 3A). The rate of 363 nmol g$^{-1}$ h$^{-1}$ for 100 mmol l$^{-1}$ D-histidine was threefold higher than that of the control [Zn(II) alone]. This effect was mimicked to a lesser extent by 100 mmol l$^{-1}$ L-histidine, which exhibited a smaller 1.5-fold stimulation. These increases were not observed at the lower amino acid concentrations tested. In

contrast to histidine, L-cysteine at 100 mmol l$^{-1}$ significantly enhanced the passage of Zn(II) into post-intestinal (blood and body) compartments (Fig. 3B). Control values of 145 nmol kg$^{-1}$ h$^{-1}$ were stimulated 227 %. This response was strongly concentration- and stereoisomer-dependent. Taurine, at 100 mmol l$^{-1}$, but not at 2 mmol l$^{-1}$, greatly reduced Zn(II) accumulation into the post-intestinal compartment, concomitant with significantly enhanced subepithelial Zn(II) accumulation.

Post-intestinal Zn(II) accumulation was analysed to determine if the effects of taurine and cysteine at 100 mmol l$^{-1}$ were compartment-specific. Taurine was found to decrease both blood and body Zn(II) accumulation, whereas the stereospecific effect of L-cysteine was solely on the plasma and erythrocyte Zn(II) accumulation rate (Fig. 4).

## Discussion

### Quantitative Zn(II) absorption: evidence of L-histidine mediated uptake

Luminal amino acids had defined and specific influences upon the intestinal uptake and absorption of Zn(II) in freshwater rainbow trout. Differences in the interaction of Zn(II) with the epithelial surface and the transport and fate of Zn(II) beyond the mucosal barrier were apparent. The most notable effect on quantitative Zn(II) absorption (absorbed and retained Zn(II) fraction) was the stereospecific action of L-histidine in maintaining Zn(II) absorption at control levels [Zn(II) alone]. At the gill epithelium it has been demonstrated that ionic Zn(II) concentration is the major determinant of Zn(II) uptake (Rainbow, 1995; Vercauteren and Blust, 1996). Moieties that chelate Zn(II) may modulate waterborne uptake by decreasing the amount of free Zn(II) available to the uptake surface. Consequently a correlation between Zn(II) uptake and the ionic Zn(II) concentration should be observed. Experiments with L-histidine did not exhibit such a relationship. While addition of 2 mmol l$^{-1}$ L-histidine resulted in an



Fig. 2. Effect of (A) histidine, (B) cysteine or (C) taurine solutions upon retained and absorbed Zn(II) fractions following perfusion of *in vivo* cannulated intestine in combination with 50 μmol l$^{-1}$ Zn(II). Retained Zn(II) fraction represents the fraction of perfused Zn(II) accumulated in the animal over the course of the 3-h perfusion (100 % of the control is equal to a retained Zn(II) fraction of 27 %), whereas absorbed Zn(II) fraction represents the proportion of perfused Zn(II) accumulated in post-epithelial compartments (100 % of the control is equal to an absorbed Zn(II) fraction of 11 %). Control data are consistent across all three plots. Values are means ± S.E.M. of 4–6 replicates for amino acid treatments, and of 10 replicates for control values. *Significant differences between control and treatment, ‡ between D- and L-stereoisomers, and § between treatment concentrations were tested at *P*<0.05, ANOVA.

insignificant decrease in quantitative Zn(II) absorption, at 100 mmol l⁻¹ L-histidine Zn(II) absorption was equivalent to control levels. This was in contrast to a significant decrease in Zn(II) uptake with the other amino acids tested. The maintenance of control levels of Zn(II) uptake corresponded to the formation of a Zn(His)₂ moiety. The significance of these data is twofold. First, in the case of the gut epithelium, uptake was not solely dependent upon the free Zn(II) species. Second, the chemical nature of the chelated species, not the chelation itself, seems to be the determining factor in the absorption of chelated Zn(II) moieties.

A specific effect of histidine upon Zn(II) uptake has been observed previously in a wide range of species and tissues. There are two theories to explain these actions. Histidine may act as a donor molecule facilitating the release of Zn(II) to a membrane transport entity (Wapnir et al., 1985; Ackland and McArdle, 1990; Bobilya et al., 1993). Alternatively the L-histidine–zinc chelate may be transported intact across the epithelium (Wapnir et al., 1983; Ashmead et al., 1985). Both these models of transport account for the stereospecificity of the observed response. Either the Zn(L-His)₂ complex had a competitive advantage in gaining access to the transport moiety, or the transporter itself exhibited enantiomer dependence. From the experiments described here it is not possible to discern the nature of L-histidine-mediated Zn(II) uptake. Significantly different slopes of Zn(II) recovered from the outflow cannula for L-histidine compared to control and D-histidine also lend support to a stereospecific mechanism of Zn(II) uptake, a mechanism that also differed from the uptake of unchelated Zn(II).

The histidine effect upon intestinal Zn(II) uptake in rainbow trout was mediated by a Zn(His)₂ species. There is compelling evidence from studies in mammalian erythrocytes that passage into cells *via* a bis-histidine Zn(II) complex is responsible for the stimulatory effects of histidine (Horn et al., 1995). Vercauteren and Blust (1996) showed a facilitatatory effect of histidine upon Zn(II) in the mussel, *Mytilus edulis*, correlated with the mono-histidine species. Uptake of Zn(II) across the blood–brain barrier of rats was also believed to be mediated by Zn(His)⁺ (Buxani-Rice et al., 1994). Where there is evidence for histidine-mediated Zn(II) uptake in mammalian intestine, Zn(His)₂ is the species implicated (Wapnir et al., 1983). While the nature of the uptake species remains controversial there is increasing evidence across a wide range of organisms, tissues and experimental systems that Zn(II) uptake can be achieved by a histidine-mediated process.

The lack of a stimulatory action of amino acids upon quantitative Zn(II) uptake in the present study
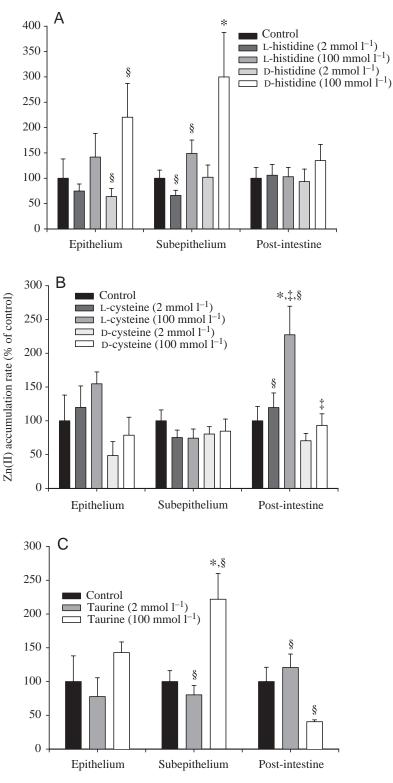


Fig. 3. Effect of (A) histidine, (B) cysteine or (C) taurine solutions upon Zn(II) accumulation rate into epithelium (scrapings), subepithelium (intestinal tissue remaining following scraping) and post-intestinal (pooled blood and body) compartments following perfusion of *in vivo* cannulated intestine in combination with 50 μmol l⁻¹ Zn(II). Values are means ± S.E.M. of 4–10 replicates. *Significant differences between control and treatment, ‡ between D- and L-stereoisomers, and § between treatment concentrations were tested at $P<0.05$, ANOVA.
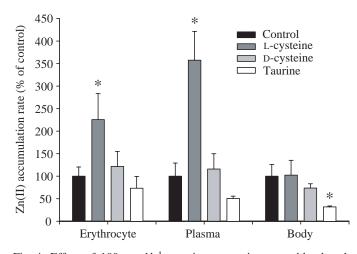
Fig. 4. Effect of $100\,mmol\,l^{-1}$ cysteine or taurine upon blood and body (carcass remaining after dissection) Zn(II) accumulation rates following perfusion of *in vivo* intestine in combination with $50\,\mu mol\,l^{-1}$ Zn(II). Values are means ± S.E.M. of 4–10 replicates. *Significant differences tested at $P<0.05$, ANOVA.

is not surprising. Dietary supplementation of low molecular mass Zn(II)-binding ligands such as cysteine and histidine enhance uptake by chelating Zn(II), removing it from other dietary constituents. Consequently Zn(II) bioavailability is increased. In the present experiments competing ligands were absent and therefore enhancement of quantitative Zn(II) uptake was not observed. Lack of any effect at this level does not necessarily equate with a lack of biological effect. Aquacultural studies have shown that Zn–methionine complexes can more easily meet piscine dietary Zn(II) requirements than Zn(II) in inorganic complexes (Paripatananont and Lovell, 1995), without altering the amount of Zn(II) absorbed (Wekell et al., 1983). The beneficial effects of organic Zn(II) chelates may be explained by altered patterns of accumulation, such as those observed by Hardy et al. (1987) and below.

### Qualitative Zn(II) absorption is modulated by luminal amino acids

Examination of qualitative changes in Zn(II) absorption provides further evidence of specific effects of amino acids on metal metabolism. Histidine stereoisomers stimulated the passage of Zn(II) into the subepithelium (intestinal tissue remaining after mucosal scraping). In contrast to the L-enantiomer effect upon quantitative Zn(II) absorption, this effect was only statistically significant in the presence of D-histidine. Interestingly Aiken and colleagues noted a similar scenario. In rat erythrocytes the effect of histidine upon uptake of Zn(II) was stereospecific (Aiken et al., 1992a); however, histidine also acted to influence body distribution of Zn(II) *in vivo* in an enantiomer-independent manner (Aiken et al., 1992b).

Such results are difficult to explain. In mammalian systems the actions of a transporter in mediating an altered tissue distribution can be eliminated, owing to strict stereospecificity of such entities (Aiken et al., 1992b). However, intestinal amino acid transport in fish is thought to be more promiscuous than that of adult mammals. For at least some amino acids, D- and L-isomers are able to share the same transporter (Huang and Chen, 1975). It is possible, therefore, that the actions of D-histidine could be, in part, mediated by a transporter of D-histidine–zinc complexes.

The effects of cysteine upon intestinal Zn(II) absorption are distinct from those of histidine. Snedeker and Greger (1983) described a similar situation in rats. Cysteine had an L-enantiomer-dependent stimulatory action upon post-intestinal Zn(II) absorption. Time-to-steady-state Zn(II) flux for L-cysteine was also greater than for the D-isomer, indicating a mechanistic difference at the uptake surface. Such a difference may again be explained by a specific transport entity or may represent a competitive advantage for L-cysteine to donate Zn(II) to a membrane moiety for uptake. The experiments described here do not allow for a distinction between these scenarios.

Cysteine infusion in rats was found to increase plasma Zn(II) levels (Abu-Hamden et al., 1981). The increase in post-intestinal Zn(II) levels in the present study was the result of enhanced Zn(II) accumulation specifically in the blood compartments. Body Zn(II) accumulation rate was unchanged, despite the fact that this compartment has reserve capacity for accumulation (Glover and Hogstrand, 2002). In the study of Abu-Hamdan (1981), the rise in plasma Zn(II) levels was concomitant with an increase in Zn(II) excretion. It is not known whether a similar effect exists in the present investigation. Enhanced post-intestinal Zn(II) may be a result of increased plasma cysteine levels, drawing Zn(II) from the epithelium into the blood where it is retained. Alternatively the passage of a cysteine–Zn(II) chelate into the blood is possible (Ashmead et al., 1985).

### Effects of taurine: evidence for reciprocal regulation of Zn(II) uptake pathways

The marked effect of taurine upon Zn(II) absorption was unexpected. Taurine was included in the study as a control, being an amino acid with low affinity for Zn(II) (Sakurai and Takeshima, 1983). Harraki and colleagues (1994) reported taurine stimulation of Zn(II) absorption in human fibroblasts. To our knowledge this report and the present study are unique in exhibiting an influence of taurine upon Zn(II) uptake. While rarely reported, such an association is not without biological relevance. Both Zn(II) and taurine are found in high concentrations in the tapetum lucidum of the retina (Sturman, 1983), mossy fibre boutons of the cerebral cortex and hippocampus (Crawford, 1983; Wu et al., 1985), and in combination both enhance membrane stability (Gaull et al., 1985).

Taurine is the only modulator of Zn(II) metabolism thus far tested in the *in vivo* perfused trout intestine that has effects on both subepithelial and post-intestinal Zn(II) accumulation. Taurine uptake was shown to be $Na^+$-dependent in flounder intestine (King et al., 1986), and the replacement of $Na^+$ with choline did not affect the pattern of Zn(II) accumulation with taurine in rainbow trout intestine (C. N. Glover and C.

Hogstrand, unpublished observations). The effect noted is therefore likely to be unrelated to taurine uptake, assuming that taurine transport is conserved between species. Taurine is known to alter the membrane binding and transport of $Ca^{2+}$ (Huxtable, 1992). Reports have shown interactions between $Ca^{2+}$ and Zn(II) in fish gill and gut absorption (e.g. Hogstrand et al., 1995, 1996) (C. N. Glover and C. Hogstrand, in preparation). The taurine-induced response may be a secondary effect due to alteration of $Ca^{2+}$ metabolism, or an action of taurine specifically upon transport or membrane interactions of Zn(II). The effect of taurine is intriguing given it is the most abundant free amino acid in intestinal mucosa of fish (Auerswald et al., 1997).

Data presented here suggest the presence of several parallel uptake pathways for Zn(II), including an amino acid-mediated process and an uptake route for inorganic Zn(II). While of obvious value, *in vitro* methods such as gut bags and isolated membrane vesicles may not discern the complex nature of intestinal Zn(II) absorption, highlighting the importance of the *in vivo* approach that we have used.

Evidence from this investigation and others (e.g. Kramer et al., 1997) suggests that interactions between amino acids and metals in the intestine are likely to have important ramifications upon the absorption of both these dietary constituents. Luminal composition therefore has the potential to modulate the oral toxicity of metals, and will also have implications for nutrition and aquaculture. The ability to maintain the uptake of essential nutrients while regulating the accumulation of potential toxicants is key to preserving life in contaminated environments.

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