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# Histidine Absorption across Apical Surfaces of Freshwater Rainbow Trout Intestine: Mechanistic Characterization and the Influence of Copper

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**Abstract** The essential amino acid histidine performs critical roles in health and disease. These functions are generally attributed to the amino acid itself, but could also be mediated by a positive effect on trace element bioavailability. Mechanistic information regarding the absorption of histidine across the gastrointestinal tract is essential for understanding the interplay between amino acid and mineral nutrients and the implications of these interactions for nutrition and toxicology. Using intestinal brush-border membrane vesicles obtained from freshwater rainbow trout, absorption of histidine over the range 0.78–780 $\mu$M was found to be saturable, with a maximal transport rate ($J_{max}$) of 9.1 ± 0.8 nmol mg protein$^{-1}$ min$^{-1}$ and a $K_m$ (histidine concentration required to reach 50% of this level) of 339 ± 68 $\mu$M. Histidine uptake was highly specific as 10-fold elevated levels of a variety of amino acids with putative shared transporters failed to significantly inhibit uptake. Elevated levels of D-histidine, however, impaired uptake of the natural L-isomer. The presence of "luminal" copper (8.3 $\mu$M) significantly increased both the $J_{max}$ and $K_m$ of histidine transport. This suggests that chelated copper–histidine species cross the brush-border epithelium through transport pathways distinct from those used by histidine alone.

**Keywords** Copper · Histidine · Amino acid · Metal chelate · Nutrient absorption · Transport kinetics · Menkes disease · Cataract · Fish

## Introduction

Histidine performs diverse and essential functions in biology. It has vital catalytic roles in proteins, is a precursor for other bioactive chemicals and is an effector molecule in its own right. Many of these actions are mediated by the imidazole functional group. This moiety can act as both an electron donor and an acceptor; thus, histidine is often a critical amino acid residue in enzyme reactive sites (Schneider, 1978). This property also confers the ability to bind metals (see below; Sundberg & Martin, 1974). As a metabolic precursor, histidine can be converted to molecules such as histamine, carnosine and anserine, all with vital roles in a myriad of physiological processes. Histamine, for example, has wide-ranging biological effects including tissue inflammation, regulation of gastric acid secretion, vasoactive effects on smooth muscle and neurological functions such as control of appetite (Babe & Serafin, 2000).

As an essential amino acid, the maintenance of histidine functions, and those of its derivatives, is dependent on absorption from the diet. A large number of transporters have been identified that are capable of facilitating transmembrane histidine movement in mammals. In fact, no fewer than seven different amino acid transport systems have been implicated in histidine uptake in a wide range of

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mammalian tissues (Bröer, 2002). This does not include transporters usually responsible for translocation of small peptides, for which histidine is also frequently a substrate (Daniel & Kottra, 2004). In nonmammalian vertebrates virtually nothing is known regarding the mechanisms of epithelial histidine transport. This is despite this amino acid being strongly implicated in the genesis of fish diseases such as cataracts (Bjerkås & Sveier, 2004), which may affect both fish health and commercial interests (Breck & Sveier, 2001; Midtlyng et al., 1999; Menzies et al., 2002). Understanding mechanisms of uptake is essential for discerning and interpreting homeostatic actions regulating uptake and delineating the possible actions of other dietary constituents on the absorptive process.

This initial aim of this study was the characterization of histidine absorption mechanisms across the apical intestinal membrane of rainbow trout (*Oncorhynchus mykiss*) using brush-border membrane vesicles (BBMVs). The brush-border membrane technique is advantageous over other preparations in that it allows careful control over luminal and cytosolic media. Additionally, its homogeneity is beneficial for delineating mechanisms that can be confounded in preparations where basolateral and paracellular uptake pathways may exist (i.e., everted sacs, intestinal sacs, Ussing-type preparations) or where central feedback mechanisms regulate uptake (i.e., in vivo).

The importance of histidine in nutrition extends beyond roles directly related to its absorption, and this was the second focus of the present study. Positive impacts of histidine supplementation in diets have been noted in the literature and attributed directly to improved histidine absorption (e.g., Kasaoka et al., 2004; Breck et al., 2005). However, given the high affinity of histidine for mineral elements, these beneficial effects may potentially be mediated by improved micronutrient absorption, facilitated by the presence of higher dietary histidine levels. Positive actions of histidine on piscine metal uptake have been supported by a number of laboratory studies that show histidine can increase uptake (e.g., Glover, Bury & Hogstrand, 2003; Nadella, Grosell & Wood, 2006) or act to redistribute minerals to body tissues where they may potentially be of greater health benefit (Glover & Hogstrand, 2002). These studies have, however, been equivocal regarding the mechanisms by which histidine achieves its actions.

A goal of the current study was to examine the effect of copper on histidine uptake. Copper is a vital nutrient, performing important roles as a cofactor in many critical biological reactions (Bury, Walker & Glover, 2003); but an excess of dietary copper is associated with toxic impacts (Clearwater, Farag & Meyer, 2002). Copper–histidine complexes are currently being employed as a treatment for the human copper deficiency syndrome Menkes disease (Deschamps et al., 20005), and recent evidence suggests that histidine stimulates copper transport across the gastrointestinal tract of fish (Nadella et al., 2006). Knowledge of histidine uptake, both alone and in the presence of copper, will enable us to discern the mechanism of trace element uptake stimulation and should provide insights into the nutritional and potentially toxicological roles of this amino acid.

## Materials and Methods

### Animals

Rainbow trout (*O. mykiss*, ~200–300 g) were obtained from Humber Springs trout farm (Orangeville, Canada) and acclimated to holding conditions (water temperature 12–13°C) at McMaster University (Hamilton, Canada) for at least 10 days before use. Fish were maintained in Hamilton City Tap water (Lake Ontario) with the following composition: $Na^+$, 0.5 mM; $Ca^{2+}$, 1 mM, $Cl^-$, 0.7 mM; hardness, 140 ppm as $CaCO_3$; pH 8. Fish were fed to satiation three times a week on commercial fish feed (Martin Mills, Elmira, Canada), comprising a standard vitamin/mineral mix with a minimum estimated histidine content of 7 g $kg^{-1}$ (National Research Council, 1993) and a measured copper content of 27 mg $kg^{-1}$ (Nadella et al., 2006). No differences in vesicle purity, integrity or transport characteristics were noted in preparations harvested from different days of the feeding regime. All animal manipulations were performed in accordance with the McMaster University Animal Utilisation Protocols.

### BBMV Preparation

Vesicles were prepared according to the protocol described by Glover et al. (2003). Intestines from two rainbow trout were dissected, flushed with saline (0.9% NaCl, 1 mM dithiothreitol, 0.5 mM ethyleneglycoltetraacetic acid [EGTA]), blotted gently and scraped on ice with glass microscope slides. Pooled scrapings from the entire length of the intestine were transferred to 35 ml of buffer A (50 mM mannitol, 2 mM EGTA, 0.5 mM $MgSO_4$, 0.1 mM phenylmethylsulfonylfluoride, pH 7.4) and homogenized with 20 strokes of a Dounce homogenizer ("loose" pestle) on ice. This homogenate was centrifuged (8,500 × *g*, 4°C, Sigma 4K15 refrigerated centrifuge; Montréal Biotechnologies, Montréal, Canada) for 15 min, and the resulting "fluffy" layer was removed and further homogenized in 30 ml of buffer B (320 mM sucrose, 10 mM 2-amino-2-[hydroxymethyl]propane-1,3-diol [Tris], pH 7.4) with a further 25 strokes of a Dounce homogenizer ("tight"

pestle). Solid $MgCl_2.6H_2O$ was added to a final concentration of 10 mM for membrane aggregation, and the homogenate was left to stand on ice for 15 min with occasional agitation. The preparation was then centrifuged at 20,000 × *g* for 10 min (4°C, Sigma 4K15), and the resulting supernatant was spun again at 45,000 × *g* for 25 min (4°C, Beckman L8M ultracentrifuge, SW28 rotor; Beckman-Coulter, Mississauga, Canada), to give the final pellet containing the enriched apical membrane. Small portions of the pellet were removed for analysis of membrane purity and related assays (see below), and the remainder was resuspended in vesicle buffer (149 mM KCl, 1 mM NaCl, 10 mM *N*-[2-hydroxyethyl]piperazine-*N*'-2-ethanesulfonic acid [HEPES]) with 30 passages through a 23-gauge needle. Protein content was determined (Bradford, 1976), and vesicle solutions were diluted to a final concentration of 1 mg protein $ml^{-1}$.

## Characterization of Membrane Vesicle Preparation

Purity of the final membrane fraction was assessed using biochemical assays. Alkaline phosphatase (Sigma, Oakville, Canada) was used as a marker of apical/brush-border enrichment and $Na^+$,$K^+$-ATPase (Bonting, Simon & Hawkins, 1961), as a marker of basolateral contamination (Glover et al., 2003). Measures of mitochondrial membrane contamination were not determined as this is rarely a problem using this isolation protocol (Glover et al., 2003).

Membrane orientation (right side–out or inside–out) was examined by measuring alkaline phosphatase activity on membrane preparations prior to and following vesiculization (Steck & Kant, 1974). Vesicle integrity was assessed by monitoring the responsiveness of the preparation to osmotic gradients (Glover et al., 2003).

## Measurement of Unidirectional Histidine and Effect of Copper

Unidirectional histidine movement into vesicles was measured by a rapid filtration method. Membrane vesicle aliquots of 35 µl (35 µg protein) were added to 125 µl of assay buffer (149 mM NaCl, 1 mM KCl, 10 mM HEPES, pH 7.4), to which was added one of seven histidine concentrations resulting in a histidine level of 0.78, 3.9, 7.8, 39, 78, 390 or 780 µM once final dilution was accounted for. Radiolabeled histidine (L-[2,5-$^3$H]histidine; Amersham Biosciences, Piscataway, NJ) was added at a label concentration of 2 µCi per reaction. Transport reactions were incubated in a water bath at 10°C for 30 s. Preliminary experiments showed that incubation times up to 30 s provided uptake rates in the linear range. Subsequently, 45-µl aliquots were added, in triplicate, to presoaked membrane filters (0.45 µM; Schleicher & Schuell, Fisher Scientific, Nepean, Canada) placed in a rapid filtration sampling manifold (Millipore, Fisher Scientific). Filters were then washed with 5 ml of stop buffer (ice-cold assay buffer, with 1 mM EDTA). After removal from the filtration apparatus, filters were added to glass scintillation vials and digested for 48 h in 1.5 M $HNO_3$, followed by a further 48 h digestion/incubation in 10 ml of scintillation fluor (Ultima Gold; Perkin-Elmer, Boston, MA). Filters were then assayed for $^3$H activity by liquid scintillation counting (Tri-Carb 2900 TR, Perkin-Elmer), using external standard ratio quench correction.

For preparations including copper, the metal was added to histidine solutions from a stock of $Cu(NO_3)_2.2½H_2O$, to give a final copper concentration of 8.3 µM. The copper was added to histidine solutions and allowed to equilibrate at 10°C for 1 min before addition of vesicles. Copper levels in the stock solution were monitored by graphite furnace atomic absorption spectrometry (SpectraAA-220; Varian, Palo Alto, CA). Copper and amino acid speciation was determined using the geochemical modeling software MINEQL+ (Environmental Research Systems, Hallowell, ME).

The effect of potential competing amino acids on L-histidine transport was also investigated. Glycine, glutamine, lysine, phenylalanine and the dipeptide glycylglycine (all reagent grade, Sigma) were added individually to extravesicular solutions at levels 10-fold greater than L-histidine. Effects were tested at two L-histidine concentrations, 0.78 and 780 µM. The impact of D-histidine (0.78, 78, 780 and 7,800 µM) on L-histidine uptake was determined at 0.78 µM L-histidine. The effect of 10-fold excess of D-histidine at L-histidine levels of 780 µM was also examined.

## Calculations and Statistical Analysis

The $^3$H activity of filters was background-corrected, using filters that had been exposed to aliquots where distilled water replaced the membrane fraction in the reaction vessel. Background-corrected counts were converted to histidine concentrations by a specific activity calculation and expressed per unit protein per minute. Michaelis-Menten uptake parameters were calculated directly from curves using Sigmaplot 8.02 (SPSS, Chicago, IL). Uptake parameters from different curves were assessed for significant differences using the parameter value and the error estimate in a conservative *t*-test (Glover, Pane & Wood, 2005). Other statistical differences were assessed by one-way analysis of variance, at the $\alpha = 0.05$ level, using an least significant difference (LSD) post-hoc test (Statistica, version 5; StatSoft, Tulsa, OK).

## Results

### Characterization of Membrane Vesicle Preparation

The membrane isolation protocol resulted in a preparation which was highly enriched with the brush-border/apical membrane. Alkaline phosphatase activity was 6.7 ± 1.8 (mean ± SEM)-fold greater in the final membrane fraction compared to the initial homogenate. Significant enrichment of the basolateral marker enzyme was also discerned, however, with $Na^+$,$K^+$-ATPase activity elevated 2.6 ± 0.2-fold. These values indicate that the isolation method was comparable with previous studies (e.g., Klaren et al., 1993).

The alkaline phosphatase activity of preparations measured prior to vesicle formation did not differ from measurements made after vesicle formation (102 ± 4%). This indicated vesicle preparations consisted entirely of vesicles in which the brush-border membrane was oriented facing out (right side–out). Altering extravesicular osmolarity exhibited a sealed, osmotically sensitive vesicle preparation. As osmolarity was raised, histidine uptake was reduced as a consequence of diminished intravesicular space (Fig. 1).

### Histidine Uptake

The uptake of histidine into rainbow trout intestinal BBMVs was time-dependent, with uptake saturating at incubation times in excess of 1 min (Fig. 2). In light of this, a period where uptake was approximately linear with time (30 s) was chosen as the standard incubation time for all following manipulations.

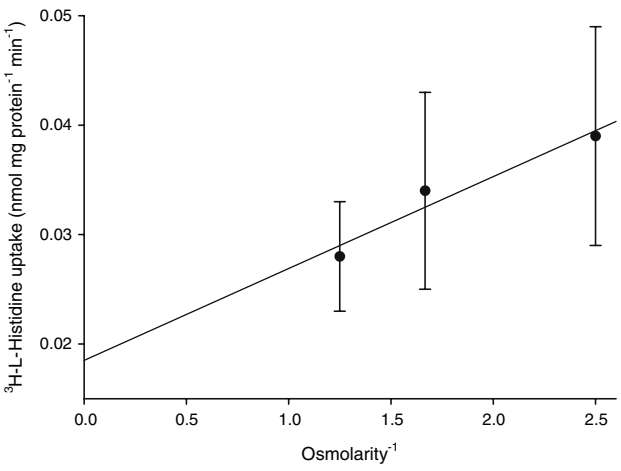


**Fig. 1** L-Histidine uptake (nmol mg protein$^{-1}$ min$^{-1}$) in intestinal BBMVs as a function of external osmolarity. Plotted points represent the means (±SEM) of three or four replicates, measured in triplicate

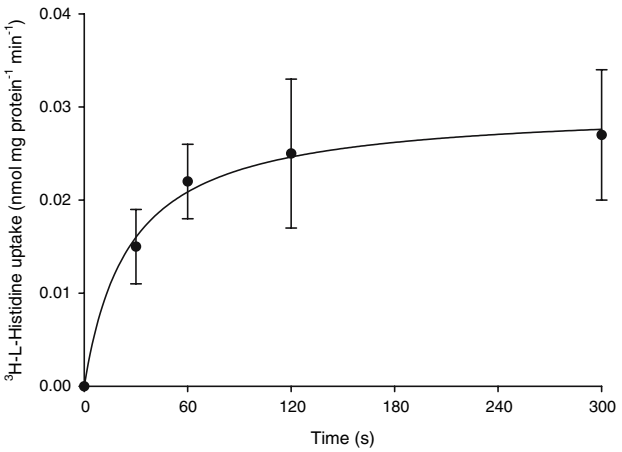


**Fig. 2** L-Histidine uptake (nmol mg protein$^{-1}$ min$^{-1}$) in intestinal BBMVs as a function of incubation time. Plotted points represent the means (±SEM) of three or four replicates, measured in triplicate

Histidine transport increased as a function of extravesicular histidine concentration, with saturation of uptake observed at the highest levels of histidine examined (Fig. 3). The pattern of uptake conformed to Michaelis-Menten kinetics, permitting calculation of affinity and capacity parameters of apical histidine absorption. The maximal rate of transport ($J_{max}$) was 9.1 ± 0.8 nmol mg protein$^{-1}$ min$^{-1}$, and the histidine concentration required to give half this level ($K_m$) was 339 ± 68 μM.

Substitution of sodium with potassium in the external medium and the subsequent diminished inward-directed sodium gradient did not inhibit histidine uptake (Fig. 4). In fact, although transport affinity ($K_m$) was unchanged at 580 ± 148 μM, maximal uptake rate was actually

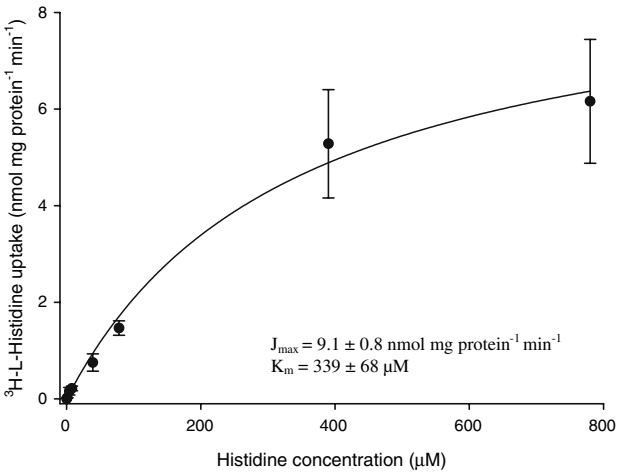


**Fig. 3** L-Histidine uptake (nmol mg protein$^{-1}$ min$^{-1}$) in intestinal BBMVs as a function of extravesicular histidine concentration (0.78–780 μM), following incubation for 30 s at 10°C. Curve fitting and calculation of Michaelis-Menten parameters performed using SigmaPlot. Plotted points represent the means (±SEM) of seven or eight replicates, measured in triplicate

significantly elevated ($J_{max}$ = 17.2 ± 2.2 nmol mg protein$^{-1}$ min$^{-1}$).

Histidine uptake was not significantly inhibited by 10-fold excess of any of the tested nonhistidine amino acids at either 0.78 μM (Fig. 5a) or 780 μM (Fig. 5b) L-histidine. D-Histidine, however, significantly impaired L-histidine absorption (Fig. 6). No significant effect of equimolar D-histidine was discerned, but L-histidine uptake was reduced at extravesicular D-histidine concentrations of 78 μM (100-fold excess). Increasing D-histidine levels had little additional effect, with ~45% of L-histidine uptake remaining recalcitrant to D-histidine levels 10,000-fold in excess. At 780 μM the uptake of L-histidine was not significantly influenced by 10-fold excess of D-histidine (Fig. 5b).

The presence of extravesicular copper (8.3 μM) significantly altered L-histidine uptake kinetics (Fig. 7). $J_{max}$ was increased to 20.2 ± 1.6 nmol mg protein$^{-1}$ min$^{-1}$, representing an increase in transport capacity, while the

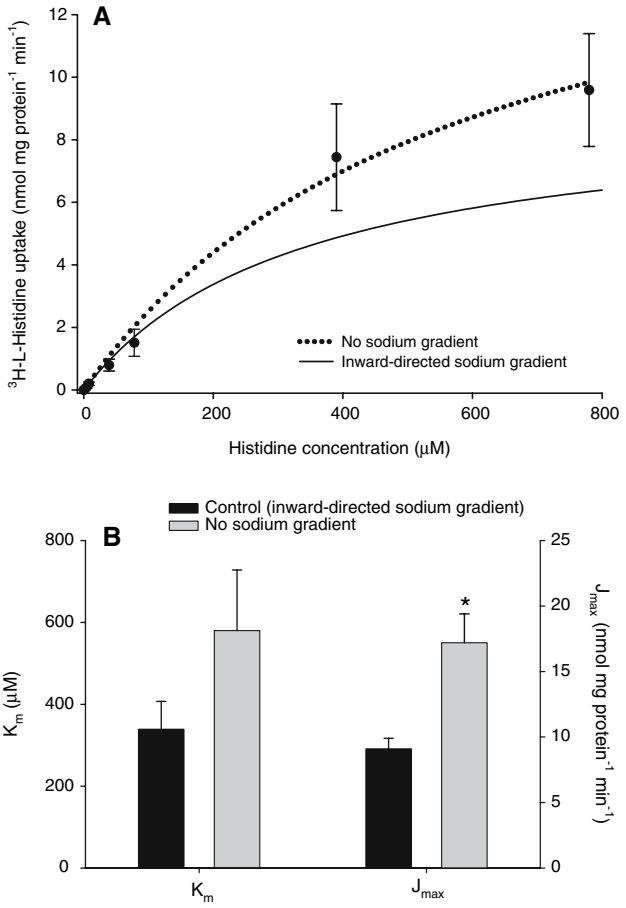


**Fig. 4** Effect of the presence (control conditions; *black bars, solid line*) or absence (*gray bars, dotted line*) of an inward-directed sodium gradient on L-histidine uptake (**a**) and derived Michaelis-Menten parameters ($J_{max}$ [nmol mg protein$^{-1}$ min$^{-1}$] and $K_m$ [μM]) (**b**). Michaelis-Menten parameters were determined directly from kinetic curves using SigmaPlot. Significant differences ($^*P < 0.05$) were calculated as described in "Materials and Methods"

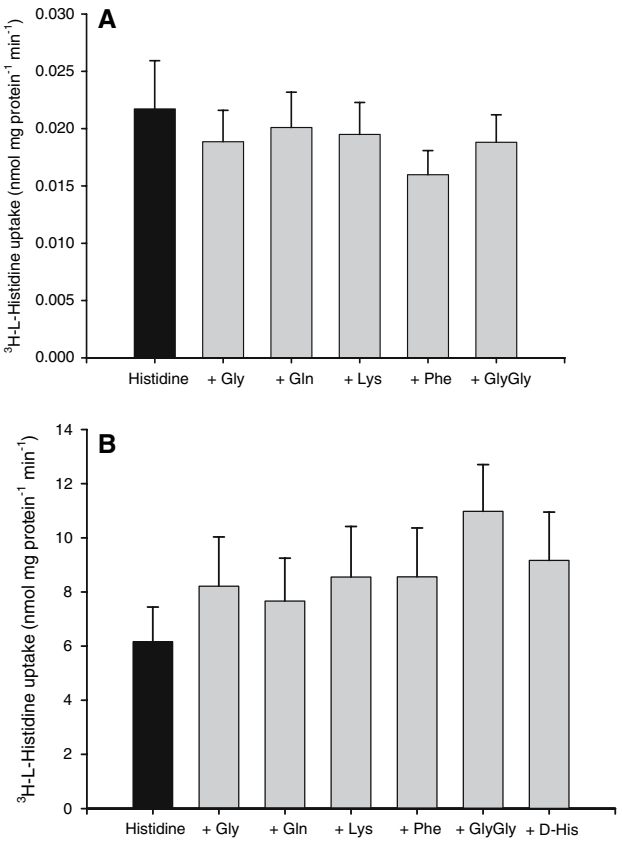


**Fig. 5** L-Histidine uptake (nmol mg protein$^{-1}$ min$^{-1}$) in intestinal BBMVs in the presence of a 10-fold excess of potential amino acid transport system competitors at L-histidine levels of (**a**) 0.78 or (**b**) 780 μM. Plotted points represent the means (±SEM) of seven or eight replicates, measured in triplicate. No significant differences were detected. Gly, glycine; Gln, L-glutamine; Lys, L-lysine; Phe, L-phenylalanine; GlyGly, glycylglycine; D-His, D-histidine

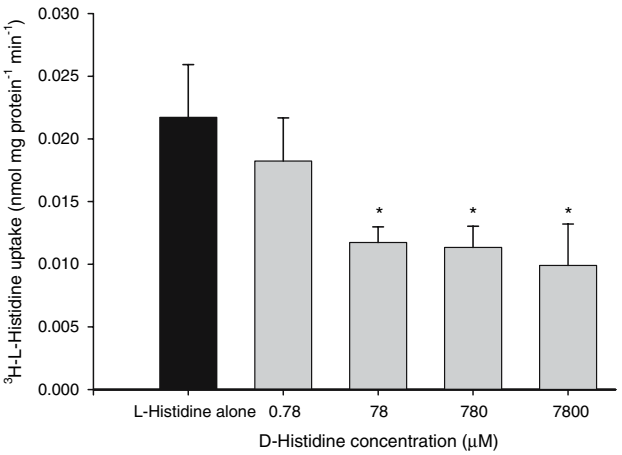


**Fig. 6** L-Histidine uptake (0.78 μM, nmol mg protein$^{-1}$ min$^{-1}$) in intestinal BBMVs in the presence of increasing concentrations (0.78 μM–7.8 mM) of D-histidine. Plotted points represent the means (±SEM) of seven or eight replicates measured in triplicate. Significant differences ($^*P < 0.05$) were determined by one-way analysis of variance, followed by LSD post-hoc analysis

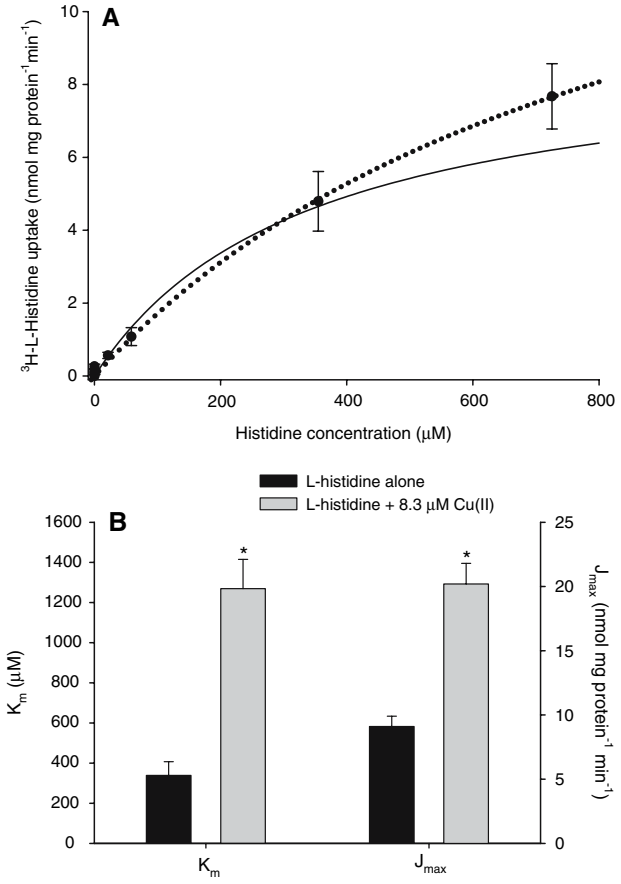


**Fig. 7** Effect of the presence (control conditions; *black bars, solid line*) or absence (*gray bars, dotted line*) of copper (8.3 μM as $Cu[NO_3]_2.2½H_2O$) on L-histidine uptake (**a**) and derived Michaelis-Menten parameters ($J_{max}$ [nmol mg protein$^{-1}$ min$^{-1}$] and $K_m$ [μM]) (**b**). Michaelis-Menten parameters were determined directly from kinetic curves using SigmaPlot. Significant differences ($^*P < 0.05$) were calculated as described in "Materials and Methods"

significant increase in $K_m$ (to 1,269 ± 146 μM) describes a reduction in L-histidine uptake affinity across the intestinal brush-border membrane of rainbow trout.

## Discussion

### Apical L-Histidine Uptake

The passage of L-histidine across the apical surface of rainbow trout intestine proceeds via a specific, saturable mechanism. This is the first study to examine the uptake of this essential amino acid across the intestine of teleost fish. The affinity of histidine uptake ($K_m$ = 339 μM) was in line with affinity constants for other amino acids across fish intestine. For example, the uptake of glycine, alanine and methionine across the brush-border of the marine teleost *Boops salpa* was described by $K_m$ values in the order of 100 μM (Bogé, Roche & Balocco, 2002). Leucine, aspartate and methionine exhibited similar transport affinities in freshwater Atlantic salmon (Bakke-McKellep et al., 2000). In contrast, affinity constants in the millimolar range have also been described in fish (e.g., Bakke-McKellep et al., 2000; Berge et al., 2004). Differences may be related to technique (membrane vesicle approaches eliminate the basolateral absorptive step) and the nature and location of the transport system being utilized by each amino acid.

The $K_m$ of the current study compares favorably with that derived for histidine in mammalian intestine (190 μM; Teillet et al., 1995). Amino acid uptake affinity constants in fish are generally lower (i.e., show higher affinity) than those derived from mammals (Ferraris & Ahearn, 1984). This higher affinity is often attributed to the relatively short gastrointestinal tract in fish, which may necessitate a higher uptake efficiency to achieve nutritional requirements (Ferraris & Ahearn, 1984). This is a pattern that also extends to the invertebrates, where affinities for histidine are approximately two orders of magnitude greater than those derived herein (insect midgut brush-border membranes 3.4 μM, Neal et al., 1996; perfused lobster intestine 6.2 μM, Conrad & Ahearn, 2005). As such, the $K_m$ of 339 μM for rainbow trout in this study is actually higher than might be expected. This may represent transporter adaptation to a diet that is heavily supplemented with histidine (~7 g kg$^{-1}$; National Research Council, 1993), reducing the need to absorb histidine as effectively as in a more histidine-restricted diet. On the basis of the uptake characteristics discerned in the present study, it is likely that the levels of histidine in fish feeds are more than sufficient to saturate the available transport systems. Measures of histidine bioavailability from commercial diets would be required to test this.

Another factor that may influence histidine bioavailability is gut pH. The present studies were performed at pH 7.4. With a $pK_a$ of 6.0, histidine speciation will alter significantly over the pH range that may be encountered along the gastrointestinal tract of fish. This could have important impacts on the transport system used to traverse the gastrointestinal epithelium (see also below).

A striking finding of the present study was the high specificity of apical histidine uptake. In a wide variety of mammalian tissues, histidine uptake has been proposed to occur by no fewer than seven amino acid transport systems (Bröer, 2002) in addition to being a substrate for most transporters of small peptides (Daniel & Kottra, 2004). A range of amino acids were tested for their impact on the absorption of histidine. Amino acids with overlapping transport specificities would be expected to inhibit histidine absorption. As exhibited in Figure 5, no significant inhibition was seen with 10-fold excess of any tested amino acid, demonstrating a transport process with high specificity. Even though D-histidine was capable of inhibiting L-

histidine absorption, approximately half of L-histidine uptake remained unaffected. This high specificity is unusual in that transport of amino acids is generally considered to be more promiscuous in fish than in mammals (Huang & Chen, 1975). This finding is not, however, without precedent in the piscine literature. A study conducted in elasmobranch gastrointestinal tract also failed to show significant inhibition of histidine transport with potentially competing amino acids (Read et al., 1960). In addition, it is worth noting that histidine uptake in lobster intestine could not be blocked by leucine, an amino acid hypothesized to move through the histidine uptake pathway in this species (Conrad & Ahearn, 2005), suggesting high specificity may be characteristic of nonmammalian histidine transport.

The nature of the histidine transporter(s) present in rainbow trout intestinal brush-border remains elusive. Based on inhibitor studies and assuming conserved transport specificities, none of the traditional amino acid transport systems appears to be implicated. The only transporter with characterized physiology that may fit the attributes described in the present study is the peptide/histidine transporter (PHT1, SLC15A4). Initially cloned from rat brain (Yamashita et al., 1997), PHT1 transcripts have also been described throughout the rat and human gastrointestinal tract (Bhardwaj et al., 2006; Herrera-Ruiz et al., 2001). This transporter facilitates the uptake of histidine and small peptides such as carnosine but does not facilitate absorption of the dipeptide Gly-Sar. It is likely that histidine uptake via PHT1 would be relatively unaffected by any of the transport substrates tested in the present study, including the dipeptide Gly-Gly. A characteristic of histidine transport through PHT1 is its pH dependence. This was not tested in the present study, but such evidence would greatly support a role for PHT1 in histidine uptake in rainbow trout intestine.

Histidine uptake via PHT1 is also sodium-independent, similar to the characteristics of absorption described herein. In fact, replacement of sodium by potassium in the extravesicular medium actually stimulated transport in the present study. Potassium-dependent amino acid transporters have been described from insect midgut (Giordana et al., 1989), but an alternative explanation may be related to sodium/proton exchange. A possible mechanism of sodium uptake in fish intestine is passage through an apical $Na^+/H^+$ exchanger (NHE). NHE3, an isoform located on the apical surface of mammalian intestine, has been described in the gut of fish (Choe et al., 2005). Decreased extravesicular sodium may have impaired proton export, and the resulting pH change at the uptake surface may have stimulated histidine uptake.

Mechanistic characterization of the amino acid transport system in nutritional studies is critical not only for assessing the nature of uptake of the amino acid of interest but also for predicting the effects of uptake on other nutrients. Knowledge of the transporters involved in facilitating the uptake of amino acids that may be heavily supplemented in diets is important in ensuring this amino acid does not competitively exclude uptake of amino acids that may share the transport system. The results of the present study show this is unlikely to be of concern, at least across the brush-border absorptive step.

## Effect of Copper on L-Histidine Uptake

The inclusion of copper in the extravesicular medium had a significant impact upon intestinal histidine uptake. The uptake affinity was lowered (increased $K_m$) but with a stimulated transport capacity (increased $J_{max}$). These results indicate that the presence of copper facilitates the passage of histidine through distinct absorptive pathways in lieu of, or in addition to, the transporters that achieve uptake in the absence of copper. Under conditions where fish receive commercial diets containing large quantities of histidine, changes in affinity ($K_m$) are likely to be less significant than changes in capacity ($J_{max}$) in dictating the overall impact of copper on histidine uptake. Consequently, it is likely that the presence of luminal copper would promote histidine uptake.

In this study, a single copper concentration was maintained and histidine uptake monitored as "luminal" histidine concentration was increased. Under this scenario, as luminal histidine level increases, histidine speciation changes from a monochelate ($Cu[His]^+$) to a bis-chelated species ($Cu[His]_2$) and eventually to conditions where free histidine predominates. This chelation effect could create both additional transport entities or impair the transport of histidine directly by removing substrate available for transport. Recalculation of kinetic parameters on the basis of free histidine (data not shown) showed affinity and capacity constants statistically similar to those based on total histidine. This suggests that the increased capacity and reduced affinity were not the consequences of altered free histidine levels but, rather, represented a redirection of transport to different apical mechanisms involving chemical species distinct from those utilized in the absence of copper.

The effect of histidine on mineral uptake in fish intestine has been the focus of some mechanistic studies (Glover & Hogstrand, 2002; Glover et al., 2003; Nadella et al., 2006), but this is the first piscine study to examine the reciprocal relationship. An understanding of histidine absorption permits a reexamination of histidine-facilitated mineral uptake. Two hypotheses exist to explain the beneficial impact of histidine on metal micronutrient accumulation in fish. Either the amino acid enhances bioavailability by

"escorting" the ionic metal to its transporter or an additional transport pathway is created by the formation of a metal–histidine complex (Glover et al., 2003). In an attempt to distinguish between these two mechanisms, Glover and colleagues (2003) examined zinc uptake in intestinal BBMVs in the presence of L- and D-isomers of histidine. Theoretically, the D-isomer, if transported at all, would be taken up with less efficiency than the natural L-isomer. Their results, showing equivalent uptake in the presence of both isomers, tended to support the escort effect (Glover et al., 2003). The findings described here, showing that D-histidine may be a transportable substrate, reignite the possibility that, for zinc at least, the effect of histidine on apical mineral uptake may be the consequence of a transported chelate. Interestingly, in preparations with the epithelium intact (in vivo perfusion, intestinal sacs) histidine-facilitated intestinal mineral uptake in rainbow trout has been shown to be stereospecific (Glover & Hogstrand, 2002; Nadella et al., 2006). This suggests that although passage across the brush-border membrane is accessible to both D and L isoforms, a later absorptive step may exhibit more stringent stereospecificity.

The transport vs. donation controversy has been addressed more directly in other studies. In lobster intestine, zinc was shown to increase the $K_m$ and $J_{max}$ of histidine absorption (Conrad & Ahearn, 2005), a similar result to that observed for copper in the present study. These authors additionally showed that zinc uptake was stimulated by histidine. On the basis of a shared set of inhibitors, it was concluded that a zinc–histidine chelate was transported across the perfused gut. Darwish and colleagues (1984) concurrently examined the uptake of histidine and copper in rat hepatocytes. Histidine was shown to stimulate copper uptake in the presence of inhibitory ligands, but the kinetic parameters of uptake were almost identical to those in the presence of copper alone. These authors concluded that histidine was not transported as part of a copper chelate and instead simply acted to facilitate copper delivery to its transporter. In a study complementary to the present one, we examined the effect of histidine on copper uptake in conditions identical to those used herein for the study of copper effects on histidine uptake (Glover & Wood, 2008). The affinity of the copper transport in the presence of histidine was indistinguishable from that described here for histidine uptake in the presence of copper, although the capacity was significantly greater. This suggests that the presence of a transportable copper–histidine complex remains a possibility in fish intestine (Glover & Wood, 2008).

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