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Iron transport across the skin and gut epithelia of Pacific hagfish: Kinetic characterisation and effect of hypoxia

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ABSTRACT

In most animals, the acquisition of the essential trace metal iron (Fe) is achieved by the gut, but in hagfishes, the skin is a nutrient absorbing epithelium, and thus may also play a role in Fe uptake. In the current study, the absorption of Fe, as Fe(II), across the intestinal and cutaneous epithelia of Pacific hagfish (*Eptatretus cirrhatus*) was investigated. Both epithelia absorbed Fe, with saturation at lower tested concentrations, superseded by a diffusive component at higher Fe exposure concentrations. Affinity constants (K_m) of 9.4 and 137 μM, and maximal Fe transport rates (J_{max}) of 0.81 and 0.57 nmol cm^{-2} h^{-1} were determined for the skin and the gut, respectively. This characterises the skin as a relatively high-affinity Fe transport epithelium. The majority of the absorbed Fe in the skin remained in the tissue, whereas in the gut, most absorbed Fe was found in the serosal fluid, suggesting distinct mechanisms of Fe handling between the two epithelia. To determine if reduced dissolved oxygen altered Fe transport, hagfish were subjected to hypoxia for 24 h, before Fe transport was again assessed. Hypoxia had no effect on Fe transport across gut or skin, likely owing to the relative lack of change in haematological variables, and thus an unaltered Fe demand under such conditions. These data are the first to kinetically characterise the absorption of a nutritive trace metal across the epithelia of hagfish and add to the growing understanding of the role of the skin in nutritive transport in this group.

1. Introduction

The absorption of nutrients is an essential process in all animals. Among vertebrates, this is a role that is restricted to the gastrointestinal epithelium, with a single exception. Hagfishes are primitive vertebrates that have the unique ability to use their cutaneous epithelium for organic nutrient uptake (Glover et al., 2011a, 2016; Stephens, 1968). In fact, the skin of hagfish performs a number of transport functions including the elimination of nitrogenous waste (Braun and Perry, 2010; Clifford et al., 2014), the uptake of the inorganic nutrient phosphorus (Schultz et al., 2014), and the potentially harmful absorption of the toxic metal nickel (Glover et al., 2015).

While there is growing evidence of the importance of the hagfish skin as a transport epithelium (Glover et al., 2013), there is remarkably little understanding of transport processes in the hagfish gastrointestinal tract (Glover and Bucking, 2015). To date, physiological evidence has shown that phosphate (Schultz et al., 2014), glucose (Bucking et al., 2011), and the amino acids L-alanine and glycine (Glover et al., 2011b) are absorbed by the gut of hagfish. However, there is nothing known regarding the absorption of nutritive trace metals across the hagfish digestive tract, or indeed, any hagfish epithelium.

Iron (Fe) is an important nutritive trace metal. It has critical functions as a co-factor in a range of enzymes and is particularly important in respiration where it acts as the oxygen-binding entity in haemoglobin. In general, Fe absorption in teleost fishes is similar to that in mammals (Bury et al., 2003). Molecular and physiological evidence suggests that apical Fe transport is achieved in the ferrous state (Fe(II)), via a proton-dependent transporter (divalent metal transporter-1; DMT1, also known as Nramp2 (SLC11A2); Cooper et al., 2006b, 2007; Dorschner and Phillips, 1999; Kwong and Niyogi, 2008; Kwong et al., 2010, 2013), which also facilitates the uptake of a number of other divalent metals (Cooper et al., 2007; Kwong and Niyogi, 2009). Basolateral transport is achieved by IREG (iron-regulated transporter, also known as ferroportin (SLC40A1); Cooper et al., 2006b; Donovan et al., 2000). The principal route of Fe uptake is likely to be the diet, due to the higher concentration and bioavailability of Fe via this route (Bury et al., 2003).

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However, although bioavailable Fe levels are very low in natural waters (Stumm and Morgan, 1996), the gills of teleost fish also play an important role in Fe acquisition (Bury and Grosell, 2003a, 2003b), especially in developing fish without viable digestive tracts (Andersen, 1997). The mechanisms of Fe absorption in hagfish, and the relative importance of the gut and alternative transport epithelia in this process, are unstudied.

A remarkable characteristic of hagfish is their extreme hypoxia tolerance (Forster, 1998). While buried in sediments, or burrowing into a decaying seafloor carcass, hagfish may experience significantly reduced environmental oxygen concentrations (Martini, 1998). In mammals, hypoxia is linked to changes in Fe metabolism (Chepelev and Willmore, 2011; Haase, 2010). For example, DMT1 contains hypoxia regulatory elements in its promotor region (Shah et al., 2009), and following hypoxia DMT1 expression increases (Li et al., 2008). Hypoxia-induced changes in Fe metabolism are mechanisms that aid in improving oxygen transport, which relies on the Fe-based respiratory pigment haemoglobin (Windsor and Rodway, 2007). Previous work has shown that hagfish epithelial transport processes are responsive to hypoxia (Bucking et al., 2011). Following exposure to 24 h of hypoxia, Pacific hagfish exhibited increases in glycine transport across the gill and the gut epithelia. This effect was accompanied by an increase in brain glycine, which is thought to induce metabolic depression and/or act as a cytoprotective mechanism (Bucking et al., 2011).

The current study sought to characterise the epithelial transport of Fe in the Pacific hagfish (*Eptatretus stoutii*). Specifically, the concentration-dependent kinetics of Fe uptake were examined in the skin and intestine utilising *in vitro* techniques. In addition, by analogy with mammals, it was hypothesised that characteristics of Fe transport in hagfish would change with hypoxia, a mechanism that might act to increase oxygen-carrying capacity of the blood and contribute to their remarkable tolerance of this phenomenon. This study highlighted key differences in the magnitude of uptake and handling of Fe between transport epithelia, but showed that transport was independent of environmental oxygen concentrations.

2. Materials and methods

2.1. Animals

Hagfish (*Eptatretus stoutii*) were collected via baited traps from Barkley Sound (Fisheries and Oceans Canada permits XR107 2012 and XR107 2013), off the west coast of Vancouver Island, near Bamfield. Following capture, fish were held in 500-L tarpaulin-covered tanks, receiving flow-through natural seawater at 12 °C, at Bamfield Marine Sciences Centre (BMSC). Hagfish were held for at least a week, unfed, prior to any experimentation. All procedures were approved by the BMSC Animal Care and Use Committee (RS-12-8; RS-13-11).

2.2. Transport assays

Hagfish (N = 6; ~70 g) were euthanised via anaesthetic overdose (2 g L^{-1} 3-aminobenzoic acid ethylester; MS222). The gut (posterior to hepatic portal vessel) and dorsal medial skin were then removed, and each was divided into six sections for Fe transport assays. For each animal, a complete kinetic curve was run with one concentration per tissue section, with sections randomised between test concentrations. For the gut, the nominal Fe concentrations tested were 2, 5, 20, 50, 200, and 500 μM, while for the skin, the nominal concentrations were 1, 2, 5, 10, 20, and 100 μM, all made up in filtered seawater (in mM: Na, 492; K, 9; Ca, 12; Mg, 50; Cl, 539; pH 8.0). These concentrations were achieved by dilution of a stock solution consisting of $FeCl_3$ and ascorbic acid in a 1:20 ratio, that was made fresh daily, and which was spiked with ~5–10 $\mu C_i\ mL^{-1}$ of ^{59}Fe (as $FeCl_3$; Perkin Elmer). The ascorbate ensured that Fe was presented to the fish as Fe(II) (Bury et al., 2001).

Gut transport was assessed using an intestinal gut sac technique (Glover et al., 2011b). Briefly, intestinal sections of ~3–5 cm were sutured closed at one end with surgical silk, and in the other was placed a short section (~3 cm) of cannula tubing (PE50; Intramedic, Clay Adams), which was flared at the inserted end. This was secured in place with surgical silk, and the cannula was used to introduce a small volume (0.5–3 mL) of the test Fe concentration in filtered sea water. The cannula was then heat-sealed and the sac was suspended in 50-mL of aerated hagfish Ringer (in mM: NaCl, 474; KCl, 8; $CaCl_2 \cdot 2H_2O$, 5; $MgSO_4 \cdot 7H_2O$, 3; $MgCl_2 \cdot 6H_2O$, 9; $NaH_2PO_4 \cdot H_2O$, 2.06; $NaHCO_3$, 41; and glucose, 5; pH 7.6). After a 2-h assay, a sample of the hagfish Ringer (serosal fluid) was taken for assessment of transported radiolabel. The gut sac was split open, rinsed in a high Fe solution (10 mM as Fe(II)) to remove any adsorbed ^{59}Fe, scraped free of any adhering mucus and epithelial tissue, and surface area was measured (see Glover et al., 2003). Tissue was then placed in a scintillation vial to which was added 2 mL of 2 N HNO_3 and the samples were digested for 48 h at 65 °C. Samples of Fe stock solutions (for determination of specific activity), the serosal fluid, and the digested tissue had scintillation fluor added (Optiphase for water samples, UltimaGold for tissue digests; Perkin Elmer), before they were counted in a liquid scintillation counter (LS6500; Beckman Coulter). Quench correction was applied based on the external standards ratio method.

Skin transport was assessed via a modified Ussing technique that has been described in detail previously (Glover et al., 2011a). Briefly, skin was stretched across the top of a 15 mL vial, and secured by screwing the lid, with an aperture cut into it, back in place. Ten millilitres of hagfish Ringer was added inside the vial, and the apparatus was secured upside-down, such that the external skin surface was immersed in an aerated bathing medium containing the appropriate radiolabelled Fe concentration in filtered seawater (prepared as described above). Transport was assessed for 2 h after which 5 mL of hagfish Ringer was removed for scintillation counting, and the skin was digested in 2 mL of 2 N HNO_3 for 48 h at 65 °C. Bathing medium, serosal fluid, and tissue digest samples were then assessed for ^{59}Fe activity as described above.

Uptake of Fe in both tissues was calculated using the following equation:

$$\text{Uptake}\left(\text{nmol cm}^{-2}\text{h}^{-1}\right) = (\text{cpm}/\text{SAct})/\text{SA}/\text{t}$$

where cpm is accumulation of ^{59}Fe radioactivity in the tissue and serosa, SAct is the specific activity (cpm $nmol^{-1}$), SA is surface area (cm^{-2}), and t is time (2 h).

2.3. Hypoxia exposure

To examine the effects of hypoxia on Fe transport, individual hagfish were put in sealed 600-mL plastic containers containing seawater, and placed in a water bath to maintain the temperature at 12 °C. Hagfish designated as normoxic (N = 7) had aeration provided through a hole in the lid, whereas chambers containing hypoxic hagfish (N = 7) were not aerated, and thus became hypoxic as the animal gradually consumed the oxygen in the chamber. After 24 h, hypoxic hagfish had all depleted oxygen partial pressure (PO_2) to levels less than 10 mmHg. Both groups were then removed, euthanised by anaesthetic overdose (2 g L^{-1} MS222), and a 1–5 mL blood sample was withdrawn from the subcutaneous caudal sinus. A heparinised capillary tube was used to draw a subsample of whole blood that was then spun at 5000 × *g* in a haemofuge, for determination of haematocrit. A 100 μL subsample was removed, added to Drabkin's reagent, and analysed for haemoglobin content (mg mL^{-1}) via measuring absorbance at 540 nm after a 24 h incubation in the dark at 4 °C. A 20 μL subsample was removed for an erythrocyte count via a haemocytometer. The remaining blood was immediately analysed for PO_2 (mmHg) via an oxygen electrode (Cameron Instruments) connected to a AM Systems Polarographic Amplifier

(Model 1900) digital dissolved oxygen meter, that was calibrated daily at the experimental temperature. Mean cell haemoglobin content (g 100 mL^{-1}), mean cell haemoglobin (μg), and mean cell volume (fL) were calculated as described by Houston (1997).

Following blood sampling, medial skin and medial intestinal sections of both normoxic and hypoxic hagfish were dissected and subjected to Fe transport assays as described above. However, only two concentrations of Fe were tested: 2 and 20 μM. These concentrations were chosen on the basis of the outcomes of the kinetic characterisation of uptake, in that they represented concentrations where uptake was dominated by a carrier-mediated component.

2.4. Data analysis

Data are expressed as means ± SEM (N) where each N represents a different hagfish. Analysis of concentration-dependent kinetic curves was performed using SigmaPlot (ver. 11.2; Systat). Raw data were fitted to linear, hyperbolic, or sigmoidal equations to identify the curve that produced the highest r^2 value. Differences in haematological measurements and Fe uptake rates between normoxic and hypoxic hagfish and differences in Fe uptake into tissue and serosal compartments were analysed via Student's unpaired two-tailed t test, or where data did not conform to normality and homogeneity of variance, via Mann–Whitney U test. Data were considered significantly different at an alpha level of 0.05.

3. Results

Gut Fe uptake (i.e. that which accumulated in the gut tissue and serosal fluid) increased with increasing exposure concentration (Fig. 1). The best fit to all raw data was a hyperbolic curve: Fe uptake = $(J_{max} \times [Fe])/(K_m + [Fe])$. Subsequent removal of the highest tested concentration increased the goodness of fit (r^2 increased from 0.50 to 0.61), indicating a saturable component at low concentrations (i.e. Michaelis–Menten kinetics), superseded by a linear, diffusive component at high concentrations. Modelling the saturable relationship over exposure concentrations up to 200 μM revealed a J_{max} (maximal rate of Fe transport) of 0.57 ± 0.20 (6) nmol cm^{-2} h^{-1}, and an affinity constant (K_m; substrate concentration required to give half J_{max}) of 137 ± 97 (6) μM.

A similar pattern to that of the gut was revealed for skin Fe uptake (Fig. 2). Again, a hyperbolic curve (Fe uptake = $(J_{max} \times [Fe])/(K_m + [Fe])$) was the best fit to all data, and with removal of the highest tested concentration, an improvement in fit was achieved (r^2 increased from 0.63 to 0.86). This indicated a saturable component at lower tested Fe concentrations (up to 20 μM), superseded by a linear component as Fe exposure concentration increased. The modelled J_{max} was 0.81 ± 0.21 (6) cm^{-2} h^{-1} and the K_m was 9.4 ± 5.2 (6) μM.

Where tested concentrations facilitated direct comparison, cutaneous Fe uptake was greater than that for intestine. For example, at an exposure concentration of 20 μM, the total absorbed (tissue + serosal fluid) Fe was 0.55 nmol cm^{-2} h^{-1} for skin, but just 0.08 nmol cm^{-2} h^{-1} for gut (Fig. 3). There were also distinct differences between skin and gut in terms of partitioning of Fe. In skin, 6–18% of total absorbed Fe was present in the serosal compartment, whereas for gut assays, this percentage was 71–91% (data not shown). In fact, despite the overall significant differences in the total Fe uptake rate, there were no significant differences in the serosal uptake rate (Fig. 3).

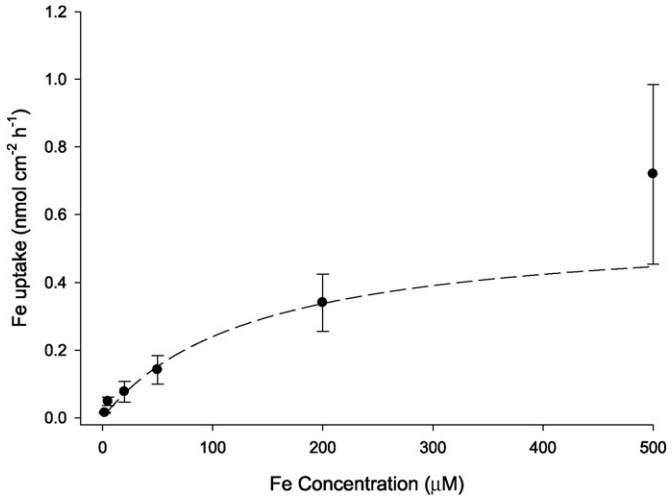

Fig. 1. Concentration-dependent uptake of Fe (nmol cm^{-2} h^{-1}) across the gut of the Pacific hagfish, as determined via an *in vitro* gut sac technique. Plotted points represent the means (±SEM) of 6 replicates. Dashed line represents the saturable component of Fe uptake $(J_{max} \times [Fe])/(K_m + [Fe])$ as determined by curve fitting analysis (SigmaPlot ver. 11.2; see Results).

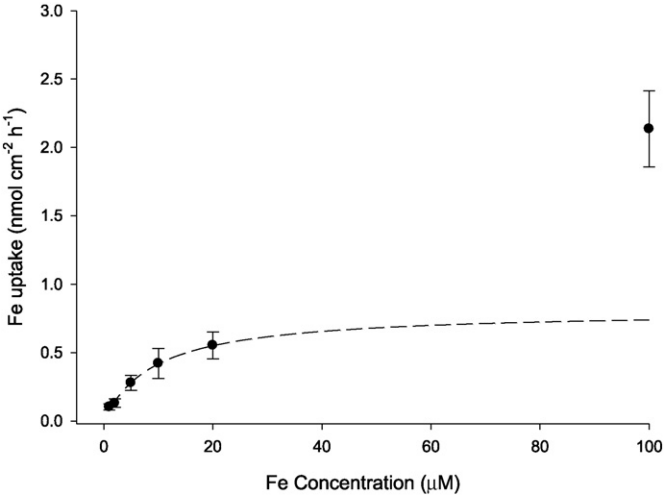

Fig. 2. Concentration-dependent uptake of Fe (nmol cm^{-2} h^{-1}) across the skin of the Pacific hagfish, as determined via an *in vitro* modified Ussing chamber technique. Plotted points represent the means (±SEM) of 5–6 replicates. Dashed line represents the saturable component of Fe uptake $(J_{max} \times [Fe])/(K_m + [Fe])$ as determined by curve fitting analysis (SigmaPlot ver. 11.2; see Results).

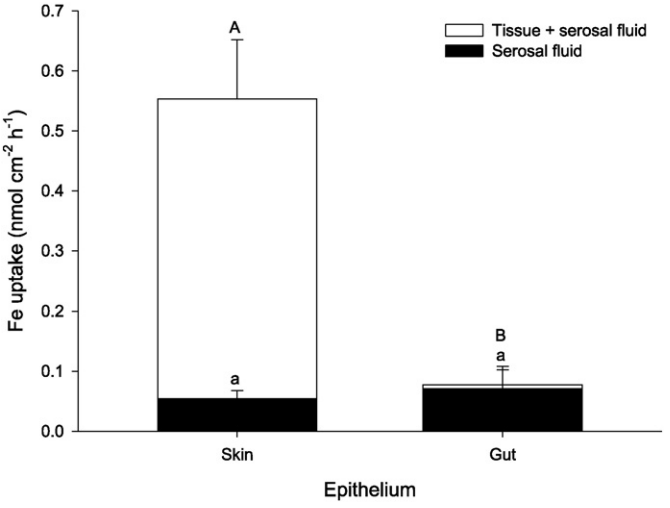

Fig. 3. Fe uptake (nmol cm^{-2} h^{-1}) into serosal or serosal + tissue compartments of Pacific hagfish skin or gut exposed to 20 μM of Fe, as determined via *in vitro* transport assays. Plotted points represent the means (±SEM) of 5–6 replicates. Serosal uptake rates sharing lowercase letters, and serosal + tissue uptake rates sharing upper case letters are not significantly different, as determined by Mann–Whitney U test, at an alpha level of 0.05.

Hagfish that were exposed to hypoxia for 24 h displayed a significantly reduced blood PO_2 relative to hagfish that were kept under identical conditions, but in aerated chambers (4 versus 78 mmHg; Fig. 4A). This exposure resulted in no other significant changes in any haematological measure (Fig 4B–G), except for red blood cell count, which was significantly reduced from 0.20×10^6 to 0.15×10^6 cells μL^{-1} (Fig. 4D). Similarly, there were no changes in Fe uptake characteristics following hypoxia exposure in either gut (Fig. 5) or skin (Fig. 6). This was true of both Fe concentrations tested (2 and 20 μM).

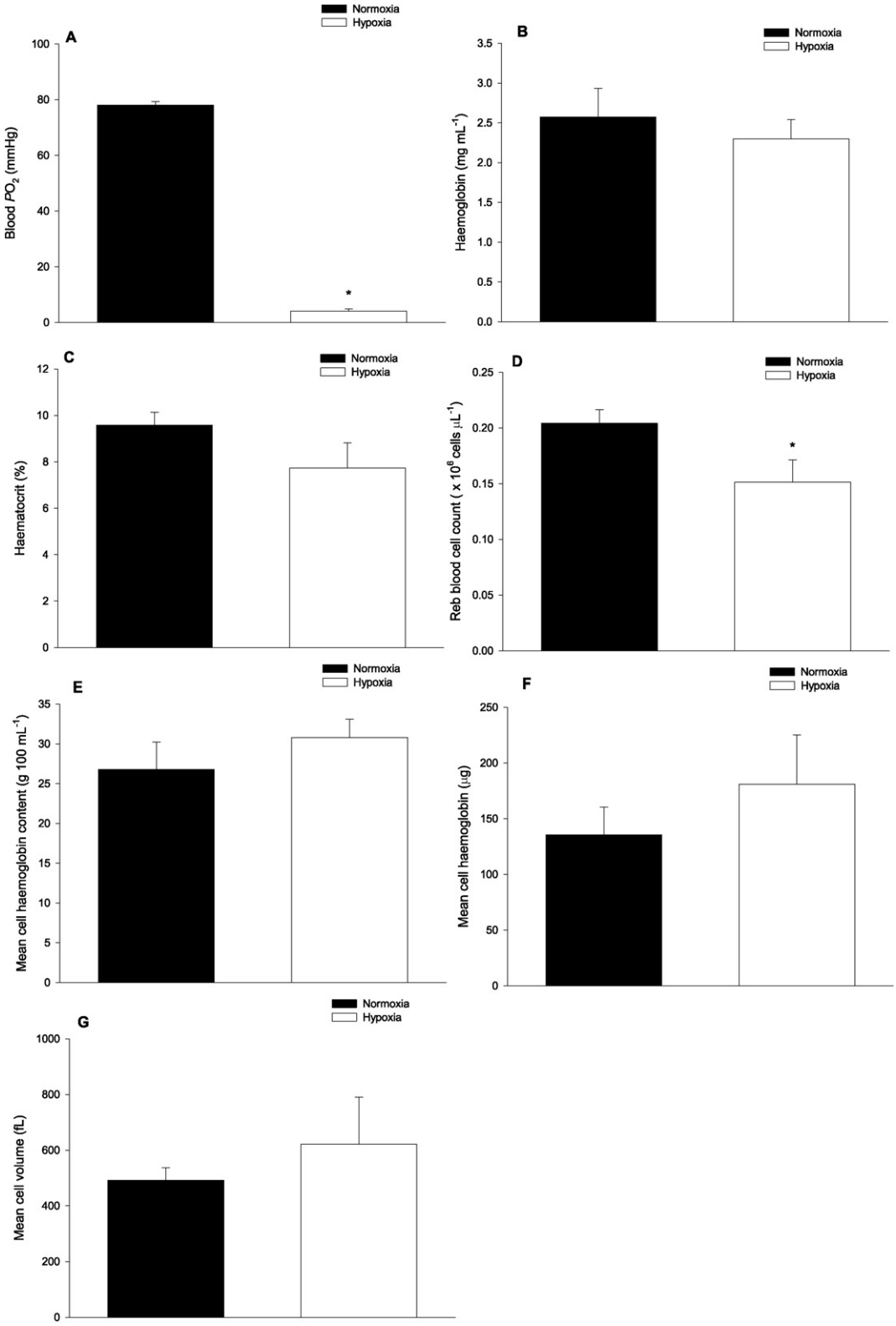

Fig. 4. Blood PO_2 (A; mmHg), haemoglobin (B; mg mL^{-1}), haematocrit (C; %), red blood cell count (D; 10^6 cells uL^{-1}), mean cell haemoglobin content (E; g 100 mL^{-1}), mean cell haemoglobin (F; μg), and mean cell volume (G; fL) in sinus blood of Pacific hagfish exposed to normoxia (black bars) or hypoxia (white bars) for 24 h. Plotted points represent the means (±SEM) of 5–7 replicates. Statistically-significant differences (*) between normoxia and hypoxia were determined by t-test or Mann–Whitney U test, at an alpha level of 0.05.

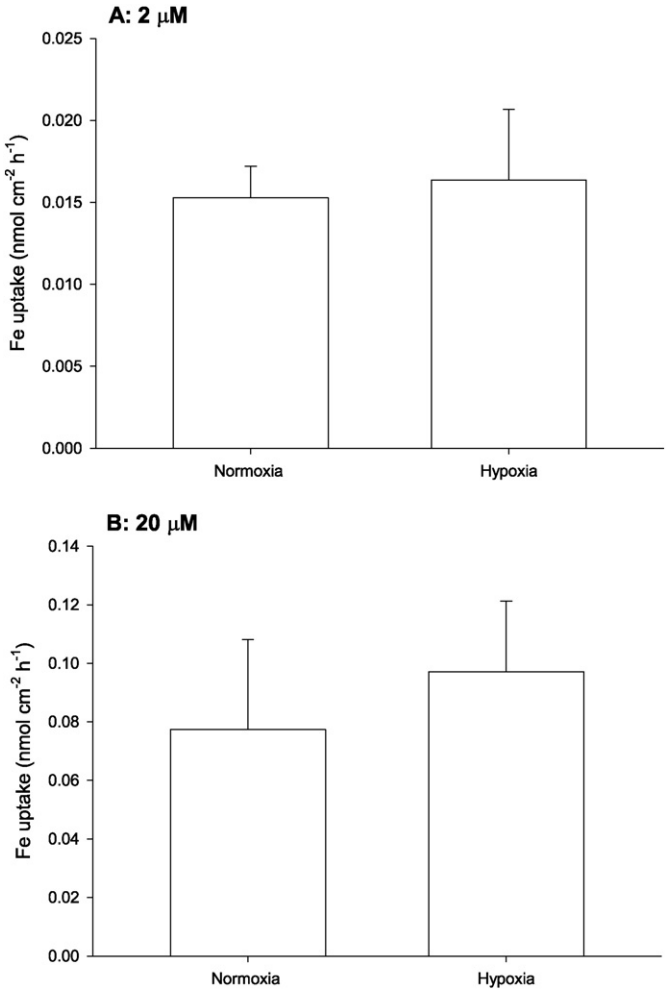

Fig. 5. Effect of 24 h hypoxia on Fe uptake rates (nmol cm^{-2} h^{-1}) across the gut of the Pacific hagfish, as determined via an *in vitro* gut sac technique at Fe exposure concentrations of 2 μM (A) or 20 μM. Plotted points represent the means (±SEM) of 5–7 replicates.

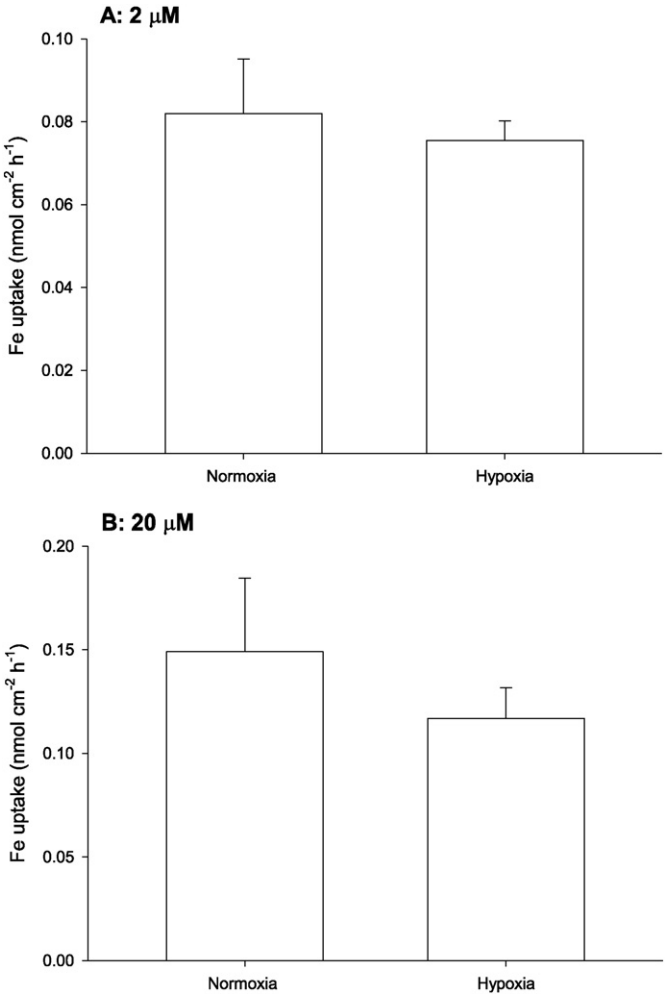

Fig. 6. Effect of 24 h hypoxia on Fe uptake rates (nmol cm^{-2} h^{-1}) across the skin of the Pacific hagfish, as determined via an *in vitro* modified Ussing chamber technique at Fe exposure concentrations of 2 μM (A) or 20 μM. Plotted points represent the means (±SEM) of 5–7 replicates.

4. Discussion

Hagfish were able to absorb Fe (as Fe(II)) across both the skin and the gut epithelia in a concentration-dependent manner, suggestive of uptake via specific transport pathways. This is the first demonstration of nutritive trace metal transport across the epithelia of hagfish. The skin was found to take up more Fe than the gut for a given Fe exposure concentration, but most Fe remained trapped in the tissue. Conversely, although rates of Fe uptake in the gut were significantly lower than those of the skin, the majority of absorbed Fe was located in the serosal fluid, indicating distinct mechanisms for handling Fe in these two tissues. In opposition to our hypothesis that hypoxia exposure would induce greater demand for oxygen carrying capacity and lead to enhanced epithelial Fe transport, no changes were observed when hagfish were exposed to reduced dissolved oxygen.

4.1. Fe transport across gut and skin epithelia

Transport of Fe across the hagfish gut was biphasic (Fig. 1). At concentrations lower than 200 μM, uptake was saturable with an uptake affinity (K_m) of 137 μM, but beyond that a component that was linear, at least over the tested concentration range, dominated. In freshwater rainbow trout gut sacs, a similar biphasic pattern of uptake was noted, although variability in data precluded calculation of kinetic parameters (Kwong and Niyogi, 2008). However, using an isolated enterocyte technique, Kwong et al. (2010) later determined an affinity constant (K_m) of 1.7–3.4 μM for Fe uptake, significantly less than that recorded for hagfish gut in the current study. This may reflect increased competition related to the higher divalent cation levels in the exposure medium of the hagfish assay (seawater) compared to the freshwater trout enterocyte assay (Cortland saline). However, it is also worth noting that the relative pattern of affinity constants here (lower Fe transport affinity in hagfish gut (i.e. higher K_m) than in teleost fish gut) is similar to that noted for amino acid transport (Glover et al., 2011b). Consequently, this could be a general pattern for nutrient uptake in the hagfish gut and may reflect the presence of the skin as an accessory nutrient absorption surface.

The current study revealed that the skin of hagfish is capable of absorbing Fe, as Fe(II), directly from the water (Fig. 2). Given that most Fe in oxic natural environments is present as insoluble Fe(III) oxides (Stumm and Morgan, 1996), the ability of hagfish skin to take up Fe(II) would seem to have limited relevance. In fact, levels of dissolved Fe in seawater are considered to be in the high picomolar to low nanomolar range (Moore and Braucherm, 2007), well below the K_m for transport across hagfish skin (9.4 μM). In teleost fish, which are capable of branchial Fe transport, this paradox between apparent lack of substrate and a specific transport system for substrate uptake is partly explained by photochemical reduction, which produces a small proportion of Fe(II) that is bioavailable to the gill (Cooper and Bury, 2007). This is unlikely to be of importance in the benthic settings inhabited by

hagfish, as these may be below the point of light penetration. However, it is thought that in teleost fish, epithelial mucus may bind Fe(III) trapping it close to epithelial reductases (Carriquiriborde et al., 2004), while Fe(III) bound to naturally occurring ligands in the water such as dissolved organic matter may also act as a source of Fe to the gills (Cooper and Bury, 2007). A similar phenomenon could also occur in hagfish skin. Importantly, it is worth noting that hagfish may burrow into anoxic mud (Martini, 1998), where Fe(II) will be a more prevalent form of Fe (Stumm and Morgan, 1996), and which would be in direct contact with the skin. Similarly, immersion into anoxic decaying carcasses may also represent a source of bioavailable Fe to the hagfish skin.

Relative to the transport of Fe across the gut, cutaneous Fe uptake was a high-affinity process, with a K_m of 9.4 μM. Affinity constants for transport of substrates across the skin of the Pacific hagfish have been determined for the toxic metal nickel (42 μM; Glover et al., 2015), the inorganic nutrient phosphorus (930 μM; Schultz et al., 2014), and the amino acids L-alanine (262 μM; Glover et al., 2011a) and glycine (465 μM; Glover et al., 2011a). Of all substrates examined to date, Fe therefore has the highest affinity for uptake. Conversely, the transport capacity (J_{max}) of 0.81 nmol cm^{-2} h^{-1} is an order of magnitude less than that of other transported nutrients (10–22 nmol cm^{-2} h^{-1}; Glover et al., 2011a, 2015; Schultz et al., 2014). While the skin of hagfish represents a relatively high affinity pathway in comparison to the hagfish gut, it is significantly lower in affinity than the gill of teleost fish. For example, Cooper and Bury (2007) measured an affinity constant of 21 nM for Fe uptake in zebrafish gill. It is likely that differences in uptake affinities between epithelia represent differences in substrate availability. The diet is likely to contain the highest concentrations of Fe, while waterborne levels are likely to be very low (see above). However, by virtue of inhabiting anoxic sediments and decaying carcasses, the environmental levels of Fe(II) available to the hagfish skin are likely to be elevated relative to those of the fish gill, thus the lower affinity for Fe uptake.

The ability of the skin of hagfish to absorb Fe is put into context by comparison of uptake rates to the hagfish gut at common tested concentrations. At 20 μM, the skin took up almost 7-fold more Fe than did the gut (Fig. 3). However, absorbed Fe partitioned differently in the two tissues. In the gut assays up to 91% of the absorbed Fe was present in the serosal fluid, but for skin, the serosal accumulation peaked at 18% of the absorbed Fe. This pattern of Fe partitioning was consistent across all tested concentrations for both tissues (data not shown). This suggests that the pathway by which Fe traverses these epithelia differs. It is notable that in mammalian systems, hephaestin, an oxidase enzyme, converts ferrous iron back to ferric iron after basolateral translocation, and this then facilitates binding to transferrin, a plasma protein that delivers Fe around the body (Garrick, 2011). It is therefore possible that the skin may lack such an oxidase, effectively trapping Fe in its ferrous form at the basolateral surface, reducing the gradient for diffusion through the IREG channel, and thus inhibiting serosal transfer. Similarly, the lack of a binding moiety in the serosal fluid may also allow for the build-up of local Fe concentrations, that may inhibit basolateral transfer. This hypothesis is supported by the work of Bury et al. (2001). These authors noted a lack of serosal fluid accumulation of Fe in *in vitro* gut transport assays, but in *in vivo* studies, Fe moved readily into the blood. They suggested the presence of hephaestin/transferrin *in vivo* was responsible for this difference (Bury et al., 2001). This was a finding supported by data examining intestinal transport of Fe in the intestine of the Gulf toadfish (Cooper et al., 2006a). However, arguing against this explanation is the fact that both assays in the current study lacked an Fe-binding moiety in the serosal fluid, yet the patterns of Fe partitioning differed significantly.

In a study of nickel transport across the skin of hagfish, a similar high proportion of metal was found to be retained in the tissue, rather than transported into the serosal fluid (Glover et al., 2015). In that study, the retention of nickel in the tissue was attributed to the presence of intracellular binding ligands prohibiting passage through the epithelium, and/or a lack of basolateral transporters to facilitate further transfer. In teleost fish and mammals, IREG is believed responsible for the passage of Fe across the basolateral membrane (Bury et al., 2003; Garrick, 2011). Based on transport data in the current study, it is hypothesised that IREG expression would be much higher in the gut than in the skin of hagfish, a scenario that requires further research.

It is important to note that the current study would require higher tested Fe concentrations in order to confirm that the second component of uptake kinetics in both skin and gut is, in fact, a linear, diffusive pathway of uptake. However, even if this component was shown to saturate, indicative of a second carrier-mediated pathway for Fe uptake, it is unlikely to be a pathway that performs a physiologically important role given that it would be relevant only at unrealistic environmental Fe concentrations.

4.2. Hypoxia did not affect Fe transport

In the current study, exposure of hagfish to hypoxia for 24 h, had no effect on Fe transport across either skin or gut. In mammalian systems, hypoxia induces increased Fe transport across the gut (Mastrogiannaki et al., 2009). However, hypoxia in mammals is also associated with a number of important haematological changes, including an increase in haemoglobin and haematocrit (Windsor and Rodway, 2007). These changes increase the demand for Fe, based on its role as the oxygen-binding moiety of haem in the respiratory pigment haemoglobin. This feedback loop is therefore a mechanism that facilitates increased oxygen delivery under environmental conditions where this vital process is impaired (e.g. high altitude). Hagfish, unlike mammals, are highly hypoxia tolerant, and in fact can withstand extended periods of anoxia (Forster, 1998). There are a number of factors likely underlying this tolerance, including a very low metabolic rate (Drazen et al., 2011; Forster, 1990), and a high affinity of hagfish haemoglobin for oxygen (Manwell, 1958). As such, hagfish appear to have a reduced need for significant changes in haematology, and consequently a reduced need for altered Fe transport. The relative lack of change in haematological variables in hypoxia-exposed hagfish has been noted previously (Bernier et al., 1996; Nikinmaa, 2001).

It is worth noting that in the current study, blood was sampled from the subcutaneous sinus. Hagfish are capable of independently regulating the sinus circulation from the central circulation (Forster, 1997), and in the current study, a decline in erythrocyte count in the sinus was recorded. This may be indicative of preferential sequestration of red blood cells in the central circulation, a mechanism that would conserve oxygen for key tissues. Similarly, following a short (1 h) hypoxia exposure, Bernier et al. (1996) noted small decreases in haematocrit and haemoglobin contents in hagfish blood, although these authors did not state whether sinus or central blood was taken. Therefore, it could be argued that collection of sinus blood may not adequately identify changes in haematological variables. However, irrespective of whether haematological measures are altered in hypoxia-exposed hagfish, it is apparent that no changes in Fe uptake occur.

5. Conclusion

The current study contributes to the growing evidence supporting the skin as an important transport epithelium in hagfish. Acting as a locus for the uptake of organic and inorganic nutrients, and toxicants, the skin is likely to play key roles in nutrition and susceptibility to pollution. It is likely that having the skin as an accessory transport surface has influenced the transport characteristics of the gut, which in general displays reduced affinities in comparison to teleost fish. However, whether the skin acts as a true uptake surface, or simply sequesters nutrients such as Fe in the skin tissue, requires further investigation. Future analysis should also identify the molecular entities underlying the transport of substrates, as such work will contribute towards an understanding of the evolution of nutrient transport systems, and whether

nutrient absorption across the skin is an ancestral vertebrate trait, or represents a specific adaptation to the unusual feeding habits of the hagfishes.

Conflict of Interest

The authors declare no conflicts of interest.

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