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Competition between selenomethionine and methionine absorption in the intestinal tract of green sturgeon (*Acipenser medirostris*)

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ABSTRACT

L-Selenomethionine (SeMet) is a dominant form of selenium (Se) found in organisms at all levels of aquatic food chains and a key source of Se bioaccumulation and ecotoxicity. In mammals, intestinal absorption of SeMet is at least partly via the Na^+-dependent neutral amino acid transporter. The mechanism of SeMet absorption and competitive effects of other dietary components on SeMet absorption in fish are unknown. Thus the *in vitro* uptake rates of L-methionine (Met) and the competitive effect of SeMet on Met absorption, an indicator that SeMet uses the same nutrient transporter(s) for absorption, in the various regions of the green sturgeon (*Acipenser medirostris*) intestine were investigated using intact tissues (a modified everted sleeve method). Intestinal tissue was incubated in Ringer's solution containing 0–10 mmol L^{-1} Met or SeMet ($n = 5$ for each substrate's concentration and intestinal region), respectively, as well as constant tracer levels of isotope-labeled Met. The data indicate that SeMet uptake was mediated by the same transporter(s) as Met and that the absorption kinetics were similar for both substrates. When there were differences in absorption they appeared to be mostly due to higher permeability (passive uptake) of the tissue for Met than for SeMet, particularly in the pyloric caeca (PC) and distal intestine (DI). Maximum rates of absorption, on the other hand, tended to be higher for SeMet than Met in the mid intestine and DI, whereas differences in affinity for the transporters varied between these tissues but were very similar in the PC. These differences may be due to differences in regional intestinal characteristics such as amount of mucus secreted and degree of tissue contraction, and/or substrate differences regarding solubility in and movement through the mucus, influence on tissue contraction, permeability through membranes or between cells, intracellular metabolism, as well as basolateral transport. Interestingly, an increasing proximal-to-distal gradient for rates of methionine and putative SeMet absorption was observed in green sturgeon which differs from the declining gradient usually observed for substrate absorption in other fish species and mammals.

1. Introduction

Among the various selenium-containing compounds, L-selenomethionine (SeMet) has been found to be a dominant form of selenium (Se) found in organisms at all levels of aquatic food chains and it has been cited as a key form of organic Se leading to Se bioaccumulation and ecotoxicity (Hamilton et al., 1990; Panter et al., 1996; Fan et al., 2002; Hamilton, 2004). The endurance of selenium through all levels of the food chain is facilitated when the sulfur in sulfur-containing amino acids is substituted with selenium and the resulting selenoamino acids are readily incorporated into primary protein structures. However, very little is known about the toxicokinetics and chronic toxicity of Se in fish species when ingested in the SeMet form. A limited number of studies have explored the chronic toxicity of SeMet in fish (Hamilton et al., 1990; Hamilton, 2004; National Research Council, 2005; Tashjian et al., 2006, 2007), however, the mechanism of SeMet absorption and competitive effects of other dietary components on SeMet absorption in fish are unknown.

Se uptake by the intestine appears to be most efficient when the element is conjugated to a sulfuric amino acid such as methionine (Panter et al., 1996; Schrauzer, 2000; Leblondel et al., 2001). Absorption of SeMet in intestinal tissue through the amino acid transporter system was first suggested by McConnell and Cho (1967). In later studies, its absorption in the gastrointestinal tract of higher organisms was found to occur mainly by the Na^+-

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dependent neutral amino acid transporter, which transports many neutral amino acids (Wolffram et al., 1989; Panter et al., 1996; Schrauzer, 2000). Additional confirmation of the analogous transport of SeMet and methionine (Met) was provided by subsequent studies (Wolffram et al., 1989; Vendeland et al., 1994; Leblondel et al., 2001), including specific contribution by symport system B^0, as well as the exchanger (antiport) system $b^{0,+}$ (Nickel et al., 2009). Evidence demonstrating similar kinetics (e.g. maximal transport velocity and the Michaelis constant) of SeMet and Met absorption has been demonstrated (Wolffram et al., 1989; Nickel et al., 2009).

The studies which have investigated the dynamics of SeMet absorption in the intestinal tissue have been conducted with mammals or mammalian-based systems (Wolffram et al., 1989; Vendeland et al., 1994; Panter et al., 1996; Schrauzer, 2000; Leblondel et al., 2001; Nickel et al., 2009). SeMet absorption dynamics in fish intestinal tissue may differ due to differences between mammals and fish in gastrointestinal morphology and physiology, as well as by other factors such as body temperature and metabolism. Green sturgeon (*Acipenser medirostris*) was chosen as the model species for this purpose due to: (1) availability; (2) similarity to the habitat, lifecycle and feeding ecology of white sturgeon (*Acipenser transmontanus*), in which elevated levels of selenium in liver and muscle tissue have been reported (Urquhart and Regalado, 1991; Linville et al., 2002); and (3) declining population numbers of the southern population of the species that lives along the pacific coast of the U.S. and Canada to the extent that the species is considered "threatened" under the U.S. Endangered Species Act (Klimley et al., 2007). A combination of biological characteristics such as longevity, late sexual maturation, and long intervals between spawning make this species especially susceptible to accumulation and toxic effects of pollutants, e.g. selenium (Adams et al., 2007; Gessner et al., 2007). A better understanding of the toxicokinetics may contribute to assessing the possible impact of pollutants on accumulation and toxicity in sturgeon, as well as aid in developing conservation measures to protect the species. The objective of this study was to determine the mechanism and kinetics of Met absorption in whole intestinal tissues, and the competitive effect of SeMet on Met absorption, an indicator that SeMet uses the same nutrient transporter(s) for absorption, in green sturgeon through the use of an *in vitro* technique.

2. Materials and methods

2.1. Organism maintenance and experimental setup

Fifty (1–2 kg) green sturgeon (*A. medirostris*) were donated by Dr. J.J. Cech Jr., Department of Fish and Wildlife Conservation Biology, University of California, Davis (UCD). The fish were from artificially spawned wild broodstocks and the hatchlings, larvae, and juveniles were reared to the appropriate size in the Center for Aquatic Biology and Aquaculture at UCD (Van Eenennaam et al., 2001). They were maintained in 620-L circular fiberglass tanks supplied with aerated well water (18–19 °C) at a flow rate of 15 L min^{-1} and fed a commercial trout feed (Silver Cup, Nelson and Sons, Murray, UT) for at least a month prior to the experiment. Care and tissue sampling of sturgeon followed protocols approved by the UCD Campus Animal Care and Use Committee.

2.2. In vitro Met absorption and competition study with SeMet

Prior to sampling, each fish was anaesthetized with MS222 (tricaine methanesulfonate) at an approximate dose of 400 mg kg^{-1} body weight. They were then euthanized by cervical dislocation before their body masses were registered and the alimentary tract was excised. L-Methionine uptake characteristics as well as competition with SeMet uptake was measured using the everted sleeve technique developed by Karasov and Diamond (1983) and modified for fish by Buddington et al. (1987) and Bakke-McKellep et al. (2000) and using tissue pieces rather than sleeves. Briefly, after death the entire post-gastric alimentary tract was rapidly removed, longitudinally opened, and rinsed in fish Ringers (see below) to free the mucosa from intestinal content. All further handling of the intestinal segments and preparation of the tissue pieces was done in ice-cold, aerated (97.5% O_2 with 2.5% CO_2) fish Ringers which contained (in mmol L^{-1}) NaCl (117), KCl (5.8), $NaHCO_3$ (25), NaH_2PO_4 (1.2), $MgSO_4$ (1.2), and $CaCl_2$ (2.5). Osmolarity, as determined by freezing point depression, was 290 mosM, and pH was adjusted to 7.4 when aerated with the O_2:CO_2 mixture. All reagents were of the purest quality available (Sigma Chemical, St. Louis, MO USA). Preliminary studies (data not shown) verified that rates of Met uptake per mg tissue did not differ between everted sleeves (tubes) and pieces of tissues mounted on the sides of rods with the serosa against the rod. Met uptake over the serosa was also found to be negligible. Therefore, tissue pieces rather than everted sleeves were used to reduce the number of animals needed for the experiment. From 10 fish, 5 fish per substrate, pieces of tissue approximately 0.5 cm × 1 cm were prepared from morphologically distinct regions of the post-gastric alimentary tract – the proximal (pyloric caecum; PC), mid (MI) and distal (spiral valve) intestine (DI) – eight tissues per region. For the PC, which in sturgeon is manifested as a discrete, complexly branched structure, the tissue was sliced sagitally to obtain two serosa-backed discs of approximately the same thickness which were then divided into four tissue sections each. For the DI, the "spiral" was carefully excised and only the outer, serosa-backed wall was used. The mid intestine was defined as the region between the discrete PC and just proximal to the spiral valve of the DI. The opened MI and DI were first cut in half longitudinally and then each half was cut into approximately 1 cm long pieces. The tissue pieces were secured by silk ligatures onto grooved, solid stainless steel rods while gently stretched, with the serosal surfaces against the rods and the mucosal surface exposed. The mounted tissues were kept in ice-cold, aerated fish Ringers until incubation commenced.

For kinetic studies of L-methionine uptake, rates of absorption were measured for Met, a substrate for a putative neutral amino acid transporter, at increasing Met concentrations in the incubation solution. For this purpose, a tracer quantity (3.6 μL) of isotope-labeled Met (L-[methyl ^{14}C] methionine, specific activity 40–55 mCi $mmol^{-1}$, radioactivity 0.1 mCi mL^{-1}; product code ARC 0345, American Radiolabeled Chemicals Inc., St. Louis, MO, USA) was added to 10 mL incubation solutions containing increasing concentrations of unlabeled L-methionine (Fisher Scientific, Pittsburgh, PA USA): 0, 0.008, 0.04, 0.2, 1.0, 2.5, 5, or 10 mmol L^{-1}. Since isotope-labeled SeMet was not commercially available, putative competition between Met and SeMet for transport into the intestinal tissue was accomplished by using tracer amounts of the same isotope-labeled Met as for the Met kinetic studies with increasing levels of unlabeled SeMet (L-selenomethionine; Fisher Scientific) in the incubation solutions: also at 0, 0.008, 0.04, 0.2, 1.0, 2.5, 5, or 10 mmol L^{-1}. The varying concentrations of the substrates Met and SeMet were prepared by isoosmotic replacement of NaCl in the fish Ringer's (see above). For both parts of the experiment, tracer ^3H-labeled polyethylene glycol (1,2-^3H PEG; MW 3500–4000; American Radiolabeled Chemicals Inc.) was added for correction of ^{14}C-Met and unlabeled Met or SeMet associated with the tissue-adherent fluids but not for passive uptake of the substrates. Therefore, absorption rates reflect both carrier-dependent and carrier-independent influx pathways (Karasov and Diamond, 1983).

Tissues from each intestinal region were selected randomly for incubation in solutions containing each substrate and substrate concentration. This was done in an attempt to attain median values

despite possible proximal-to-distal gradients in absorption rates within an intestinal region. Tissue preparation was performed as quickly as possible to ensure viability *ex vivo*. Beginning 30 min after death, the tissues were incubated for 5 min in aerated Ringers containing no nutrients at the experimental temperature of 20 °C. The tissues were then suspended in tubes containing 20 °C Ringers with a specific concentration of Met or SeMet. Preliminary studies (data not shown) verified that 4 min incubations were appropriate for studying Met absorption by the PC and DI; whereas 8 min incubation times were needed for the MI before equilibration with the amino acid and PEG occurred, most likely due to the thick mucosa and large amount of mucus produced by this region. The incubation solutions were aerated and stirred to maintain tissue viability and reduce unstirred layer effects (Karasov and Diamond, 1983).

After the incubation the tissue pieces were blotted carefully on absorbent paper, removed from the rods, weighed, solubilized (Soluene 350, PerkinElmer Life and Analytical Sciences, Boston, MA, USA), scintillant added (Hionic Fluor, PerkinElmer Life and Analytical Sciences), and radioactivity detected by dual channel liquid scintillation counter (Packard Model 1900A Tri-Carb Liquid Scintillation Analyzer, Perkin Elmer, Waltham, MS, USA). The tissues were not rinsed following incubation because the diffusion coefficient of amino acids exceeds that of PEG. Thus more Met/SeMet than PEG could be removed from tissue-adherent fluids during a rinse, and this would lead to inaccuracies in the correction for adherent fluid (see Karasov and Diamond, 1983).

Due to time constraints and the need to preserve tissue viability, the weights of the whole intestinal regions from the fish used for the absorption and competition studies were not measured. Therefore, the intestinal tracts of an additional five fish, which cohabited with the previously sampled fish in the same tank, and thus held under the same environmental conditions and fed the same feed, were excised, chyme carefully removed, connective and adipose tissue dissected from the serosal surfaces, and the tissue gently blotted to remove adherent water before the weights of each region were recorded to calculate intestinal region weights relative to the body weights of the fish.

2.3. Calculations

Rates of absorption in nmol min^{-1} were calculated following Karasov and Diamond (1983) and expressed as functions of weight of the incubated tissue pieces. As an approach to verify the presence of a saturable, i.e. transporter-mediated, component of absorption, the competition between the labeled tracer (^{14}C-Met) and unlabeled Met was assessed by calculating the accumulation of tracer (^{14}C-Met) in tissue within each intestinal region for each concentration of unlabeled Met by using a slightly modified equation to that used to calculate absorption rate. Similarly, competition between tracer ^{14}C-Met and unlabeled SeMet for a common transporter was assessed. Furthermore, ratios were calculated for the tissue accumulation of tracer when present alone relative to when it was in the presence of 10 mmol L^{-1} unlabeled nutrient. Ratios greater than 1.0 therefore indicate that tracer accumulation was reduced in the presence of 10 mmol L^{-1} unlabeled nutrient. Theoretically, the higher the accumulation ratio, the higher the relative importance of a saturable transport component compared to non-saturable absorption. If there is no competition between the labeled and unlabeled substrate for transporter sites and absorption increases with concentration in a linear fashion, accumulation ratios will have a value of 1.0.

From five additional fish from the same group, wet weights of the various whole intestinal regions were measured and related to their body weights to calculate mean somatic indices. Assuming a situation of equal intake of Met and SeMet with the diet, total intestinal absorptive capacities per day relative to body weight were estimated by summing the products of rates of absorption at 1.0 mmol L^{-1} (assuming this concentration approximates mean concentrations existing in the intestinal lumen based on feed intake and methionine content in diets; see below), regional wet weight, and molecular weights for Met and SeMet. The amount of amino acids present in the gut following a meal (intake) was calculated by estimating a feed intake of 1% of body weight, and 2.5% (on a dry matter basis) of methionine in fish meal (Anderson et al., 1995).

2.4. Statistics

Kinetic parameters (V_{max}, apparent K_m, passive permeability coefficient) for Met absorption were calculated by non-linear regression analysis (Enzfitter, R. Leatherbarrow, Elsevier, BIOSOFT, UK). The data were fit to an equation for a single transporter with a passive component to account for non-saturable influx. Kinetic parameters were based on the lowest order equation that provided the best fit after examination of the residuals from predicted values. Results from the *in vitro* studies are presented as means ± S.E.M. (standard error of the mean) for each substrate, concentration and intestinal region ($n = 5$). ANOVA using JMP 6.0.0 (SAS Institute Inc., Cary, NC, USA) was used to detect effects of intestinal region and substrate on rates of absorption and tracer accumulation. Comparisons across means among treatments were performed using the Tukey honestly significant difference test. For all statistical comparisons, $p < 0.05$ was accepted as the critical level for significance.

3. Results

3.1. Intestinal dimensions

The mass (absolute and relative to body weight) and lengths of the intestine (post-gastric alimentary tract) and each of the intestinal regions of the five green sturgeon sampled for this purpose are given in Table 1. The MI was the longest and heaviest region which proximally was equipped with a thick mucosa (mean wall thickness 3.2 ± 0.2 mm) that decreased distally (mean 1.8 ± 0.2 mm). For the DI, the spiral contributed an average of 55% of the DI mass. The wall of the DI was substantially thinner than that of the proximal MI and slightly thinner than the distal MI; mean wall thickness of proximal DI 1.6 ± 0.1 mm and distal DI 1.1 ± 0.1 mm.

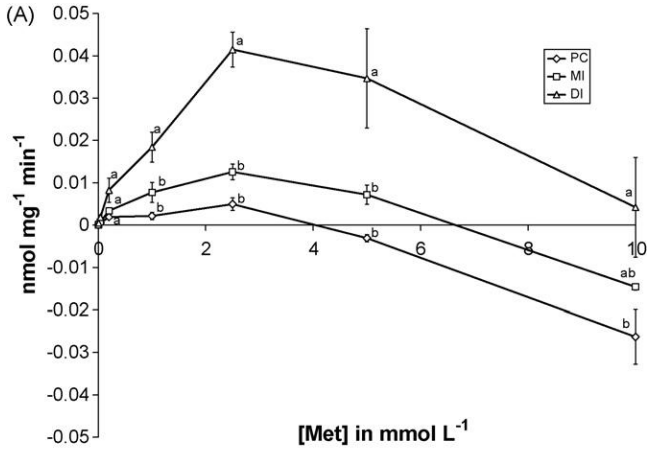
3.2. In vitro methionine uptake

Rates for total Met absorption were significantly ($p < 0.05$) higher in the DI, than in the MI and PC (Fig. 1A and B) at most concentrations. Even at the lower concentrations, 0 (^{14}C-labeled

Table 1
Body and intestinal dimensions of green sturgeon.

		Somatic indices (g kg^{-1} body weight)
Body weight, g	1552 ± 83	
Fork length, cm	64 ± 1	
Total intestinal weight, g	23.6 ± 1.4	15.3 ± 0.8
PC	3.7 ± 0.2	2.4 ± 0.1
MI	12.9 ± 0.7	8.4 ± 0.5
DI	7.0 ± 0.6	4.5 ± 0.4
Total intestinal length, cm	27.8 ± 0.3	
PC	3.4 ± 0.1	
MI	14.1 ± 0.3	
DI	11.3 ± 0.4	

Values are means ± S.E.M. (standard error of the mean); $n = 5$. PC: pyloric caecum; MI: mid intestine; DI: distal intestine.



(B)

	[Met] 0 (^{14}C-Met only)	0.008	0.04
PC	$9.1\times10^{-6} \pm 2.3\times10^{-6}$ b	$1.0\times10^{-4} \pm 1.3\times10^{-5}$ b	$5.0\times10^{-4} \pm 1.6\times10^{-4}$ b
MI	$1.2\times10^{-5} \pm 2.6\times10^{-6}$ b	$1.8\times10^{-4} \pm 7.6\times10^{-5}$ b	$7.7\times10^{-4} \pm 8.4\times10^{-5}$ ab
DI	$3.3\times10^{-5} \pm 5.8\times10^{-6}$ a	$4.9\times10^{-4} \pm 8.1\times10^{-5}$ a	$1.9\times10^{-3} \pm 5.3\times10^{-4}$ a

Fig. 1. (A) Total *in vitro* absorption rates of L-methionine (Met) in nmol mg^{-1} tissue and min^{-1} as a function of substrate concentration ([Met] in mmol L^{-1}) measured in intact tissue from the pyloric caecum (PC; ◊), mid intestine (MI; □), and distal intestine (DI; △) of green sturgeon. The curves reflect the summed absorption by transporter-mediated and non-mediated (passive) components. Values are mean absorption rates ± standard error of the mean (S.E.M.; $n = 5$). Different letters indicate significant differences ($p < 0.05$) between intestinal regions within each concentration. (B) The table below the figure shows the mean absorption rates measured at Met concentrations 0 mmol L^{-1} (^{14}C-labeled Met only), 0.08 and 0.04 mmol L^{-1}.

Met only), 0.08 and 0.04 mmol L^{-1}, the same trend was observed (Fig. 1B). Absorption rates at or above Met concentrations of 2.5 mmol L^{-1} started to decrease in all regions. The relationships between total absorption and concentration up to 2.5 mmol L^{-1} in all three regions were curvilinear (Figs. 1A and 2A–2C). The use of a model equation for a transporter with a passive component provided a better fit than a linear model. Furthermore, tracer accumulation ratios were greater than 1.0 (Table 2), indicating a saturable component of absorption. The slopes of the lines from the linear relationships were used to estimate passive, carrier-independent absorption (Fig. 2A–C).

Maximum rates of uptake (V_{max}) of Met were calculated to be highest in the MI, intermediate in the DI and lowest in the PC (Table 2). On the other hand, apparent affinity constants (K_m) indicated that affinity of Met for the transporter(s) was lowest in the MI (high K_m value) and higher in the PC and DI (low K_m values).

Table 2
Kinetic parameters[a] of L-methionine (Met) and L-selenomethionine (SeMet) absorption pathways in intestinal tissue of green sturgeon.

	V_{max}	Apparent K_m	P	Accumulation ratio
Met				
PC	1.93	0.081	1.20	16.6 ± 10.7
MI	6.36	0.260	2.72	4.7 ± 1.6
DI	5.51	0.069	14.31	3.6 ± 0.3
SeMet				
PC	1.65	0.082	0.12	18.2 ± 5.9
MI	9.63	0.150	2.25	7.7 ± 2.4
DI	18.41	0.507	5.34	6.5 ± 1.5

[a] Maximum rates of absorption (V_{max}; pmol mg^{-1} min^{-1}), apparent affinity constants (apparent K_m; mmol L^{-1}) and permeability coefficients (P; pmol mg^{-1} min^{-1}) as estimated using the software program EnzFitter (BIOSOFT, UK). Accumulation ratios (means ± S.E.M.; $n = 5$) were calculated as described in Section 2. PC: pyloric caecum; MI: mid;intestine; DI: distal intestine.

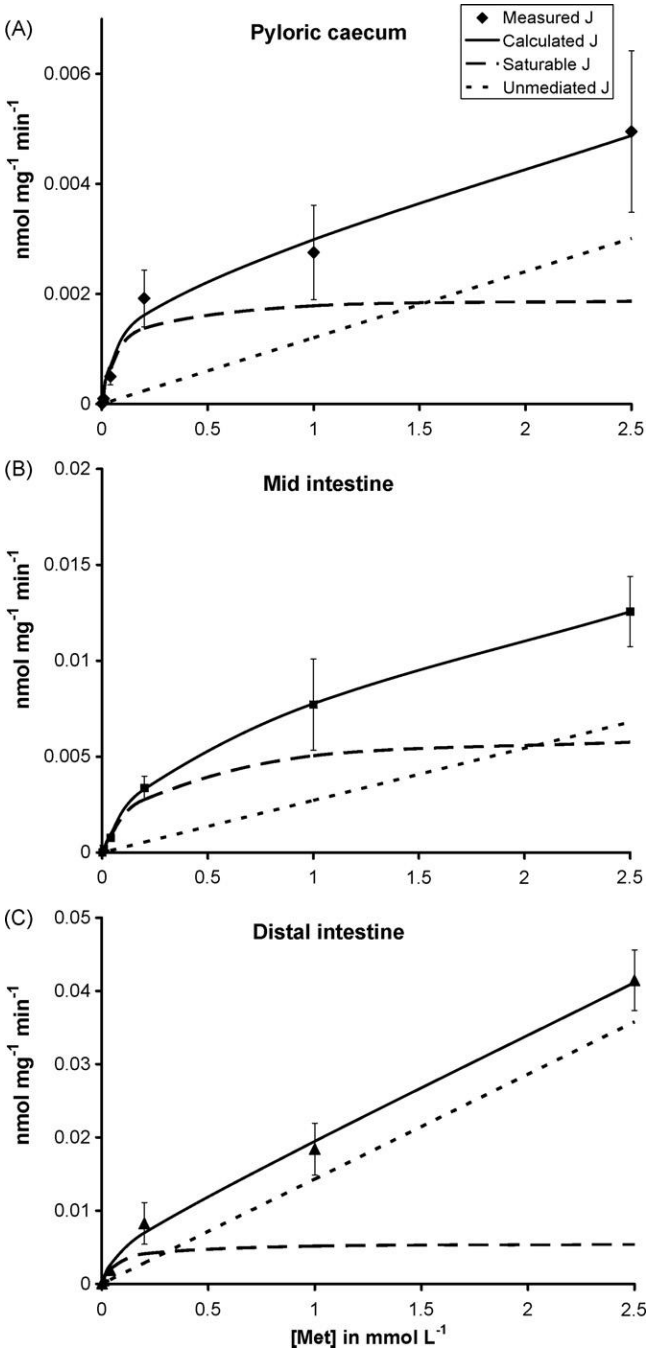


Fig. 2. Measured total methionine absorption rate in nmol mg^{-1} tissue and min^{-1} (measured J; solid points; means ± S.E.M.; $n = 5$) as compared to total calculated J (solid line), the latter calculated from the kinetic parameters for methionine absorption V_{max}, K_m and P (given in Table 2), as a function of methionine concentration ([Met] in mmol L^{-1}) in the pyloric caecum (A, top), mid intestine (B, middle) and distal intestine (C, bottom). Using the same kinetic parameters, putative saturable (transporter-mediated absorption; dashed line) and unmediated (dotted line) components of total absorption for the three intestinal regions are also indicated. Only data from [Met] of 0–2.5 mmol L^{-1} are given since measured uptake for 5 and 10 mmol L^{-1} declined toward 0 or less (see Fig. 1). Note that unmediated absorption appears to contribute more to total absorption in the distal intestine compared to the two more proximal regions.

The linear component of absorption, defined as the permeability coefficient (P), was highest in the DI, intermediate in the MI and lowest in the PC. As a consequence, the non-saturable component contributed most to total absorption in the DI whereas in the more proximal regions it represented an increased proportion of total

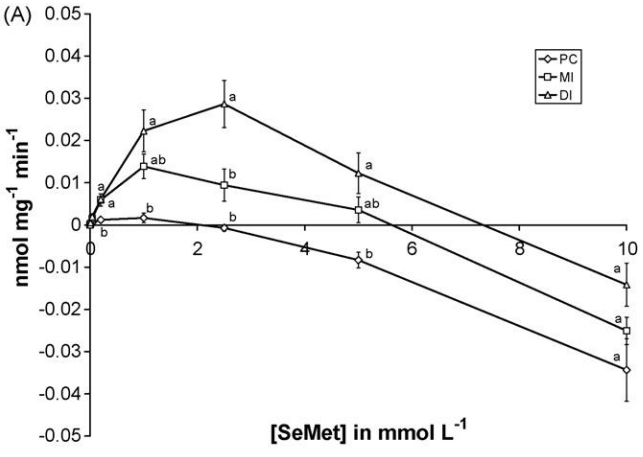
Table 3
Tissue accumulation of ^{14}C-labeled L-methionine in the presence of increasing concentrations of the substrates L-methionine (Met) or L-selenomethionine (SeMet) in intestinal tissue of green sturgeon.

Intestinal region	Substrate concentration (mmol L^{-1})	^{14}C-Met accumulation (DPM mg^{-1})		*p*-value
		Met	SeMet	
PC	0.008	3.46 ± 0.47	4.89 ± 1.67	n.s.
	0.04	3.51 ± 1.08	4.14 ± 0.67	n.s.
	0.2	3.00 ± 0.76	1.89 ± 0.28	n.s.
	1	0.64 ± 0.26	0.51 ± 0.35	n.s.
	2.5	0.56 ± 0.17^{a}	−0.09 ± 0.09^{b}	*0.0090*
	5	−0.16 ± 0.05^{a}	−0.53 ± 0.12^{b}	*0.0202*
	10	−0.73 ± 0.18	−1.14 ± 0.25	n.s.
MI	0.008	12.78 ± 5.29	23.30 ± 4.32	n.s.
	0.04	10.73 ± 1.17	22.55 ± 5.30	*0.0610*
	0.2	9.95 ± 1.80	18.75 ± 4.71	n.s.
	1	4.56 ± 1.40	8.54 ± 1.80	n.s.
	2.5	2.84 ± 0.41	2.46 ± 1.00	n.s.
	5	0.77 ± 0.24	0.45 ± 0.39	n.s.
	10	−0.81 ± 0.04^{a}	−1.66 ± 0.21^{b}	*0.0045*
DI	0.008	16.87 ± 2.82	20.86 ± 4.35	n.s.
	0.04	12.98 ± 3.67	14.45 ± 4.75	n.s.
	0.2	12.17 ± 4.15	9.67 ± 0.58	n.s.
	1	5.44 ± 1.04	6.87 ± 1.53	n.s.
	2.5	4.69 ± 0.47	3.74 ± 0.73	n.s.
	5	1.87 ± 0.63	0.78 ± 0.30	n.s.
	10	0.12 ± 0.33	−0.47 ± 0.17	n.s.

Mean ^{14}C-Met accumulation values ± standard error of the mean (S.E.M.; $n = 5$). Different letters indicate significant differences ($p < 0.05$) between ^{14}C-Met accumulation when in the presence of Met or SeMet at each concentration and intestinal region. The *p*-values were generated following one-way analysis of variance (ANOVA). PC: pyloric caecum; MI: mid intestine; DI: distal intestine; n.s.: not significant.

absorption at substrate concentrations at or above 1.5–2 mmol L^{-1} (Fig. 2).

Accumulation of labeled (^{14}C) Met (as measured by radioactivity given in disintegrations per minute and mg tissue) decreased with increasing unlabeled methionine concentration in the incubation solution (Table 3). This indicates competition between ^{14}C-Met and unlabeled Met for a limited number of transporters. At concentrations higher than 1–5 mmol L^{-1} (depending on tissue) the transporters were more or less saturated, not allowing any considerable amount of labeled Met into the tissue.



(B)

	[SeMet]		
	0 (^{14}C-Met only)	*0.008*	*0.04*
PC	$8.8\times10^{-6} \pm 1.5\times10^{-6}$ ^{b}	$1.2\times10^{-4} \pm 4.2\times10^{-5}$ ^{b}	$5.6\times10^{-4} \pm 9.1\times10^{-5}$ ^{a}
MI	$2.4\times10^{-5} \pm 3.1\times10^{-6}$ ^{a}	$2.9\times10^{-4} \pm 5.4\times10^{-5}$ ^{ab}	$1.5\times10^{-3} \pm 3.6\times10^{-4}$ ^{a}
DI	$2.9\times10^{-5} \pm 4.4\times10^{-6}$ ^{a}	$5.2\times10^{-4} \pm 1.1\times10^{-4}$ ^{a}	$1.9\times10^{-3} \pm 6.4\times10^{-4}$ ^{a}

Fig. 3. (A) Putative *in vitro* absorption rates of L-selenomethionine (SeMet) in nmol mg^{-1} tissue and min^{-1} as a function of substrate concentration ([SeMet] in mmol L^{-1}) measured in intact tissue from the pyloric caecum (PC; ◊), mid intestine (MI; □), and distal intestine (DI; △) of green sturgeon. Values are mean calculated absorption rates using the differences in ^{14}C-labeled Met accumulation in the tissues when unlabeled Met was replaced with unlabeled SeMet in the incubation solutions. Vertical bars indicate standard error of the mean (S.E.M.; $n = 5$). Different letters indicate significant differences ($p < 0.05$) between intestinal regions within each concentration. (B) The table below the figure shows the mean absorption rates measured at SeMet concentrations of 0, 0.08 and 0.04 mmol L^{-1} and statistical analysis.

3.3. Competition between SeMet and Met

Concentrations of SeMet in the incubation solution above 0.04 mmol L^{-1} caused the accumulation of ^{14}C-labeled Met in tissues to decrease dose-dependently (Table 3). This indicates competition between ^{14}C-Met and unlabeled SeMet for transport pathways. When tissue accumulation of ^{14}C-labeled Met was compared in the presence of Met or SeMet at each concentration for each intestinal region, few significant differences were detected (Table 3). However, when there were differences (at 2.5 and 5 mmol L^{-1} in PC, 10 mmol L^{-1} in MI), SeMet inhibited ^{14}C-Met accumulation more (lower DPM mg^{-1} values) than Met. This was a general trend for unlabeled Met and SeMet at higher concentrations (≥0.2 mmol L^{-1} for the PC and DI and ≥2.5 mmol L^{-1} for the MI). At lower concentrations Met tended to inhibit ^{14}C-Met accumulation more than SeMet. When the entire data set was analyzed independent of region and concentration, Met tended to inhibit ^{14}C-Met accumulation (mean 5.55 DPM mg^{-1}) more than SeMet (mean 7.47 DPM mg^{-1}; S.E.M. 0.75; $p = 0.0720$).

3.4. In vitro SeMet uptake

Due to the few significant differences between inhibition of Met and SeMet on ^{14}C-Met accumulation, putative SeMet absorption rates were calculated. For this purpose, the relative differences in the tracer accumulation data obtained when unlabeled Met was replaced with unlabeled SeMet in the incubation solutions (Table 3) was calculated. In turn, these numbers were related to the total absorption data for Met.

As was observed for regional differences in Met absorption, the calculated rates for total putative SeMet absorption were also often significantly ($p < 0.05$) higher in the DI, than in the PC, whereas they

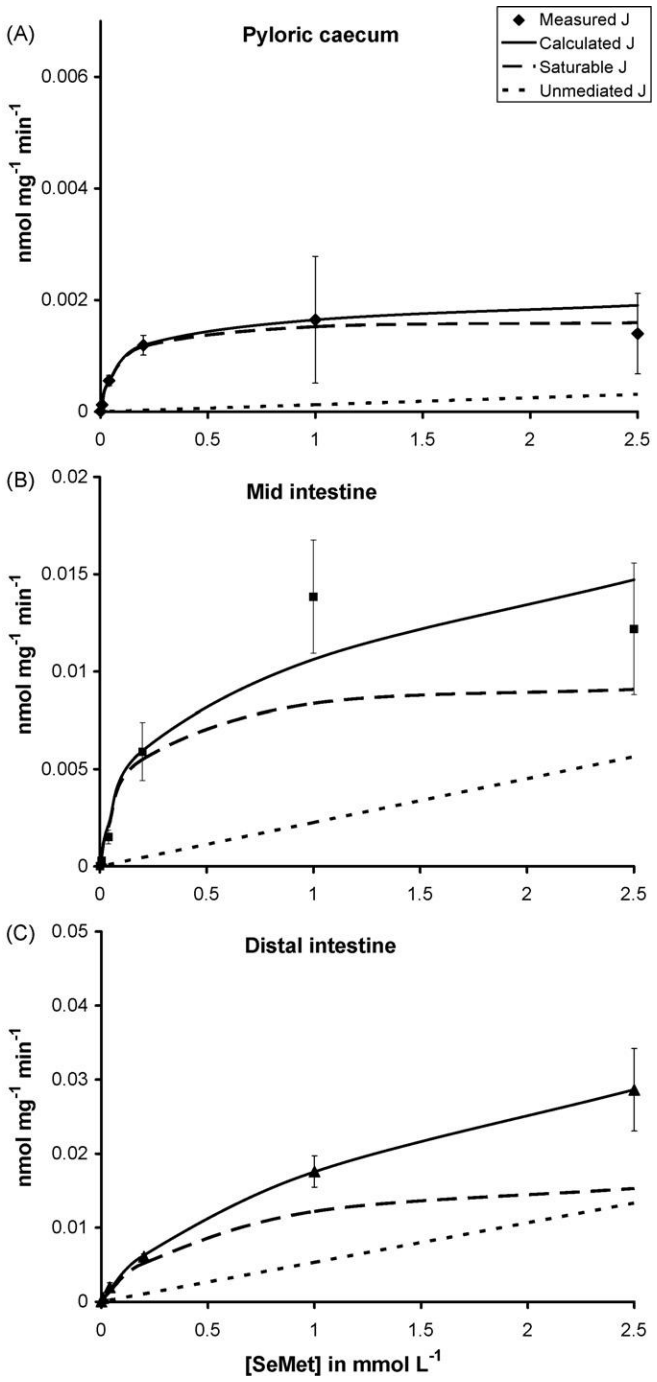


Fig. 4. Putative total selenomethionine absorption rate in nmol mg^{-1} tissue and min^{-1} (measured *J*; solid points; means ± S.E.M.; $n = 5$) as compared to total calculated *J* (solid line), the latter calculated from the kinetic parameters for putative selenomethionine absorption V_{max}, K_m and *P* (given in Table 2), as a function of selenomethionine concentration ([SeMet] in mmol L^{-1}) in the pyloric caecum (A, top), mid intestine (B, middle) and distal intestine (C, bottom). Using the same kinetic parameters, putative saturable (transporter-mediated absorption; dashed line) and unmediated (dotted line) components of total absorption for the three intestinal regions are also indicated. Only data from [SeMet] of 0–2.5 mmol L^{-1} are given since calculated uptake for 5 and 10 mmol L^{-1} declined toward 0 or less (see Fig. 3). Note that saturable absorption pathway(s) appears to contribute most to total absorption of SeMet in all three intestinal regions.

were generally intermediate in the MI (Fig. 3A and B). At the lower concentrations, 0 (^{14}C-labeled Met only), 0.08 and 0.04 mmol L^{-1} (Fig. 3B), as well as at 10 mmol L^{-1} SeMet (Fig. 3A), the trend was still evident but significant differences were often not detected, especially between uptake rates observed in the mid and distal intestinal tissues. Absorption rates above SeMet concentration of 1 mmol L^{-1} in the PC and MI and 2.5 mmol L^{-1} in the DI started to decrease. The relationships between total absorption and concentration up to 2.5 mmol L^{-1} were curvilinear (Figs. 3 and 4A–C). The use of a model equation for a transporter with a passive component provided a better fit than a linear model. Furthermore, tracer accumulation ratios were greater than 1.0 (Table 2), indicating a saturable component of absorption. The slopes of the lines from the linear relationships were used to estimate passive, carrier-independent absorption (Fig. 4A–C).

Maximum rates of uptake (V_{max}) were calculated to be highest in the DI, intermediate in the MI and lowest in the PC (Table 2). On the other hand, apparent affinity constants (K_m) indicated that affinity of SeMet for the transporter(s) was lowest in the DI (high K_m value), intermediate in the MI and highest in the PC (low K_m value). The permeability coefficient (*P*) was highest in the DI, intermediate in the MI and lowest in the PC. As a consequence, the non-saturable component contributed more to total absorption in the DI than in the more proximal regions (Fig. 4A–C).

As for the tracer accumulation data, when differences between Met and SeMet total absorption rates were detected, they were at 2.5 and 5 mmol L^{-1} in PC and 10 mmol L^{-1} in MI. At these points, Met absorption rates were higher than SeMet. When the entire data set was analyzed independent of region and concentration, Met tended to have a higher rate of absorption (mean 0.0045 nmol mg^{-1} min^{-1}; S.E.M. 0.0014) than SeMet (mean 0.0011 nmol mg^{-1} min^{-1}; S.E.M. 0.0013; $p = 0.0820$). Comparing the kinetic parameters (Figs. 2 and 4; Table 2) revealed that differences between Met and putative SeMet total absorption rates appeared to be mostly due to lower permeability of the tissue for SeMet than for Met, particularly in the PC and DI. Maximum rates of absorption (V_{max}), on the other hand, tended to be higher for SeMet than Met in the MI and DI, whereas differences in affinity for the transporters (K_m) varied between these tissues but were very similar in the PC.

Assuming an equal intake of Met and SeMet with the diet and as reflected by differences in absorption rates at 1 mmol L^{-1} for

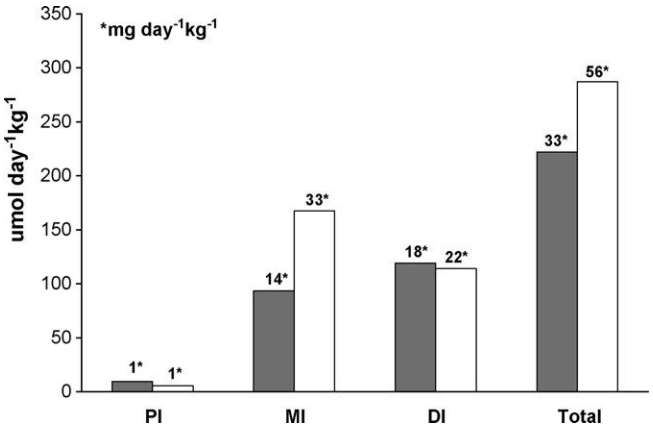


Fig. 5. Putative absorption capacity per day (μmol day^{-1} kg^{-1} body weight; (*) given in mg day^{-1} kg^{-1} body weight above each column) of L-methionine (grey bars) and L-selenomethionine (white bars) in the pyloric caecum (PC), mid intestine (MI), distal intestine (DI) and total intestinal tract (total) as calculated based on weights of each intestinal region relative to body weight (somatic indices; see Table 1), and the rates of absorption at substrate concentrations of 1.0 mmol L^{-1} (approximate concentration of methionine present in the gut assuming a feed intake of 1% of body weight and 2.5% methionine in fishmeal [Anderson et al., 1995]).

the two substrates, calculated absorption capacity (Fig. 5) for Met was lower than for SeMet. The total absorption capacity by the intestine and per kg fish for Met appears to be about 222 μmol d^{-1} (33 mg d^{-1}) versus 287 μmol d^{-1} (56 mg d^{-1}) for SeMet. Regarding contribution of each intestinal region to total Met and SeMet absorption capacity, the PC contributes about 4% and 2%, MI 42% and 58%, DI 54 and 40%, respectively.

4. Discussion

The data strongly suggest that intestinal SeMet carrier-mediated transport is via the same apical membrane transporter(s) as Met in green sturgeon. Whether there may be more than one transporter involved is not known and whether the transporter(s) for neutral amino acids such as Met are the same in fish as in mammals has not been specifically investigated in green sturgeon or any other fish species. Nonetheless, Se absorption in the form of SeMet appears to occur relatively efficiently and may be an important source of Se, both in nutrition and toxicity, for green sturgeon. Recently SeMet transport mechanisms were more closely investigated using intestinal Caco-2 cell lines and oocytes expressing specific mammalian brush border amino acid transporters (Nickel et al., 2009). The data revealed that SeMet was taken up by the intestinal transporters for neutral amino acids symport system B^0, as well as the exchanger (antiport) $b^{0,+}$. Further studies are needed to confirm the existence of these transporters and their contribution to SeMet absorption in fish.

It would be expected, however, that SeMet ingestion would occur with other amino acids and nutrients. The presence of other amino acids in the intestinal content, which are transported through the same amino acid transporters as SeMet, could result in competition at influx sites, possibly reducing absorption of SeMet across the intestinal tissue. The lower affinity of SeMet relative to Met to transporter binding sites in the DI, where most of the substrates appear to be absorbed, may be an indication that competition for binding sites may lead to lower net transport of SeMet when in the presence of similar or higher levels of competing amino acids with higher affinity, such as Met. The importance of the competitive effects at transporter sites to overall SeMet assimilation would also be dependent on the relative contribution of diffusion (unmediated uptake) to total intestinal SeMet absorption. As Figs. 2 and 4 as well as the estimated permeability constants P (Table 2) show, unmediated uptake accounts for a smaller proportion of total absorption of SeMet than Met. Not surprisingly, the maximum rate of absorption constant (V_{max}) was then higher for SeMet than Met in the MI and DI. Together the foregoing data indicate that transporter-mediated uptake accounts for a large proportion of total SeMet assimilation. This may help explain the higher total absorptive capacity of SeMet compared to Met calculated at a presumed substrate concentration of 1 mmol L^{-1} of both substrates in the lumen. More accurate information regarding absorptive capacities of the various intestinal regions as well as competition between Met and SeMet for absorption may be gained with *in vivo* studies.

Since *in vitro* transport kinetics (K_m and V_{max}) of Met and SeMet have been reported to be very similar in isolated cellular models such as brush border membrane vesicles (Wolffram et al., 1989), as well as intestinal cell cultures and oocytes expressing specific transporters (Nickel et al., 2009), any differences in Met and putative SeMet absorption rates and kinetic values found in the current study may be due to factors affecting absorption in whole tissue. These factors may include differences in regional intestinal characteristics such as the amount of mucus secreted and degree of tissue contraction, and/or substrate differences regarding solubility in and movement through the intestinal mucus, influence on tissue contraction, permeability through membranes or between cells, intracellular metabolism, as well as basolateral transport. These factors are a source of variation in the data set. However, they also mimic natural conditions *in vivo* and the data presented may therefore be more practically applicable than that acquired from isolated cell models or other *in vitro* methods.

Intestinal absorption of nutrients of any kind does not appear to have been a subject of investigation in green sturgeon previously. Some data exist for white sturgeon, *A. transmontanus* (Buddington et al., 1987), also using the everted sleeve method, in which *in vitro* glucose and proline absorption were studied but not methionine. Values reported for the neutral imino acid proline V_{max} and apparent K_m (Buddington et al., 1987) were higher by at least two orders of magnitude than the values reported for methionine in the present study. Compared to methionine absorption rates reported in Atlantic salmon (*Salmo salar*) using the same method (Bakke-McKellep et al., 2000), the absorption in green sturgeon is lower by one to two orders of magnitude. Buddington et al. (1987) observed a similar difference for proline absorption in rainbow trout (*Oncorhynchus mykiss*) versus white sturgeon and attributed it to differences in natural food preferences – rainbow trout being carnivorous and therefore have a higher protein/amino acid requirement than omnivorous white sturgeon. The occurrence of zero or negative absorption values at higher concentrations (≥5 mmol L^{-1}) of methionine were not reported in salmon or trout (Bakke-McKellep et al., 2000; Nordrum et al., 2000) nor for proline in white sturgeon (Buddington et al., 1987), although they were observed for glucose absorption in salmon and trout at 10 mmol L^{-1} (Nordrum et al., 2000). Negative values are difficult to explain but indicate a saturation of influx pathways and possibly even a net secretion of nutrient. The involvement of multiple transporters in the movement of Met and SeMet across the brush border membrane (Nickel et al., 2009), including exchangers such as the system $b^{0,+}$ exchanger described in other animal systems (see review by Bröer, 2008; Nickel et al., 2009), may also help explain negative uptake values. The $b^{0,+}$ exchanger mediates the influx of cationic amino acids in exchange for neutral amino acids, such as methionine, which are then recycled back into the epithelial cells by system B^0. Depending on the presence of these types of transport system in green sturgeon, coordination with the activity of basolateral amino acid transporters, these various transporter systems' kinetic characteristics, membrane potentials, solute concentrations present in the lumen, and intracellular metabolism of the solute, an apparent net secretion of methionine or a ^{14}C-labeled metabolite cannot be excluded. In addition, the secretion of mucus, which may bind or disturb the movement of substrate, may also be a factor. Regional differences in the amount of mucus secreted, especially evident in the MI, may also account for the different kinetic data obtained for the various regions. The large variation experienced in total absorption rates and tracer accumulation, especially at low substrate concentrations, may indicate individual or intraregional variation in the number of transporters present in the intestinal mucosa. Individual differences may be related to body size or feed intake whereas intraregional variation in transporter numbers may vary from proximal to distal.

Regarding regional differences in apparent substrate absorption, an interesting observation made in this study was the increasing proximal-to-distal gradient for rates of methionine and putative SeMet absorption rather than a declining gradient usually observed for nutrient absorption in fish and mammals (Ferraris and Ahearn, 1984; Collie, 1985; Buddington et al., 1987; Ferraris et al., 1989; Bakke-McKellep et al., 2000; Nordrum et al., 2000). Currently, it is not known whether the increasing proximal-to-distal gradient in absorption rates for Met and SeMet is connected to a corresponding increase in transporter distribution from the proximal to distal regions. Nor if this is only the case for Met/SeMet or whether similar trends exist for the absorption of other nutrients. The question

arises whether the observed apparent gradient matches a corresponding increase in the concentration of transporter substrates – glucose, amino acids and small peptides – along the intestinal tract as digestion progresses, or whether it is a strategy to efficiently absorb remaining nutrients as substrate concentrations decrease in the chyme in more distal regions as absorption takes place. However, not only transporter numbers, but also diffusion distance for nutrients and other substrates to transporters may be a factor. As suggested for white sturgeon (Buddington and Doroshov, 1986; Buddington et al., 1987; Gawlicka et al., 1995) and Adriatic sturgeon *A. naccarii* (Cataldi et al., 2002), morphological and physiological characteristics of the sturgeon intestine indicate that absorptive activities mainly occur deep in the complex mucosa of the anterior regions as well as in more distal intestinal regions. It has been suggested that this may have developed to fully take advantage of their durophagous feeding habits (consumption of hard or hard-shelled organisms). In this case, nutrients may be released more distally along the intestine in sturgeon compared to animals that feed on more easily digestible feed items. Furthermore, the PC with its branched or "glandular" organization, as described in other sturgeon species (Buddington and Doroshov, 1986; Domeneghini et al., 1999), presented a challenge when preparing the tissue for this study. Following the dissection (sagital cut and portioning), a proportion of the tissue exposed to the substrates was most likely not intact intestinal mucosa that could effectively transport nutrients. Moreover, the MI has a thick, complex mucosa, especially the proximal part, with numerous goblet cells bordering the lumen (Buddington and Doroshov, 1986; Domeneghini et al., 1999; Gisbert and Doroshov, 2003) which resulted in an observable, thick mucus layer. These properties may create physical barriers which slow substrate movement to the brush border membrane of the enterocytes where the transporters are located. All these factors may contribute to the relatively low absorption rates in general and particularly in the more proximal intestinal regions. In any case, the distribution of transporters as related to actual contribution of the various intestinal regions to *in vivo* substrate assimilation would be an interesting area for further study.

5. Conclusions

SeMet appears to be transported by the same intestinal transporter(s) as Met in green sturgeon. Competition between SeMet and Met for influx pathways indicate that the absorption of selenium in the form of Se-amino acids can be inhibited by the presence of amino acids using the same transporters and other absorption routes. The consequences of this for *in vivo* bioaccumulation of selenium in the animal merit investigation.

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