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Effects of chronic dietary selenomethionine exposure on repeat swimming performance, aerobic metabolism and methionine catabolism in adult zebrafish (*Danio rerio*)

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ABSTRACT

In a previous study we reported impaired swimming performance and greater stored energy in adult zebrafish (*Danio rerio*) after chronic dietary exposure to selenomethionine (SeMet). The goal of the present study was to further investigate effects of chronic exposure to dietary SeMet on repeat swimming performance, oxygen consumption (MO_2), metabolic capacities (standard metabolic rate [SMR], active metabolic rate [AMR], factorial aerobic scope [F-AS] and cost of transport [COT]) and gene expression of energy metabolism and methionine catabolism enzymes in adult zebrafish. Fish were fed SeMet at measured concentrations of 1.3, 3.4, 9.8 or 27.5 μg Se/g dry mass (d.m.) for 90 d. At the end of the exposure period, fish from each treatment group were divided into three subgroups: (a) no swim, (b) swim, and (c) repeat swim. Fish from the no swim group were euthanized immediately at 90 d and whole body triglycerides, glycogen and lactate, and gene expression of energy metabolism and methionine catabolism enzymes were determined. Individual fish from the swim group were placed in a swim tunnel respirometer and swimming performance was assessed by determining the critical swimming speed (U_{crit}). After both U_{crit} and MO_2 analyses, fish were euthanized and whole body energy stores and lactate were determined. Similarly, individual fish from the repeat swim group were subjected to two U_{crit} tests ($U_{crit\text{-}1}$ and $U_{crit\text{-}2}$) performed with a 60 min recovery period between tests, followed by determination of energy stores and lactate. Impaired swim performance was observed in fish fed SeMet at concentrations greater than 3 μg Se/g in the diet. However, within each dietary Se treatment group, no significant differences between single and repeat U_{crits} were observed. Oxygen consumption, SMR and COT were significantly greater, and F-AS was significantly lesser, in fish fed SeMet. Whole body triglycerides were proportional to the concentration of SeMet in the diet. While swimming resulted in lesser concentrations of glycogen in the body, exposure to SeMet in the diet had no significant effect on glycogen content. Exposure to SeMet significantly down-regulated mRNA abundance of protein tyrosine phosphatase 1B (PTP 1B) in muscle, and β-hydroxyacyl coenzyme A dehydrogenase (HOAD), sterol regulatory element binding protein 1 (SREBP 1) and methionine adenosyltransferase 1 alpha (MAT 1A) in liver of adult zebrafish. Overall the results of this study suggest chronic exposure of adult zebrafish to SeMet in the diet can cause both cellular and organismal effects that could affect fitness and survivability of fish.

1. Introduction

Among essential trace elements, selenium (Se) is reported to have a narrow margin between beneficial and toxic effects (Janz et al., 2010). For example, fish require dietary Se concentrations of 0.5–1.0 μg Se/g dry mass (d.m.) to maintain normal physiological homeostasis. However, concentrations >3 μg Se/g d.m. in the diet can result in bioaccumulation of Se and cause toxicosis (Lemly, 1997; Janz et al., 2010). Concentrations of Se in both terrestrial and aquatic environments are increasing due to expansion of mining (coal, uranium, phosphate), power generation (coal-fired power plants) and agriculture (Janz et al., 2010; Janz, 2011). Selenium enters aquatic ecosystems as inorganic selenate and selenite from both point and non-point sources, and these oxyanions are sufficiently soluble to be mobile in water. In aquatic ecosystems, primary producers, such as algae, can convert inorganic forms of Se

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into organic forms, such as selenomethionine, selenocysteine and selenoproteins. Such organic forms have greater potential to bioaccumulate and be transferred through trophic webs than inorganic Se (Fan et al., 2002). Selenomethionine (SeMet) is the dominant form of selenium (Se) present in food (Fan et al., 2002). Since the molecular structure of SeMet resembles the essential amino acid methionine, absorption and accumulation of SeMet and subsequent incorporation into proteins occurs in a concentration dependent fashion (Behne et al., 1991; Bakke et al., 2010). In aquatic ecosystems contaminated with excess concentrations of Se, 50–70% of total Se occurs in both primary producers and consumers as SeMet (Fan et al., 1998, 2002; Phibbs et al., 2011). Although primary producers and invertebrates accumulate substantial concentrations of SeMet, they are tolerant of SeMet. In contrast, oviparous species, including fish, birds, and amphibians are more susceptible to toxic effects of SeMet (reviewed in Janz et al., 2010; Janz, 2011), and hence there is a need to investigate mechanisms of toxicity of dietary SeMet to oviparous species.

Exposure of adult fishes to SeMet in the diet is known to cause developmental abnormalities and mortality in F1 larval fish (Lemly, 1997; Janz et al., 2010). In adult female fish, SeMet is deposited into eggs during vitellogenesis. Subsequent utilization of SeMet in yolk by developing embryos causes abnormalities and mortality of larval fish. Exposure of juvenile fishes to SeMet via the diet has also been reported to attenuate physiological responses to stressors and to modulate endocrine function (Teh et al., 2004; Wiseman et al., 2011a,b). A general notion among aquatic toxicologists is that adult fish are tolerant of chronic exposure to SeMet. Although acute toxicity of Se to adult fishes has been investigated at concentrations that are not environmentally relevant, less information is available on chronic Se toxicity in adult fishes. A previous study demonstrated that dietary exposure of adult zebrafish (*Danio rerio*) to SeMet in the diet resulted in lesser swimming performance, caused accumulation of stored energy and resulted in greater production of cortisol and greater body mass (Thomas and Janz, 2011).

Swimming performance is an important fitness trait in wild fish as it is closely linked to food acquisition, predator avoidance, reproduction, schooling and migration (Hammer, 1995; Plaut, 2001) and has been used as an ecologically relevant endpoint for the assessment of sublethal toxicity to fish (Little and Finger, 1990). The most commonly used test of swimming performance is critical swimming speed (U_{crit}) (Brett, 1964), which challenges fish with incremental changes in velocity until exhaustion. In addition to single exercise U_{crit}, studies have also investigated repeat U_{crit}, where fish are subjected to two successive U_{crit} tests ($U_{crit\text{-}1}$ and $U_{crit\text{-}2}$) with a short recovery period, normally 40–60 min, between tests (Jain et al., 1998; McKenzie et al., 2007). Repeat swimming performance is commonly used to investigate rates of recovery of fish after exposure to a stressor and is considered a more sensitive indicator of fish health than single exercise U_{crit} (Jain et al., 1998). Recovery ratio ($U_{crit\text{-}2}/U_{crit\text{-}1}$) is a relevant measure of the ability of fish to recover from exhaustive exercises (Jain et al., 1998). Stressor exposures have been shown to cause impaired performance in repeat swimming trials and a lesser recovery ratio (Jain et al., 1998; McKenzie et al., 2007).

Oxygen consumption (MO_2) is a measure of metabolic activity of fish and is often measured in combination with U_{crit}. Alteration in MO_2 is an indicator of stress in fish and a number of toxicants including Se are reported to alter MO_2 in fish (Lemly, 1993; Scott and Sloman, 2004). Swimming respirometry has been used by both fish physiologists and toxicologists to investigate metabolic capacities such as standard metabolic rate (SMR), which is the minimal maintenance metabolic rate of unfed fish, active metabolic rate (AMR), which is the metabolic rate of fish at maximum sustainable velocity in U_{crit} tests, aerobic scope (AS), which is the difference between AMR and SMR, factorial aerobic scope (F-AS), which is the ratio of AMR to SMR, and cost of transport (COT), which is a measure of the energetic cost of swimming (Videler, 1993; Webber et al., 1998; Shingles et al., 2001; Claireaux et al., 2006; McKenzie et al., 2007; Killen et al., 2007). Stressors, including temperature and chemicals have been shown to alter both metabolic expenditure and energetic cost of swimming in fish (Shingles et al., 2001; Claireaux et al., 2006; McKenzie et al., 2007). Triglycerides (triacylglycerols) and glycogen are the major forms of stored energy in fish and are usually used during swimming. In fish, both sustained and prolonged swimming are powered by slow oxidative red muscle fibres and are fuelled primarily by aerobic catabolism of triglycerides (Hammer, 1995; Moyes and West, 1995). Critical swimming speed is a measure of prolonged aerobic swimming capacity of fish, and triglycerides are used as the primary energy source up to approximately 80% of U_{crit} (Hammer, 1995; Moyes and West, 1995; Plaut, 2001). Alternatively, burst swimming and swimming speed beyond 80% U_{crit} in fish are powered by fast glycolytic white muscle fibres, where the majority of energy comes from anaerobic catabolism of glycogen (Hammer, 1995; Moyes and West, 1995). Lactate, a by-product of anaerobic energy catabolism, accumulates in muscle during burst swimming and serves as a measurable indicator of anaerobic metabolic activity.

Greater accumulation of stored energy was reported in zebrafish and rainbow trout (*Oncorhynchus mykiss*) exposed to augmented dietary SeMet (Thomas and Janz, 2011; Wiseman et al., 2011b) and in native fishes collected from Se-impacted field sites (Kelly and Janz, 2008; Driedger et al., 2009; Goertzen et al., 2012). Stressor exposures have been shown to alter energy metabolism by either impairing MO_2 or altering activity or expression of energy metabolism enzymes (Rajotte and Couture, 2002; McClelland et al., 2006; Goertzen et al., 2011, 2012). Commonly studied enzymes that are associated with aerobic metabolism are citrate synthase (CS) and β-hydroxyacyl coenzyme A dehydrogenase (HOAD). Citrate synthase is a key enzyme involved in the citric acid cycle whereas HOAD is involved in metabolism of triglycerides. Altered expressions or activities of CS and HOAD have been reported to occur after exposure to stressors (Rajotte and Couture, 2002; McClelland et al., 2006; Goertzen et al., 2011, 2012). In mammals, exposure to Se via the diet has been shown to result in greater accumulation of triglycerides and cause up-regulation of expression or activities of enzymes, including, protein tyrosine phosphatase 1B (PTP 1B), fatty acid synthase (FAS) and a transcription factor, sterol regulatory element binding protein 1 (SREBP 1), involved in synthesis of fatty acids (Mueller et al., 2008, 2009a,b). In addition, restricted dietary intake of methionine has been reported to cause greater activity of FAS and accumulation of triglycerides in liver of fish (Espe et al., 2010). Methionine is an important precursor in the synthesis of S-adenosylmethionine (SAM), which is an important donor of methyl groups in multiple methyltransferase reactions. Methionine adenosyltransferase (MAT) is an enzyme responsible for synthesis of SAM. Expression of methionine adenosyltransferase 1 alpha (MAT 1A), a liver-specific MAT gene, can be used as a measure of methionine catabolism, steady-state SAM production and methylation (Lu, 2000; Mato et al., 2002). Exposure to either selenite or selenocystine has been shown to deplete SAM and inactivate MAT in mammalian liver (Hoffman, 1977; Hasegawa et al., 1996). Exposure of mammals to dietary Se has been shown to alter both metabolism of triglycerides and catabolism of methionine, but to date no such studies have been conducted in fishes. Since our previous study showed reduced swimming performance and greater accumulation of stored energy in adult zebrafish exposed to elevated dietary SeMet (Thomas and Janz, 2011), the objectives of present study were to further investigate effects of chronic dietary SeMet exposure on repeat swimming performance, and to elucidate possible mechanisms of SeMet induced impairment of swimming

performance and greater accumulation of stored energy in adult zebrafish.

2. Materials and methods

2.1. Test chemical and test species

Seleno-L-methionine (purity >98%) was purchased from Sigma–Aldrich (Oakville, ON, Canada). Adult zebrafish were purchased from a local supplier and housed in an environmental chamber with controlled temperature (28.0 ± 1.0 °C) and photoperiod (14 h light and 10 h dark). Mean standard length of fish was 2.8 cm and ranged from 2.6 to 3.1 cm. Fish were introduced (25 fish/tank) into twelve 40 L glass aquaria with continuous aeration and filtration, after which treatments were randomly assigned to aquaria, with $n = 3$ replicate aquaria per treatment. Fish were acclimated for 3 weeks to laboratory conditions, and during this time they were fed Nutrafin® basic flake food (Hagen Inc., Montreal, QC, Canada).

2.2. Diet preparation and experimental design

Nominal concentrations of 3, 10 or 30 μg Se/g d.m. in the form of SeMet were added to Nutrafin flake food as described previously (Thomas and Janz, 2011). The control diet was prepared by adding an equivalent volume of water without SeMet to food. Food was lyophilized in a freeze dryer (Dura-Dry™ MP, FTS Systems, Stone Ridge, NY, USA). Freeze dried diets were crushed into flakes and stored at −20 °C in air tight containers. Representative samples of these diets were collected for determination of total concentrations of Se.

All methods applied in the present study were approved by the University of Saskatchewan's Animal Research Ethics Board (protocol # 20030076), and adhered to the Canadian Council on Animal Care guidelines for humane animal use. Fish were fed twice daily (5% body weight/d ration) with either control or SeMet spiked foods for 90 d. Fish were allowed to feed for 2 h, after which excess food was siphoned from the aquarium bottom. During the feeding experiment, 75% of water was renewed from each aquarium every day. Water samples ($n = 1$ from each aquaria) for quantification of dissolved Se were collected 5 h after feeding on day 30 of the feeding experiment. These water samples ($n = 3$ from each treatment) were filtered using 0.45 μm disposable filters, acidified to pH < 2 using ultra-pure nitric acid, and stored in 25-mL high density polyethylene (HDPE) bottles at 4 °C until Se analysis. After 90 d, fish from each treatment group were divided into three subgroups: (a) no swim, (b) swim, and (c) repeat swim. Fish from the no swim group were euthanized immediately at 90 d and whole body Se levels ($n = 3$–5), and whole body concentrations of triglycerides, glycogen and lactate ($n = 6$–7) were determined. In addition, liver and muscle ($n = 4$) were dissected from adult fish in each treatment group, immediately flash-frozen using liquid nitrogen, and stored at −80 °C until gene expression analyses of energy metabolism and methionine catabolism enzymes. Individual fish from the swim group were placed in a swim tunnel respirometer and swimming performance was determined using the critical swimming speed (U_{crit}) method (Brett, 1964). After both U_{crit} and MO_2 analyses, fish were euthanized and whole body energy stores and lactate were determined. Similarly, individual fish from the repeat swim group were subjected to two U_{crit} tests ($U_{crit\text{-}1}$ and $U_{crit\text{-}2}$) performed with a 60 min recovery period between tests, followed by determination of whole body energy stores and lactate.

2.3. Quantification of selenium

Total concentrations of Se in fish, food and water samples were measured by use of inductively coupled plasma-mass spectrometry (ICP-MS) at the Toxicology Centre (University of Saskatchewan, Saskatoon, SK, Canada). Prior to analysis to determine concentrations of Se, whole body fish and samples of food were lyophilized and homogenized by use of a mortar and pestle. Moisture content of whole zebrafish was 73.7 ± 1.0%. Aliquants of 100 mg of homogenized samples were cold digested in Teflon vials by use of 5 mL of ultra-pure nitric acid and 1.5 mL of hydrogen peroxide. After digestion, samples were concentrated on a hot plate (<75 °C) and reconstituted in 5 mL of 2% ultrapure nitric acid. Reconstituted samples were stored at 4 °C until quantification. A LOQ of 0.5 μg Se/g was determined using method blanks. Selenium recovery was determined using certified reference material (TORT-2, lobster hepatopancreas, NRC, Ottawa, ON, Canada).

2.4. Oxygen consumption and swim performance

Consumption of oxygen and swim performance was conducted in a modified Blazka-type, variable speed, miniature swim tunnel respirometer with a DAQ-M control device and AutoResp™ 1 software (Loligo Systems, Tjele, DK). The system consists of a 170 mL swim tunnel submerged in a 20 L buffer tank supplied with 28.5 ± 0.1 °C aerated water from a 20 L heated water bath circulator (VWR International, Mississauga, ON, Canada). Measurement of the rate of consumption of oxygen (MO_2) was performed by automated intermittent-flow respirometry in loops of 10 min. Each loop consisted of a 5 min measuring phase followed by a 4 min flushing phase and a 1 min waiting phase. Concentrations of oxygen (O_2) were measured using a fibre optic oxygen dipping probe which was connected to a Fibox 3 minisensor oxygen meter (Precision Sensing GmbH, Regensburg, DE). AutoResp™ 1 software was used to calculate MO_2 and the detailed MO_2 measuring principle is explained elsewhere (Steffensen et al., 1984).

To establish a stabilized minimal rate of metabolism for adult zebrafish after being introduced in the swim tunnel respirometer, fish were acclimated for 1.5–2 h at a minimal water velocity of 0.8 BL/s (body length per second). Fish were not fed 24 h prior to MO_2 and U_{crit} analyses. In the U_{crit} experiment, individual fish were subjected to step-wise increments in swimming velocity (2.7 BL/s every 20 min) until exhaustion. On occasions where fish stopped swimming and rested at the downstream portion of the swim tunnel for the first time, water velocity was reduced for 15 s to initiate swimming and then the velocity was returned to the previous set point. In the repeat U_{crit} experiment, fish were subjected to two successive U_{crit} tests ($U_{crit\text{-}1}$ and $U_{crit\text{-}2}$) with a 60 min recovery period between tests. Critical swimming speed was calculated using the equation previously described (Brett, 1964). Fish cross sectional area was less than 5% of swim tunnel cross sectional area, hence U_{crit} values were not corrected for solid blocking effect. Critical swimming speed values were corrected for standard body length of each individual fish, and thus U_{crit} values were represented as body lengths per second (BL/s). The recovery ratio of individual fish from the repeat swim test was calculated by dividing the results of the two swim tests ($U_{crit\text{-}2}/U_{crit\text{-}1}$) (Jain et al., 1998). Two MO_2 measurements were determined in each water velocity increment, and the average MO_2 value was used for statistical analysis. After the U_{crit} and MO_2 analyses, fish were euthanized using an overdose of MS 222 (1 g/L) and stored at −80 °C for subsequent whole body triglyceride, glycogen and lactate analyses. Prior to storage, total body length, wet weight, and condition factor were determined for each fish.

2.5. Determination of SMR, AMR, F-AS and COT

Standard metabolic rate (SMR) was calculated by extrapolating consumption of O_2 back to a water velocity of zero. This was done from a plot of swimming speed (m/s) versus MO_2 (mg O_2/kg/h) and

use of nonlinear, curve fitting regression analysis (Webber et al., 1998; Shingles et al., 2001). Active metabolic rate (AMR) is defined as the MO_2 at maximum sustainable speed in the U_{crit} test. The factorial aerobic scope (F-AS) was calculated as AMR/SMR (Webber et al., 1998; Shingles et al., 2001; Killen et al., 2007). Cost of transport (J/kg/m) was calculated by multiplying MO_2 (mg O_2/kg/s) by an oxycaloric value of 14.1 J/mg O_2 and then dividing by the corresponding swimming speed (m/s) (Videler, 1993).

2.6. Quantification of triglycerides and glycogen

Concentrations of triglycerides in whole body of adult zebrafish samples were measured by use of a kit prepared by Sigma–Aldrich (Oakville, ON, Canada), which follows the McGowan et al. (1983) method. A glycerol solution was used for the standard curve. This method has been previously validated in our laboratory for measuring triglycerides in whole fish homogenates (Kelly and Janz, 2008). Concentrations of glycogen in whole body adult zebrafish were measured by use of use a modified version of the method of Gŏmez-Lechŏn et al. (1996). Glycogen assay reagents were purchased from Sigma–Aldrich (Oakville, ON, Canada). The standard curve was created using purified Type IX bovine liver glycogen. This method has been previously validated in our lab for use in whole fish homogenates (Goertzen et al., 2011, 2012).

2.7. Lactate assay

Whole body concentrations of lactate were measured by use of a commercially available kit according to the manufacturer's instructions (Eton Bioscience Inc., San Diego, CA, USA). The kit method is based on reduction of tetrazolium salt INT (2-p-iodophenyl-3-p-nitrophenyl-5-phenyl tetrazolium chloride) to formazan (a dark red coloured product), and absorbance was measured at 490 nm.

2.8. Real-time PCR

Expression of mRNA for genes coding for enzymes or transcription factor of interest were quantified by use of quantitative polymerase chain reaction (Q-PCR). Total RNA was extracted from approximately 30 mg of liver using the RNeasy Plus Mini Kit (Qiagen, Mississauga, ON, Canada) and muscle using RNeasy Lipid Tissue Mini Kit (Qiagen) according to the manufacturer's instructions. Purified total RNA was quantified using a NanoDrop ND-1000 spectrophotometer (NanoDrop Technologies, Wilmington, DE, USA). Integrity of RNA was checked on a 1% denaturing formaldehyde–agarose gel with ethidium bromide and visualized under ultraviolet (UV) light on a VersaDoc 4000MP imaging system (Bio-Rad, Mississauga, ON, Canada). Purified RNA samples were stored at −80 °C until synthesis of cDNA. A QuantiTect® Reverse Transcription Kit (Qiagen) was used to synthesis cDNA from 1 μg total RNA. The cDNA samples were stored at −20 °C until further analysis.

Quantitative real-time PCR (Q-PCR) was performed in 96-well PCR plates by use of an ABI 7300 Real-Time PCR System (Applied Biosystems, Foster City, CA, USA). Gene specific primers were designed against target genes by use of Primer 3 software, and the sequences of primers are shown (Table 1). A separate 45 μL PCR mixture consisting of Power SYBR Green master mix (Applied Biosystems), an optimized volume of cDNA, gene specific primers, and nuclease free water was prepared for each cDNA sample and primer pair. A final reaction volume of 20 μL was transferred to each well and reactions were performed in duplicate. The PCR mixture was denatured at 95 °C for 10 min before the first PCR cycle. The thermal cycle profile was as follows: denature for 10 s at 95 °C and extension for 1 min at 60 °C for a total of 40 PCR cycles. Optimal qPCR conditions were established by determining the efficiency of each qPCR assay by with a standard curve of serially diluted cDNA standards. Target gene mRNA abundance was quantified by normalizing to the expression of elongation factor 1α (EF 1α) according to the Mean Normalized Expression (MNE) method of Simon (2003).

Table 1
Gene-specific primer sequences for the quantitative real time PCR used in this study.

Target	Accession #	Sequence (5′–3′)	Annealing temp.
EF 1α	NM_131263.1	F: CTTCAACGCTCAGGTCATCA R: CGGTCGATCTTCTCCTTGAG	60
FAS	XM_682295	F: TGAAGCGGAGGCAGAAA R: CAAGCAGTGGCGTAAGG	60
SREBP 1	NM_001105129.1	F: ACATGGCCCTCAAAATGAAC R: GTCTGAAGCTGGAGGAGTGG	60
PTP 1B	NM130924	F: CTTCACCGAGAGCATCACAA R: GTTCGTCGGGTTGTTCATTT	60
HOAD	NM_001003515.1	F: CTGGTGGTGGAGGCTATTGT R: ACGTGTTGCTTGCGAATATG	60
CS	BC045362	F: ATCCGTTTCCGTGGTTACAG R: AGACAGCCAACTGACCTGCT	60
MAT 1A	NM_199871.1	F: ATGCAGTTCTTGACGCACAC R: TGGTGTCTCGCACAATCTTC	60

2.9. Statistical analyses

All data were tested for normality by use of the Shapiro–Wilk test and homogeneity of variance was investigated by use of Levene's test (SigmaStat 3.1, SPSS Inc., Chicago, IL, USA). Data that did not meet the assumptions for parametric statistical procedures were log 10 transformed. Non-transformed data are shown in all figures. Significant differences in total Se concentrations in foods, water and whole body fish as well as total length, wet mass, condition factor, SMR, AMR, F-AS and recovery ratios, and mRNA abundance of energy metabolism and methionine catabolism enzymes of fish fed control or SeMet spiked diets were tested by use of one-way ANOVA followed by the Holm–Sidak post hoc, multiple range test.

Effects of the Se treatment factor (main effect) and swim challenge factor (swim versus repeat swim) on U_{crit} of adult fish, and Se treatment factor and swim status factor (no swim, swim and repeat swim) on whole body triglyceride, glycogen and lactate were tested by use of two-way ANOVA. There were no interactions between factors for all two-way ANOVAs. When two-way ANOVA showed significant differences, Holm–Sidak post hoc tests were performed to compare differences between control versus SeMet exposed treatment groups, and no swim versus swim or repeat swim groups. At each incremental water velocity tested in U_{crit} analysis, repeated measures ANOVA (RM-ANOVA) followed by Holm–Sidak post hoc test was used to test for differences in MO_2 and COT of adult fish fed control food or SeMet. Since *t*-tests did not reveal significant differences between U_{crit} of swim and first U_{crit} ($U_{crit\text{-}1}$) of repeat swim groups within each dietary treatment group, those values were combined for subsequent statistical analysis. A similar approach was followed for both MO_2 and COT data. Data were expressed as mean ± S.E.M. Differences were considered statistically significant at $p \leq 0.05$.

3. Results

3.1. Concentrations of selenium

The total concentrations of Se in non-spiked (control) and spiked foods (nominal concentrations 3, 10 and 30 μg/g d.m.) were 1.3,

Table 2
Total Se concentrations in food and whole fish (μg/g, dry mass), mortality and morphometrics of adult zebrafish fed control or SeMet-spiked foods for 90 d. Data are mean ± S.E.M of $n = 3–5$ samples for food and fish Se analyses and $n = 20–21$ for morphometrics.

Dietary Se (μg/g d.m.)	Fish Se (μg/g d.m.)	Mortality (%)	Total length (mm)	Wet mass (g)	Condition factor
1.3 ± 0.01	1.6 ± 0.06	14.7 ± 3.5	38.1 ± 0.3	0.50 ± 0.02	0.90 ± 0.03
3.4 ± 0.09*	4.0 ± 1.00	20.0 ± 2.3	36.7 ± 0.5	0.49 ± 0.03	0.99 ± 0.03
9.8 ± 0.24*	7.6 ± 2.58	26.7 ± 3.5	37.5 ± 0.5	0.56 ± 0.03	1.07 ± 0.07*
27.5 ± 1.02*	11.2 ± 1.72*	34.7 ± 3.5*	36.4 ± 0.6	0.55 ± 0.02	1.14 ± 0.04*

Condition factor = (wet mass/total length^3) × 100,000.
* Significantly different from the control group using one-way ANOVA followed by Holm–Sidak post hoc test ($p < 0.05$).

3.4, 9.8 and 27.5 μg Se/g d.m., respectively (Table 2). Total concentrations of Se in spiked foods were significantly greater than the control diet ($p < 0.05$). Concentrations of dissolved Se in water from the 1.3, 3.4, 9.8 or 27.5 μg Se/g d.m. fed fish aquaria were 0.3, 0.3, 0.5 and 1.1 μg/L, respectively. Concentrations of dissolved Se in all treatment groups were comparable to the control group with the exception of dissolved Se in aquaria of the greatest SeMet group ($p < 0.05$). Whole body concentrations of Se in adult zebrafish fed 1.3, 3.4, 9.8 or 27.5 μg Se/g d.m. were 1.6, 4.0, 7.6 and 11.2 μg Se/g d.m., respectively. Although concentrations of Se in adult fish increased with increasing concentrations of SeMet in the diet, only fish fed the greatest concentrations of SeMet had significantly greater whole body concentrations of Se when compared to the control ($p < 0.05$).

3.2. Survival and growth

Mean mortality and condition factor in fish fed greatest concentrations of dietary SeMet were greater than the controls (Table 2). Mean mortality in fish fed 1.3, 3.4, 9.8 or 27.5 μg Se/g d.m. was 14.7%, 20.0%, 26.7% and 34.7%, respectively. Mortality was significantly greater for fish fed the 27.5 μg Se/g d.m. than for the controls ($p < 0.05$). There were no statistically significant differences in total length or body mass between fish fed SeMet or control diets. However mean condition factors of fish fed the two greatest concentrations of dietary SeMet (1.07 and 1.14) were significantly greater when compared to the control group (0.90) ($p < 0.05$).

3.3. Swim performance and oxygen consumption

Swimming performance was significantly reduced by the dietary Se treatment factor ($p = 0.003$ for Se treatment factor in two-way ANOVA; Fig. 1) but not the swim challenge factor ($p = 0.717$ for swim challenge factor in two-way ANOVA; Fig. 1). The U_{crit} for adult zebrafish fed diets augmented with SeMet was significantly lesser than that of fish fed the control diet. There were no differences in U_{crit} between swim (U_{crit-1}) and repeat swim (U_{crit-2}) fish within each treatment group. Recovery ratios for adult zebrafish fed 1.3, 3.4, 9.8 or 27.5 μg Se/g d.m. were 0.99 ± 0.05, 1.03 ± 0.03, 0.94 ± 0.05 and 0.98 ± 0.32, respectively. Recovery ratios for fish fed SeMet were not significantly different from that of the control group.

Mean MO_2 of both control and fish fed elevated SeMet were directly proportional to swimming speed (Fig. 2). Mean routine MO_2, which is MO_2 measured at the least water velocity, of fish fed SeMet were significantly greater than that of the control group ($p < 0.05$). Fish fed elevated SeMet had significantly greater MO_2 than fish fed the control diet at all incremental water velocities tested with the exception of 0.17 and 0.48 m/s ($p < 0.05$; Fig. 2). Mean fatigue MO_2 of fish fed 1.3, 3.4, 9.8 or 27.5 μg Se/g d.m. was 2572 ± 162.4, 2553 ± 124.1, 2305 ± 106.2 and 2238 ± 161.7 mg O_2/kg/h, respectively. There were no significant differences in fatigue MO_2 among control and fish fed elevated SeMet.

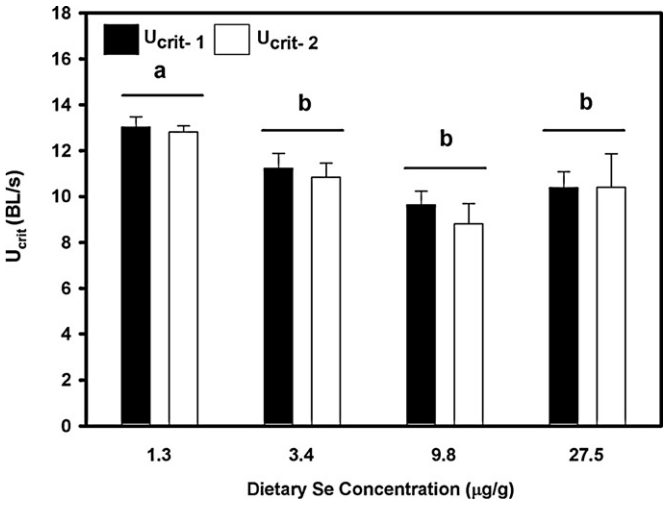

Fig. 1. Critical swimming speed (U_{crit}) in body lengths per second (BL/s) of adult zebrafish fed control (1.3 μg Se/g d.m.) or SeMet spiked diets (3.4, 9.8, and 27.5 μg Se/g d.m.) for 90 d. Solid bars are single exercise critical swimming speed (U_{crit-1}) and open bars are repeat critical swimming speed (U_{crit-2}). Critical swimming speed was significantly altered by the dietary Se treatment factor ($p = 0.003$) but not the swim challenge factor ($p = 0.717$) in two-way ANOVA. Different lowercase letters denote a significant effect of dietary SeMet treatments on U_{crits}. Data are mean ± S.E.M. of $n = 12–14$ for U_{crit-1} and $n = 6–7$ for U_{crit-2}.

3.4. SMR, AMR, F-AS and COT

Elevated dietary SeMet exposure significantly altered metabolic capacities of adult zebrafish (Fig. 3). Fish fed greater SeMet had significantly greater SMR when compared to fish fed the control diet

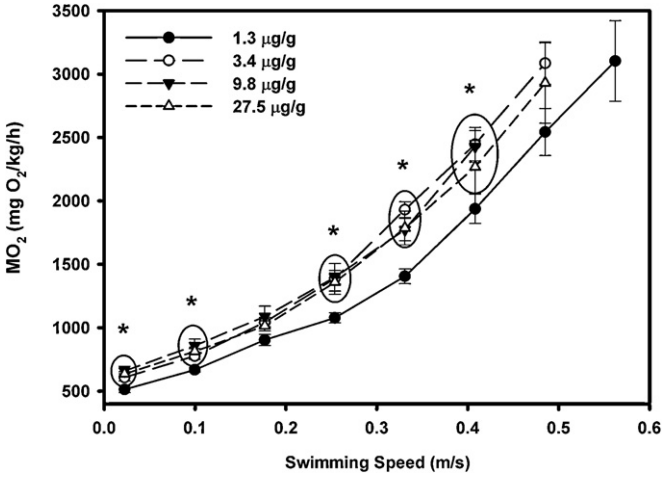

Fig. 2. Oxygen consumption (MO_2) versus swimming speed in adult zebrafish fed control (1.3 μg Se/g d.m.) or SeMet spiked diets (3.4, 9.8, and 27.5 μg Se/g d.m.) for 90 d. *Significantly different from control group using repeated measures ANOVA followed by Holm–Sidak post hoc tests ($p < 0.05$). Data are mean ± S.E.M. of $n = 12–14$ fish.

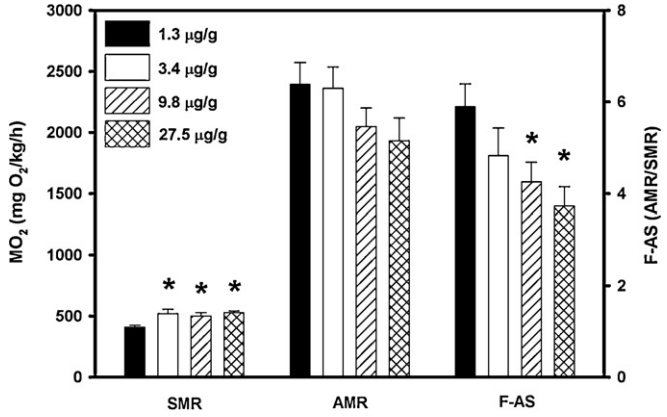

Fig. 3. Metabolic capacities (standard metabolic rate [SMR; the minimal maintenance MO_2 of unfed fish], active metabolic rate [AMR; the MO_2 at the maximum sustainable swimming speed in U_{crit}] and factorial aerobic scope [F-AS; the ratio of AMR to SMR]) of adult zebrafish fed control (1.3 μg Se/g d.m.) or SeMet spiked diets (3.4, 9.8, and 27.5 μg Se/g d.m.) for 90 d. *Significantly different from control group using one-way ANOVA followed by Holm–Sidak post hoc tests ($p < 0.05$). Data are mean ± S.E.M. of $n = 12–14$ fish.

($p < 0.05$). Although AMR was lesser in fish fed 9.8 and 27.5 μg Se/g d.m., those values were not significantly different from the control group. Alternatively, F-AS of fish fed 1.3, 3.4, 9.8 or 27.5 μg Se/g d.m. was 5.98, 4.82, 4.25 and 3.73, respectively, and F-AS in fish fed the two greatest concentrations of SeMet were significantly lesser than fish fed the control diet ($p < 0.05$; Fig. 3). Compared to control fish, cost of transport was consistently greater in fish fed diets that had been augmented with SeMet. Cost of transport was significantly greater in fish fed 3.4, 9.8 or 27.5 μg Se/g d.m. compared to control fish at water velocities of 0.09, 0.25, 0.33 or 0.40 m/s ($p < 0.05$; Fig. 4).

3.5. Whole body triglycerides and glycogen

Concentrations of triglycerides were significantly altered by both the dietary Se treatment factor and the swim status factor ($p < 0.001$ for Se treatment factor and $p = 0.043$ for swim status factor in two-way ANOVA; Fig. 5). Whole body concentrations of triglycerides were significantly greater in fish fed 9.8 or 27.5 μg Se/g d.m. compared to fish fed the control diet (Fig. 5). Although

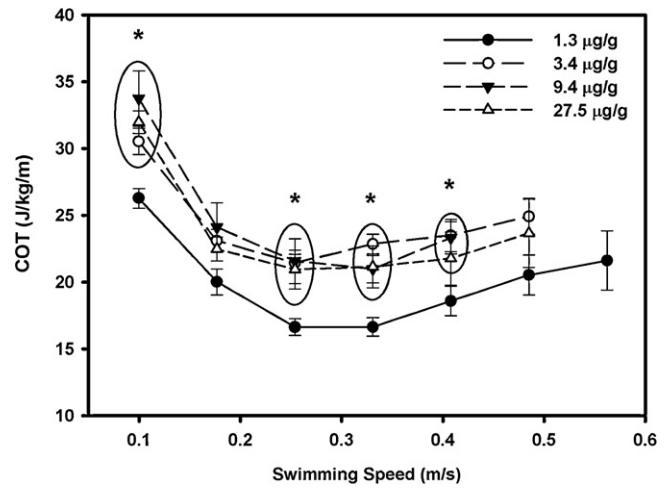

Fig. 4. Cost of transport (COT) as a function of swimming speed in adult zebrafish fed control (1.3 μg Se/g d.m.) or SeMet spiked diets (3.4, 9.8, and 27.5 μg Se/g d.m.) for 90 d. *Significantly different from control group using repeated measures ANOVA followed by Holm–Sidak post hoc tests ($p < 0.05$). Data are mean ± S.E.M. of $n = 12–14$ fish.

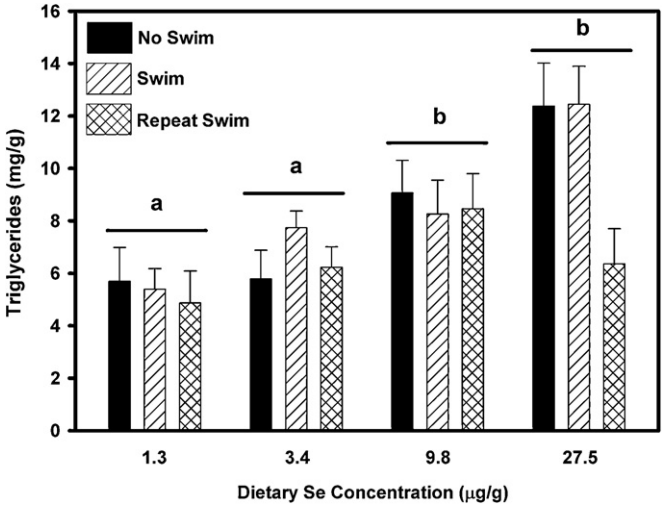

Fig. 5. Whole body triglycerides in adult zebrafish fed control (1.3 μg Se/g d.m.) or SeMet spiked diets (3.4, 9.8, and 27.5 μg Se/g d.m.) for 90 d. Solid, stripped and crossed bars represent whole body triglycerides in fish from no swim (fish withheld from swim tests), swim (fish subjected to single U_{crit}) and repeat swim (fish subjected two U_{crits}) groups, respectively. Both dietary Se treatment factor and swim status factor had significant effects on concentration of whole body triglycerides in fish ($p < 0.001$ for dietary Se treatment factor and $p = 0.043$ for swim status factor in two-way ANOVA). Different lowercase letters denote a significant effect of dietary Se treatments on concentration of whole body triglycerides in adult fish. Post hoc tests did not show any significant differences of swim status factor on whole body triglycerides in respective dietary Se treatment groups. Data are mean ± S.E.M. of $n = 6–7$ fish.

two-way ANOVA showed a significant effect of swim status factor on concentrations of whole body triglycerides in fish, post hoc tests were unable to demonstrate statistically significant differences between no swim versus swim or repeat swim fish within respective dietary Se treatment groups.

The dietary Se treatment factor was not associated with variance of the concentration of glycogen in whole bodies of fish ($p = 0.193$ for the dietary Se factor in two-way ANOVA; Fig. 6), but whole body

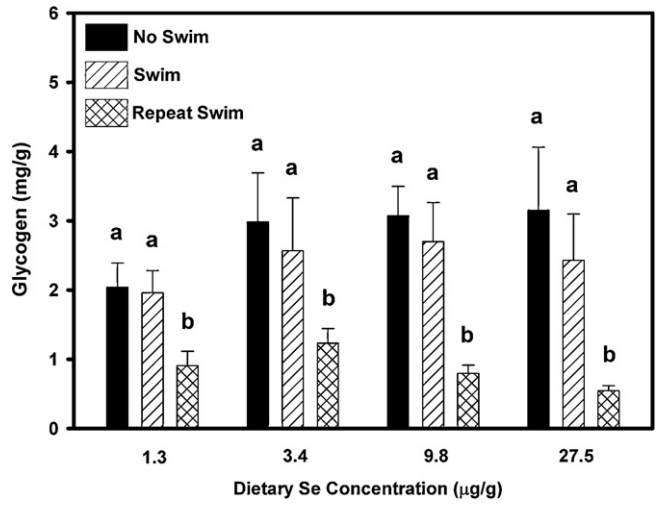

Fig. 6. Whole body glycogen in adult zebrafish fed control (1.3 μg Se/g d.m.) or SeMet spiked diets (3.4, 9.8, and 27.5 μg Se/g d.m.) for 90 d. Solid, stripped and crossed bars represent whole body glycogen in fish from no swim (fish withheld from swim tests), swim (fish subjected to single U_{crit}) and repeat swim (fish subjected two U_{crits}) groups, respectively. For whole body glycogen, $p = 0.193$ for dietary Se treatment factor and $p < 0.001$ for swim status factor in two-way ANOVA. Different lowercase letters denote significant differences in concentrations of whole body glycogen between no swim versus repeat swim groups in respective Se treatment groups (Holm–Sidak post hoc tests; $p < 0.05$). Data are mean ± S.E.M. of $n = 6–7$ fish.

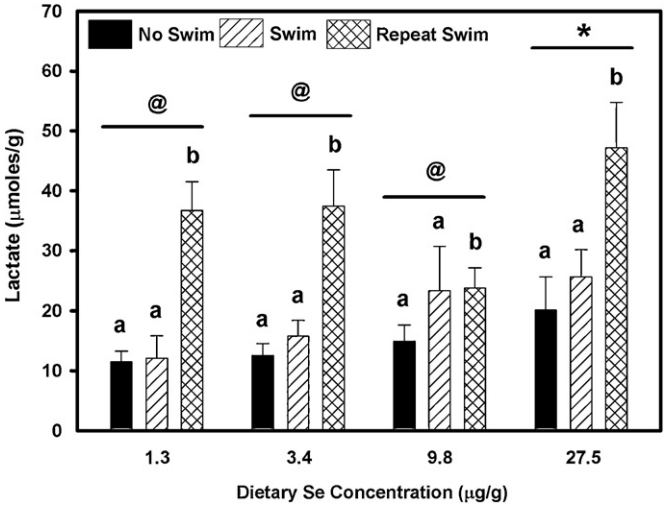

Fig. 7. Whole body lactate concentrations in adult zebrafish fed control (1.3 μg Se/g d.m.) or SeMet spiked diets (3.4, 9.8, and 27.5 μg Se/g d.m.) for 90 d. Solid, stripped and crossed bars represent whole body lactate in fish from no swim (fish withheld from swim tests), swim (fish subjected to single U_{crit}) and repeat swim (fish subjected two U_{crits}) groups, respectively. Two-way ANOVA showed significant effects of both dietary Se treatment factor ($p = 0.022$) and swim status factor ($p < 0.001$) on whole body lactate concentrations. Different symbols (@, *) denote significant differences in whole body lactate between control and fish fed SeMet spiked diets, and different lowercase letters denote significant differences in whole body lactate concentrations between no swim versus repeat swim fish in respective dietary Se treatment groups (Holm–Sidak post hoc tests; $p < 0.05$). Data are mean ± S.E.M. of $n = 6$–7 fish.

concentrations of glycogen were significantly associated with the swim status factor ($p < 0.001$ in two-way ANOVA; Fig. 6). When compared within each concentration of SeMet in the diet, significantly lesser whole body concentrations of glycogen were observed in repeat swim fish when compared to no swim fish ($p < 0.05$; Fig. 6).

3.6. Whole body lactate

Whole body concentrations of lactate in adult zebrafish were significantly altered by both the dietary Se treatment and the swim status factors ($p = 0.022$ for dietary Se treatment factor and $p < 0.001$ for swim status factor in two-way ANOVA; Fig. 7). Fish fed the greatest concentrations of SeMet showed significantly greater whole body lactate accumulation when compared to fish fed the control diet ($p < 0.05$; Fig. 7). Within each concentration of SeMet in the diet, whole body concentration of lactate was significantly greater in repeat swim fish when compared to no swim fish ($p < 0.05$; Fig. 7). Mean whole body concentrations of lactate in repeat swim fish were 36.7, 37.5, 23.8 and 47.2 μmol/g in fish fed 1.3, 3.4, 9.8 or 27.5 μg Se/g d.m., respectively. These values were between 1.5 and 4 times greater than concentrations of lactate in corresponding no swim fish (Fig. 7).

3.7. Abundances of mRNA of HOAD, CS, SREBP 1, PTP 1B, FAS and MAT 1A

Abundances of mRNA of HOAD, CS, SREBP 1, PTP 1B, FAS and MAT 1A in liver and HOAD, CS, SREBP1, PTP 1B and FAS in muscle of adult zebrafish were determined after chronic dietary SeMet exposure. Abundances of mRNA of SREBP 1, HOAD and MAT 1A were significantly down-regulated in liver of fish fed the two greatest concentrations of SeMet (9.8 or 27.5 μg Se/g d.m.) when compared to fish from the control group ($p < 0.05$; Figs. 8A, B and 9). Significant down-regulation of mRNA abundances of PTP 1B was observed in muscle of fish fed diets augmented with SeMet compared to control fish ($p < 0.05$; Fig. 8C). Abundance of mRNA of CS was up-regulated in muscle of fish fed 9.8 μg Se/g d.m. ($p < 0.05$; Fig. 8D). There were no statistically significant differences in abundances of mRNA of CS, PTP 1B and FAS in liver and HOAD, SREBP 1 and FAS in muscle of adult zebrafish exposed to SeMet when compared to control fish (data not shown).

4. Discussion

The present study is the first to investigate repeat swimming performance, metabolic capacities (SMR, AMR and F-AS) and gene expression of energy metabolism and methionine catabolism enzymes in adult zebrafish after chronic dietary exposure to elevated SeMet. Concentrations of Se used in this study were environmentally relevant and had been previously shown to elicit sublethal effects in adult zebrafish (Thomas and Janz, 2011). Accumulation of SeMet from diets to adult zebrafish occurred in a concentration-dependent manner. Previous studies reported similar accumulation of Se by the Sacramento splittail (*Pogonichthys macrolepidotus*), white sturgeon (*Acipenser transmontanus*), and adult zebrafish exposed to dietary SeMet (Teh et al., 2004; Tashjian et al., 2006; Thomas and Janz, 2011). In addition, since SeMet is the dominant form of Se present in food, the dietary concentrations of SeMet and bioaccumulation of Se in the present study represent values commonly observed in fish inhabiting aquatic ecosystems that are contaminated with Se (Janz et al., 2010).

Greater condition factor in fish fed diets that had been augmented with SeMet in the present study was in contrast to a previous study, where an increase in total length and wet mass but no change in condition factor were observed after exposure to dietary SeMet (Thomas and Janz, 2011). Dietary SeMet has been reported previously to cause changes in the morphology of fishes. Lesser body mass, fork length and condition factor were observed in Sacramento splittail and white sturgeon that had been fed SeMet (Teh et al., 2004; Tashjian et al., 2006), whereas dietary exposure of cutthroat trout (*Oncorhynchus clarki bouvieri*) to SeMet did not affect body mass (Hardy et al., 2010). Significantly lesser body mass and fork length, and greater condition factor were observed in rainbow trout that had been exposed to dietary SeMet (8.47 μg Se/g d.m.) (Wiseman et al., 2011a), whereas in a second study (Wiseman et al., 2011b) greater body mass and condition factor were observed but there was no statistically significant effect on fork length.

The observation in the present study that, compared to fish fed the control diet, mortality was significantly greater in fish fed the greatest concentration of SeMet (27.5 μg Se/g d.m.) is consistent with the results of previous studies. Greater mortality was observed when zebrafish were fed 26.6 μg Se/g d.m. as SeMet (Thomas and Janz, 2011). A similar result was observed when Sacramento splittail were fed 26.0 and 57.6 μg Se/g d.m. in the form of SeMet for 9 months (Teh et al., 2004) and bluegill (*Lepomis macrochirus*) fed 6.5 and 26 μg Se/g d.m. in the form of SeMet for 60 d (Cleveland et al., 1993).

Since swimming performance integrates many physiological processes, it is commonly used to study whole organismal effects after sublethal exposure to chemicals (Scott and Sloman, 2004). Dietary SeMet exposure had pronounced effect on swimming performance of adult zebrafish. Fish fed greater concentrations of SeMet had reduced swimming performance than the controls. In order to make sure that fish from all treatment groups were able to swim at their maximum swimming capacity before the onset of fatigue, we compared fatigue MO_2 of fish. No significant differences in fatigue MO_2 among fish exposed to control and greater concentrations of SeMet indicated that fish from all treatment groups were able to swim their maximum swimming capacity before the onset of fatigue. Previous studies have reported impaired swimming performance in adult zebrafish exposed to elevated dietary SeMet and aqueous exposure to 2,4-dinitrophenol (Thomas and Janz, 2011;

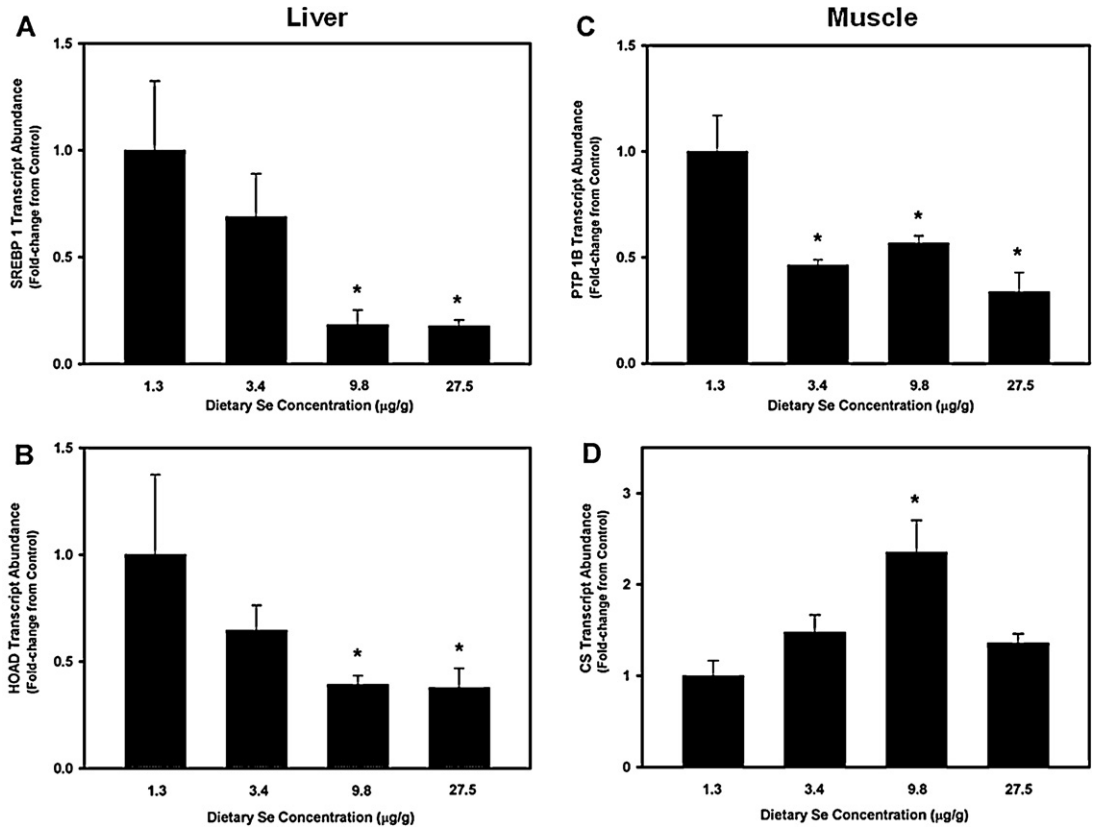

Fig. 8. mRNA abundance of (A) sterol regulatory element binding protein 1 (SREBP 1) and (B) β-hydroxyacyl coenzyme A dehydrogenase (HOAD) in liver, and (C) protein tyrosine phosphatase 1B (PTP 1B) and (D) citrate synthase (CS) in muscle of adult zebrafish fed control (1.3 μg Se/g d.m.) or SeMet spiked diets (3.4, 9.8, and 27.5 μg Se/g d.m.) for 90 d. Transcript abundance was determined by quantitative real-time PCR. *Significantly different from control group using one-way ANOVA followed by Holm–Sidak post hoc tests ($p < 0.05$). Data are mean ± S.E.M. of $n = 4$ liver or muscle samples.

Marit and Weber, 2011). The mean U_{crit} value of zebrafish fed the control diet in the present study (13.5 BL/s) was greater than U_{crit} values reported previously in control fish by Thomas and Janz (2011; 9 BL/s) and Marit and Weber (2011; 5 BL/s). The observed differences in U_{crits} among these studies in adult zebrafish could be due to differences in acclimation time and U_{crit} protocol adopted in the present study and in those reported by Thomas and Janz (2011) and Marit and Weber (2011). For example, differences in incremental velocity and time interval between velocity increments in U_{crit} tests have been shown to alter swimming performance of fish (reviewed in Hammer, 1995).

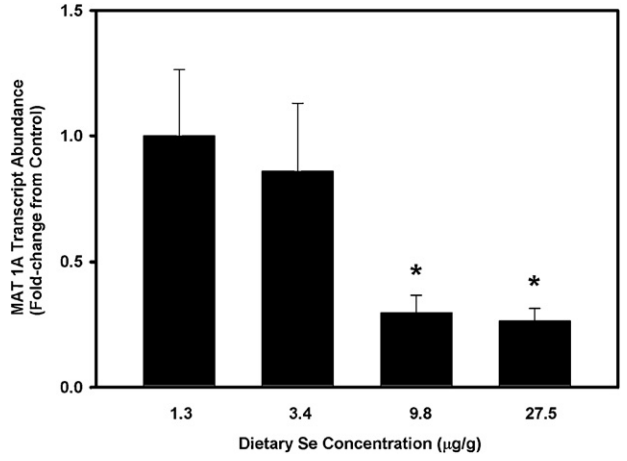

Fig. 9. mRNA abundance of methionine adenosyltransferase 1 alpha (MAT 1A) in liver of adult zebrafish fed control (1.3 μg Se/g d.m.) or SeMet spiked diets (3.4, 9.8, and 27.5 μg Se/g d.m.) for 90 d. Transcript abundance was determined by quantitative real-time PCR. *Significantly different from control group using one-way ANOVA followed by Holm–Sidak post hoc tests ($p < 0.05$). Data are mean ± S.E.M. of $n = 4$ liver samples.

Abnormal swimming behaviours were observed in bluegill sunfish exposed to waterborne selenite, and in Sacramento splittail and white sturgeon fed SeMet (Cleveland et al., 1993; Teh et al., 2004; Tashjian et al., 2006). Although fish fed enriched concentrations of SeMet in the present study had significantly impaired U_{crit}, their recovery ratios were not different from fish fed the control diet. The recovery ratio ($U_{crit\text{-}2}/U_{crit\text{-}1}$) is a relevant measure of the ability of fish to recover from exhaustive exercises (Jain et al., 1998). These results suggest that exposure to SeMet only reduces U_{crit} of adult zebrafish and exposure to SeMet is less likely to alter the ability of fish to recover from exercise stress. Similar results were observed in chub (*Leuciscus cephalus*) collected from metal contaminated sites (McKenzie et al., 2007). An explanation for the observed lesser U_{crit} of adult zebrafish exposed to SeMet in the present study is impaired muscle function. Exposure to sublethal concentrations of copper has been shown to reduce U_{crit} in brown trout (*Salmo trutta*) by impairing contraction of muscle fibres (Beaumont et al., 2000). Selenomethionine can substitute for methionine in muscle protein (Stadtman, 1974) and such inappropriate amino acid insertion could possibly cause dysfunction of muscle. Exposure to augmented concentrations of SeMet has been reported to result in lesser amplitude of tail-beat of adult zebrafish (Thomas and Janz, 2011). Since amplitude of tail-beat is functionally related to contraction of musculature of the tail, a lesser amplitude of tail-beat of fish fed SeMet indirectly supports the hypothesis that the cause

of this is dysfunction of muscle. In addition, catabolism of SeMet has been reported to induce oxidative stress in cells (Palace et al., 2004) and oxidative stress has been shown to impair contraction of muscle fibres (Musarò et al., 2010). Taken together, these results suggest that dietary exposure to SeMet has the potential to cause impaired muscle function. However, further studies are needed to test the hypothesis that SeMet directly causes muscle dysfunction in fish.

An alternate explanation for lesser U_{crit} of zebrafish fed SeMet is altered aerobic metabolism. Since U_{crit} is a measure of aerobic swimming capacity of fish, alteration of aerobic metabolism could negatively affect swimming performance. Both MO_2 and activity or expression of key aerobic energy metabolizing enzymes (e.g., CS and HOAD) provides relevant information on aerobic metabolism in fish. Altered aerobic metabolism has been shown to result in impaired swimming in fish collected from metal-contaminated sites and fish exposed to organic and inorganic pollutants (Shingles et al., 2001; Rajotte and Couture, 2002; Pane et al., 2004; Marit and Weber, 2011). In the present study greater values for SMR, exercise MO_2, and COT in fish fed SeMet indicated greater requirements for energy, during both routine activities and forced swimming by those fish to maintain homeostasis, compared to fish fed a control diet. Cray fish (*Procambarus acutus*) and bullfrog (*Rana catesbeiana*) collected from metal-contaminated sites were reported to have higher SMR than reference sites (Rowe et al., 1998, 2001). Elevated SMR may indicate greater energy requirements of organisms to repair toxicant-induced tissue damage and/or eliminate toxicants (Calow, 1991). Both AS and F-AS are commonly calculated from swimming respirometry studies to determine the aerobic capacity of fish (Priede, 1985; Killen et al., 2007). F-AS is the ratio of AMR to SMR and it measures aerobic capacity of fish in a non-mass-specific basis (Killen et al., 2007). Thus, F-AS was used instead of AS to compare aerobic capacity of fish. Significantly lesser values for F-AS in fish fed the two greatest concentrations of dietary SeMet indicates that those fish had a reduced capacity to support aerobic physiological functions such as swimming. Elevated SMR of fish fed SeMet may also contribute to the observed changes in U_{crit}, FAS and COT.

During aerobic swimming fish utilize triglycerides as their major energy source (Hammer, 1995; Moyes and West, 1995). Although zebrafish fed SeMet had significantly greater whole body concentrations of triglycerides, they appeared to not utilize this source of energy during swimming as efficiently as control fish. As explained below, down regulation of expression of HOAD and MAT 1A in liver of fish fed elevated SeMet could have altered triglyceride catabolism and transport. During anaerobic metabolism, fish use glycogen as a major source of energy and as a result lactate is accumulated in muscle (Hammer, 1995; Moyes and West, 1995). In large-bodied fishes, lactate in blood plasma is often determined as a measure of anaerobic energy metabolism, whereas in small-bodied fish such as zebrafish, whole body or muscle lactate concentrations can be determined as a surrogate marker of anaerobic energy metabolism (Sancho et al., 1996; Beaumont et al., 2000; McClelland et al., 2006). Swim performance, temperature and exposure to both organic and inorganic chemicals have been reported to cause greater concentrations of lactate in blood plasma or whole body of fishes (Sancho et al., 1996; Beaumont et al., 2000; McClelland et al., 2006). In the present study, whole body concentrations of lactate were proportional to the concentration of SeMet in the diet, where zebrafish fed the greatest concentration of SeMet had significantly greater whole body concentrations of lactate, compared to control fish. The observation of lesser F-AS, greater accumulation of triglycerides and greater accumulation of lactate in fish fed greater concentrations of SeMet provide further evidence that those fish had an impaired ability to perform aerobic metabolism. Taken together, the lesser U_{crit} of adult zebrafish fed SeMet is most likely related to altered aerobic metabolism.

The observed greater accumulation of whole body triglycerides in fish fed greater concentrations of dietary SeMet in the present study was agreeable to previous studies. Greater accumulation of triglycerides was reported in zebrafish and rainbow trout exposed to augmented dietary SeMet (Thomas and Janz, 2011; Wiseman et al., 2011b) and in several species of fish collected from Se-impacted field sites (Kelly and Janz, 2008; Driedger et al., 2009). To elucidate potential mechanisms of triglyceride accumulation in fish exposed to elevated SeMet, we investigated gene expression of key enzymes (FAS, PTP 1B and HOAD) and a transcription factor (SREBP 1) involved in triglyceride metabolism. Elevated dietary SeMet exposure resulted in down regulation of mRNA abundance of SREBP 1 and HOAD in liver and PTP 1B in muscle of adult zebrafish. However, there were no differences in mRNA abundance of FAS in both liver and muscle of zebrafish fed elevated dietary SeMet. In contrast, dietary Se exposure in mammals has been shown to increase expression or activities of PTP 1B, FAS and SREBP 1 (Mueller et al., 2008, 2009a,b). It is unclear why the present study observed significant down regulation of SREBP 1 and PTP 1B in fish fed elevated SeMet. However the observed down regulation of HOAD in liver of adult zebrafish exposed to elevated dietary SeMet could potentially result in triglyceride accumulation. β-Hydroxyacyl coenzyme A dehydrogenase is a key mitochondrial enzyme involved in β-oxidation of fatty acids. Impaired β-oxidation of fatty acids is shown to increase triglyceride accumulation in both fish and mammals (Fromenty et al., 1990; van den Thillart et al., 2002).

Since elevated SeMet exposure is reported to limit methionine uptake and its insertion into protein, we investigated the role of dietary SeMet exposure in methionine catabolism in adult zebrafish. mRNA expression of the liver-specific MAT gene, methionine adenosyltransferase 1 alpha (MAT 1A), was significantly down regulated in adult zebrafish fed greater than 9 μg Se/g d.m. in the form of SeMet. To the best of our knowledge, this is first study to report down regulation of MAT 1A in fish after chronic dietary SeMet exposure. Exposure to either selenite or selenocystine has been reported to deplete SAM and inactivate MAT in mammalian liver (Hoffman, 1977; Hasegawa et al., 1996). Reduced cellular methionine concentration and elevated accumulation of s-adenosyl homocysteine, a by-product of methylation, are reported to inhibit expression or activity of liver specific MAT in mammals (Lu, 2000; Mato et al., 2002, 2008). Limited MAT 1A expression is an indicator of both reduced SAM production and impaired methylation (Lu, 2000; Mato et al., 2002). Liver is a major organ responsible for synthesis of triglycerides and methylation plays an important role in transport of triglycerides out of liver (Kerai et al., 1999). Restricted dietary intake of methionine and reduced SAM levels have been shown to increase hepatic triglyceride accumulation in both mammals and fish (Kerai et al., 1999; Rinella et al., 2008; Espe et al., 2010). Based on these results, we postulate that down regulation of both HOAD and MAT 1A in zebrafish liver following elevated dietary SeMet exposure in the present study caused greater accumulation of triglycerides. Both methylation and SAM are vital for regulation of gene expression, transport of triglycerides and biotransformation of toxicants including SeMet (Daniels, 1996; Chiang et al., 1996; Lu, 2000; Kobayashi et al., 2002). More extensive studies are needed to investigate the role of SeMet exposure in methylation, regulation of gene expression, transport of triglycerides and biotransformation of toxicants in fish.

In summary, environmentally relevant concentrations of SeMet in the diet of adult zebrafish can cause effects at both cellular and organismal levels of organization. Lesser swimming performance of adult fish fed SeMet could alter food acquisition, predator avoidance and migration, and such effects could negatively affect fitness and survivability of fish inhabiting aquatic ecosystems that are contaminated with Se. Impaired aerobic metabolism in fish exposed to greater concentrations of SeMet in the diet might be responsible

for poorer swimming performance. Lesser hepatic β-oxidation of fatty acids and methionine catabolism in fish fed SeMet might have caused triglyceride accumulation in liver. Selenomethionine-induced down-regulation of MAT 1A can impair production of SAM in the liver, and such an effect could possibly reduce cellular methylation reactions. Fish fed the two greatest concentrations of dietary SeMet had better condition factors than fish fed control diet. This result suggests that condition factor of fish is not a good determinant of assessing overall fish fitness after exposure to SeMet.

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