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Cardiometabolic response of juvenile rainbow trout exposed to dietary selenomethionine

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ABSTRACT

Selenium (Se) is considered an essential trace element, involved in important physiological and metabolic functions for all vertebrate species. Fish require dietary concentrations of 0.1–0.5 μg Se/g dry mass (d.m.) to maintain normal physiological and selenoprotein function, however concentrations exceeding 3 μg/g d.m. have been shown to cause toxicity. As Se is reported to have a narrow margin between essentiality and toxicity, there is growing concern surrounding the adverse effects of elevated Se exposure caused by anthropogenic activities. Previous studies have reported that elevated dietary exposure of fish to selenomethionine (Se-Met) can cause significant cardiotoxicity and alter aerobic metabolic capacity, energy homeostasis and swimming performance. The goal of this study aims to further investigate mechanisms of sublethal Se-Met toxicity, particularly potential underlying cardiovascular and metabolic implications of chronic exposure to environmentally relevant concentrations of dietary Se-Met in juvenile rainbow trout (*Oncorhynchus mykiss*). Juvenile rainbow trout were fed either control food (1.3 μg Se/g d.m.) or Se-Met spiked food (6.4, 15.8 or 47.8 μg Se/g d.m.) for 60 d at 3% body weight per day. Following exposure, ultrahigh resolution B-mode and Doppler ultrasound was used to characterize cardiac function *in vivo*. Chronic dietary exposure to Se-Met significantly increased stroke volume, cardiac output, and ejection fraction. Fish fed with Se-Met spiked food had elevated liver glycogen and triglyceride stores, suggesting impaired energy homeostasis. Exposure to Se-Met significantly decreased mRNA abundance of citrate synthase (CS) in liver and serpin peptidase inhibitor, clad H1 (SERPINH) in heart, and increased mRNA abundance of sarcoplasmic reticulum calcium ATPase (SERCA) and key cardiac remodelling enzyme matrix metalloproteinase 9 (MMP9) in heart. Taken together, these responses are consistent with a compensatory cardiac response to increased susceptibility to oxidative stress, namely a decrease in ventricular stiffness and improved cardiac function. These cardiac alterations in trout hearts were linked to metabolic disruption in other major metabolic tissues (liver and skeletal muscle), impaired glucose tolerance with increased levels of the toxic glucose metabolite, methylglyoxal, increased lipid peroxidation in skeletal muscle, development of cataracts and prolonged feeding behaviour, indicative of visual impairment. Therefore, although juvenile rainbow trout hearts were apparently able to functionally compensate for adverse metabolic and antioxidant changes after chronic dietary exposure Se-Met, complications associated with hyperglycemia in mammalian species were evident and would threaten survival of juvenile and adult fish.

1. Introduction

Selenium (Se) is considered as an essential trace element, involved in important physiological and metabolic functions for all vertebrate species. Fish require dietary concentrations of 0.1–0.5 μg Se/g dry mass (d.m.) to maintain normal physiological and selenoprotein function, however concentrations exceeding 3 μg/g d.m. have been shown to cause toxicity (Janz, 2012). This represents the paradoxical nature of Se, as there is a narrow margin between essentiality and toxicity. Oviparous vertebrates such as birds and fishes are among the most

Abbreviations: AV, atrioventricular; CSI, cardiosomatic index; CAT, catalase; CS, citrate synthase; d.m., dry mass; EDV, end-diastolic volume; EF1α, elongation factor 1α; ESV, end-systolic volume; f_H, contractile rate; GPX1a, glutathione peroxidase 1a; GST-pi, glutathione-S-transferase pi class; GTT, glucose tolerance test; hERG, human Ether-à-go-go-Related Gene; HOAD, β-hydroxyacyl coenzyme A dehydrogenase; HSI, hepatosomatic index; MMP, matrix metalloproteinase; MNE, mean normalized expression; MO2, metabolic oxygen consumption; Q, cardiac output; ROS, reactive oxygen species; Se, selenium; Se-Cys, selenocysteine; Se-Met, selenomethionine; SERCA, sarcoplasmic reticulum calcium ATPase; SERPINH, serpin peptidase inhibitor, clad H1 protein; SOD, superoxide dismutase; T2D, type 2 diabetes; U_{crit}, critical swimming speed; VB, ventriculobulbar; Vs, stroke volume

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sensitive organisms, as selenium is deposited into eggs during vitellogenesis, and transferred to developing embryos during yolk resorption (Lemly, 1997; Janz et al., 2010). Selenium is a naturally occurring element, commonly found in areas of sedimentary rock depositions, in particular black shale, phosphate and coal deposits (Presser et al., 2004; Maher et al., 2010). Coal-fired power plants, petroleum refineries, as well as agricultural and mining industries, can greatly exacerbate the loading of Se into the environment (Lemly, 2004; Maher et al., 2010; Janz, 2012). Water soluble inorganic Se, in the form of selenite and selenate, are taken up by primary producers and microorganisms, then biotransformed into organoselenoproteins, predominantly selenomethionine (Se-Met) and selenocysteine (Se-Cys) (Fan et al., 2002). Upon ingestion, there is a trophic transfer of organic Se amongst primary consumers, secondary consumers and finally higher order consumers including birds, fish, and humans. As a result, there is a risk of bioaccumulation and biomagnification of organic Se. Se-Met is the predominant dietary source of Se available to fish (Fan et al., 2002). Due to the structural similarity between Se-Met and the amino acid methionine, Se-Met can avoid biotransformation and be directly integrated into any methionine-containing protein in a non-specific, dose-dependent manner (Behne et al., 1991). In addition, due to its biotransformation to selenoxides, such as methylselenol, excessive Se-Met uptake has been shown to cause oxidative stress *in vitro* (Palace et al., 2004; Spallholz et al., 2004). While the underlying toxicodynamic mechanisms behind Se toxicity are not fully understood, it is generally agreed that protein dysfunction and oxidative stress are fundamental culprits (Ma et al., 2012).

Using zebrafish (*Danio rerio*) as a model test organism, elevated exposure to Se-Met has been reported to cause significant cardiotoxicity as well as alter aerobic metabolic capacity, respiration, energy homeostasis, swimming performance and increased incidence of early life stage deformities and mortalities (Tashjian et al., 2006; Thomas and Janz, 2011; Thomas et al., 2013; Arnold et al., 2016; Pettem et al., 2017). Conversely, there is a lack of research investigating the potential role of cardiovascular dysfunction associated with such responses in rainbow trout exposed to elevated chronic dietary Se exposure. Previous studies have shown that the oxygen consumption (MO_2) of fish exposed to Se were consistently greater than that of control fish (Scott and Sloman, 2004; Thomas and Janz, 2011; Thomas et al., 2013). Metabolic rate and cardiac output are closely integrated, allowing the heart to adjust for increases in oxygen demand, and any alterations to these responses could lead to impaired aerobic performance (MacKinnon and Farrell, 1992). Whether the Se-Met-mediated increases in oxygen demand can be met by increased cardiac output in juvenile trout is unknown. In a previous study using zebrafish and similar diets, a significant down-regulation of matrix metalloproteinase 2 (MMP2) mRNA transcript abundance was observed in hearts of the elevated Se-Met exposed fish (Pettem et al., 2017). MMPs are a class of proteolytic enzymes that have important vascular and cardiac remodelling properties (Seliktar et al., 2001). Decreased cardiac MMP expression has been reported along with increased collagen content (fibrosis) specifically in rainbow trout (Keen et al., 2017).

Glycogen and triglycerides are the two main forms of energy stored in fish and are predominantly used in burst and prolonged swimming, respectively (Hammer, 1995; Moyes and West, 1995). Fish fed a range of excess Se-Met in controlled laboratory settings, as well as those collected from Se-contaminated sites, exhibited elevated levels of stored triglycerides and glycogen (Bennett and Janz, 2007; Wiseman et al., 2011; Thomas et al., 2013; Pettem et al., 2017). In animal models of human disease, excess dietary selenium has been shown to impair insulin-regulated carbohydrate and lipid metabolism through a mechanism involving reactive oxygen species to produce diabetes (Steinbrenner, 2013; Wang et al., 2014). Moreover, prolonged hyperglycemia is associated with increased circulating levels of the toxic glucose metabolite, methylglyoxal, in mammals (Desai et al., 2010; Adolphe et al., 2012; Kalapos, 2013). Methylglyoxal, in turn, is known to promote oxidative stress and is a precursor to advanced glycation end products responsible for development of diabetic complications such as cataracts (Desai et al., 2010; Kalapos, 2013). In fish, even in the absence of overt diabetes, inadequate mobilization of these energy stores could lead to impaired swimming and cardiovascular function. We hypothesized that a similar link exists between excess selenium, impaired energy mobilization, cardiotoxicity, development of impaired glucose control, oxidative stress and methylglyoxal leading to complications such as cataracts in fish.

The overall goal of this study was to further investigate sublethal mechanisms of Se toxicity in juvenile rainbow trout, particularly potential underlying cardiometabolic and energy storage implications of chronic exposure to environmentally relevant concentrations of dietary Se-Met. Cardiac function was assessed *in vivo* using ultrahigh resolution ultrasonography, followed by quantification of energy stores in heart, liver and muscle tissues, and mRNA transcript abundance of selected genes involved in aerobic metabolism, cardiac function, and oxidative stress. Glycemic control was assessed using an intraperitoneal glucose challenge, with measurement of blood glucose, levels of the toxic glucose metabolite, methylglyoxal, and lipid peroxidation using thiobarbituric acid reactive substances (TBARS) assay. Finally, fish were evaluated for development of cataracts and behavioural evidence of visual impairment.

2. Materials and methods

2.1. Test compound

Seleno-L-methionine (≥98% purity) was purchased from Sigma-Aldrich (Oakville, ON, Canada).

2.2. Test species

All female rainbow trout eggs were purchased from a commercial supplier (Troutlodge, Sumner, WA, USA) and reared in the Aquatic Toxicology Research Facility at the Toxicology Centre, University of Saskatchewan. Eggs were hatched and fish reared for 12 months, then 240 juvenile (yearling) rainbow trout (size ranging from 80 to 100g) were randomly selected from this colony and placed into four 719L tanks (60 fish/tank). These tanks were supplied with continuous aeration (11 ± 0.5 mg/L dissolved oxygen), running water with a flow rate of 3.5L/min, controlled temperature (12 ± 1 °C) and photoperiod (14 h light: 10 h dark). Fish were then acclimated for 2 weeks to these conditions and fed commercial trout pellet food (Martin Classic Sinking Fish Feed, Martin Mills Inc., Elmira, ON, Canada) twice daily prior to the beginning of the dietary exposure with daily 50% water changes. Experiments were conducted according to procedures approved by the University of Saskatchewan Animal Care and Use committee according to the Canadian Council on Animal Care guidelines.

2.3. Diet preparation

Nominal concentrations (3, 10 and 30 μg/g d.m.) of Se in the form of Se-Met were dissolved in nanopure deionized distilled water, added to ground trout pellets, and mixed thoroughly for approximately 20 min. The control diet followed the above process with an equal volume of water, without the addition of Se-Met. Diets were then fed through a meat grinder, cut into individual pellets and placed in a 55 °C drying oven for 24 h until water had been removed from all the diets. Food was then stored at 4 °C for the remainder of the experiment. Representative samples of each diet were taken for total Se analysis using inductively coupled plasma-mass spectrometry (ICP-MS) at the Toxicology Centre (University of Saskatchewan, Saskatoon, SK, Canada).