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Effects of 17-β Estradiol Exposure on Metallothionein and Fat Soluble Antioxidant Vitamins in Juvenile Lake Trout (*Salvelinus namaycush*)

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Concern regarding the ability of aquatic contaminants to disrupt endocrine functions in exposed organisms has increased over the last two decades. Compounds that mimic the effects of estrogen are most widely studied (Kime 1998) and have been identified in many aquatic environments at concentrations that are known to cause reproductive abnormalities (Folmar et al. 2000). A variety of biochemical, behavioural and physiological surrogate measures of exposure to estrogens are currently available to toxicologists. However, there are no examinations of the effects of estrogenic compounds on protective antioxidant compounds like vitamins A and E, and relatively few studies that have considered the effects of exposure on levels of the metal binding protein, metallothionein (MT), in fish.

In fish, maternally derived vitamins in eggs are essential for normal embryonic development (Cowey et al. 1985). Since estrogens can affect the metabolism of vitamins A and E in mammals (Demacker et al. 1991, Mooij et al. 1991), it is relevant to examine this possibility in fish. MT concentrations can be affected by estrogen exposure (Gerpe et al. 2000). MT may also decline prior to spawning, probably to facilitate the release of essential metals needed to stabilize membrane structures that are required to produce and export the primary yolk protein precursor, vitellogenin (Olsson et al. 1990). In this study, the effects of 17-β estradiol on vitamins A and E and on MT were investigated in lake trout tissues after 3 weekly injections of either ethanol/corn oil (control) or 5mg 17-β-estradiol kg^{-1} bodyweight.

MATERIALS AND METHODS

Juvenile lake trout (*Salvelinus namaycush*) (260 ± 15g) were acclimated in 200 L fiberglass tanks receiving 1 L of aerated and de-chlorinated Winnipeg city tap water per minute (7 fish per tank) for 10 days. Temperatures were maintained between 11.5 and 13.1°C and dissolved oxygen was at least 90% saturation at all times. During acclimation and dosing periods of the experiment, fish were fed a

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diet of commercial dry pellet feed (Martin Feed Mills; Elmira, Ontario) at a ration of 1% per day. Following the acclimation period, fish were anesthetized with pH buffered (=7.0) tricaine methanesulfonate (MS222) (0.38 g/L) until fin movement ceased (<2 min). They were then injected with either corn oil:ethanol (8:2 v/v) (control, n=7) or corn oil:ethanol containing 5 mg 17-β-estradiol kg^{-1} wet bodyweight (n=14). Identical doses were given 7 and 14 days after the initial doses. After each dose was administered blood was obtained (500 µl) from the caudal vein with a pre-heparinized syringe (50,000 U/ml) and centrifuged at 3000 g to obtain plasma, which was frozen at -90°C and protected from light until analysis. Following these procedures, fish were returned to their original tanks and recovered within 3 minutes. Twenty-one days after the first injection, fish were anesthetized individually by immersion in MS222 (0.8 g/L). When all fin movement had ceased (<2min), fish were removed from the anesthetic, blotted dry, weighed and measured. Liver and gonad were dissected and weighed to obtain liver somatic (LSI=Liv Wt/Bdwt X 100) and gonadal somatic indexes (GSI = Gonad Wt/Bdwt X 100). A sub-sample of liver tissue was fixed in Bouin's solution for histological analysis. The rest of the tissues were frozen in sterile plastic bags and stored at -90°C for analysis of vitamins and metallothionein.

Vitamins A and E in plasma, liver, and kidney, and pro-vitamin A esters in liver and kidney were measured using the reverse-phase HPLC method of Palace and Brown (1994). MT was determined using a Hg-saturation assay (Klaverkamp et al. 2000) and glycogen in liver was measured according to Montgomery (1957). After 24 hours in Bouin's, liver tissue was washed in several changes of 70 % ethanol for 3 days, and then embedded in paraffin. Sections were cut at 6 µm, affixed to glass slides and stained with Harris' hematoxylin and eosin. All data are presented as Mean ± SEM. Statistical comparisons between groups were performed using two sample t-tests with α=0.05.

RESULTS AND DISCUSSION

Condition factor (=Bdwt/Length^3 X 100) was not different between the control group and the 17-β estradiol treated fish following the 21-day experiment (Table 1). However, liver somatic index was elevated by more than 54% in the treated group compared with the control fish, probably as a result of induced synthesis of the primary egg yolk protein precursor, vitellogenin, within liver cells (Kime 1998). Gonadal somatic index was also elevated by 29% in the female fish exposed to estradiol compared to their controls. Although the same trend appears in male fish, insufficient numbers in the control group did not allow statistical comparisons of the GSI means. GSI would be expected to increase in females as vitellogenin, produced in the liver, becomes sequestered into oocytes from the plasma (Kime 1998). Furthermore, glycogen-rich areas of the hepatocytes, which do not stain well and impart a pale homeogenous color to large portions of the tissue sections, appeared to be less prominent in the livers of fish treated with estradiol. In fact, lower glycogen content of the livers from estradiol treated fish

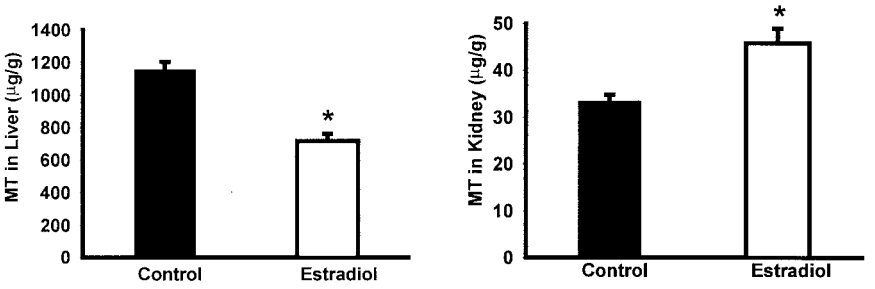

Figure 1. Metallothionein in liver and kidney of lake trout injected with corn oil:ethanol (control) or corn oil:ethanol containing 5 mg/kg 17 β-estradiol.

Table 1. General somatic parameters of lake trout injected with corn oil:ethanol (control) or corn oil:ethanol containing 5 mg/kg 17 β-estradiol.

	n	Condition Factor	Liver Somatic Index (LSI)	Gonadal Somatic Index (GSI)
Control Group	7	0.93 ± 0.02	0.71 ± 0.05	(F) 0.158 ± 0.026 (M) 0.040 (n=2)
Estradiol Group	14	0.96 ± 0.02	1.10 ± 0.05*	(F) 0.222 ± 0.009* (M) 0.133 ± 0.05

* Significanlty different from the control group based on two sample t-test results ($p<0.05$).

were confirmed (48.9 ± 6.7 mg/g for controls, 16.6 ± 3.6 mg/g for estradiol treated, $p<0.05$) in subsequent biochemical analyses.

MT concentrations were lower in the livers of 17β-estradiol treated fish than in the livers of control fish ($p<0.05$) (Fig. 1). Lower concentrations of the essential metal copper (57%) and the trace metal contaminant cadmium (26%), were also found in the livers of 17-β treated fish (data not shown) ($p<0.05$). In contrast to the liver, kidney MT levels of were significantly higher in treated fish compared to the controls ($p<0.05$) (Fig. 1). Whereas lower concentrations of hepatic MT in response to estrogen treatment have been documented in fish, kidney levels of the protein have not normally been altered (Olsson et al. 1990). However, previous mammalian studies suggest that MT production in the kidney following estradiol exposure may arise indirectly from enhanced uptake of metals by the kidney (Nishiyama et al. 1987). In the current study, copper concentrations were

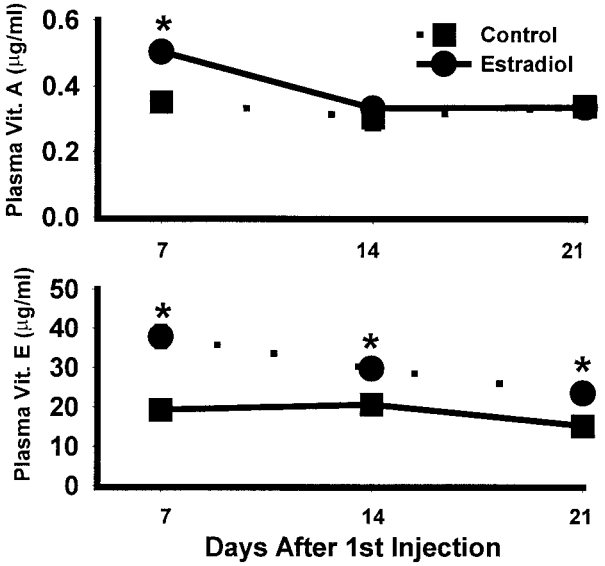

Figure 2. Vitamins A and E in plasma of lake trout injected with corn oil:ethanol (control) or corn oil:ethanol containing 5 mg/kg 17 β-estradiol. * significantly different from the control group at the same sample time based on two sample t-test results ($p<0.05$).

also elevated in the kidneys of estradiol exposed fish (51%) compared with the control fish, supporting the aforementioned hypothesis. Cadmium and zinc concentrations were not significantly different in kidneys of the two groups of fish ($p<0.05$).

Plasma vitamin A (retinol) was elevated in the estradiol group after 7 days, but returned to control values by 14 days (Fig. 2). However, vitamin E (tocopherol) remained elevated throughout the 21-day exposure period ($p<0.05$). Higher concentrations of fat-soluble vitamins have also been reported in the plasma of mammals following administration of oral contraceptives (Thurnham et al. 1999).

Livers from the estradiol treated group had significantly lower concentrations of vitamin A (retinol and dehydroretinol) (Fig. 3). Concentrations of the predominant storage form of vitamin A, retinol palmitate, were not affected 21 days post injection (data not shown). Lower vitamin E (tocopherol) was also found in the liver of estradiol treated fish (Fig. 3). The liver serves as a storage organ for vitamins A and E, mobilizing its stores when plasma concentrations of the vitamins decline (Palace et al. 1999). Given the significant increases in

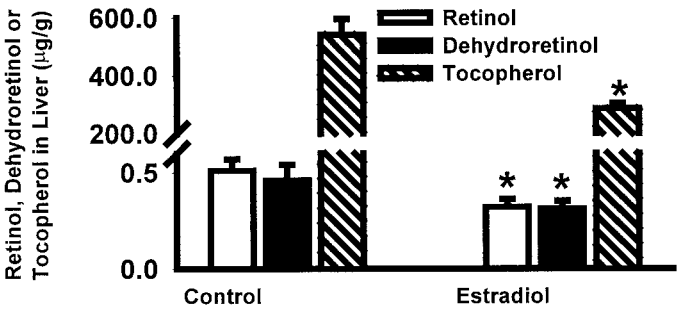

Figure 3. Vitamin A isoforms (retinol and dehydroretinol) and vitamin E (tocopherol) in liver of lake trout injected with corn oil:ethanol (control) or corn oil:ethanol containing 5 mg/kg 17 β-estradiol. * significantly different from the control group based on two sample t-test results ($p<0.05$).

plasma vitamins A and E that were evident in the estradiol treated fish, it appears likely that the increase in fat soluble plasma vitamin concentrations results from a loss of these vitamins from the liver. Estradiol had no effect on concentrations of any of the vitamins in the kidney (data not shown).

Results from this study indicate that freshwater fish exposed to estrogenic compounds may have altered vitamin and metallothionein tissue concentrations. These biochemical effects may increase the susceptibility of fish exposed to complex effluents that contain both estrogenic compounds and toxic metals or contaminants that can generate free radical injury, such as PCBs.

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