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Thyroid Responses in Rats Fed Diets Formulated with Great Lakes Coho Salmon

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In earlier papers we reported thyroid hyperplasia and goiters together with clinical signs of hypothyroidism in male rats fed diets of Great Lakes coho salmon (Oncorhynchus kisutch) (SONSTEGARD & LEATHERLAND 1979a, LEATHERLAND & SONSTEGARD 1980). In these preliminary studies, the rats were fed a total fish diet without supplementation of any kind. The present paper reports on the effects of Great Lakes coho salmon on thyroid function in rats when the fish was given as part of a formulated diet together with rat chow, vitamin, mineral, and lipid supplementation. The study is a further attempt to assess the possible health hazards of using Great Lakes salmon for human consumption and an attempt to monitor the level of goitrogenic xenobiotics in the flesh of these fish.

MATERIALS AND METHODS

Sexually-mature coho salmon were captured during their potamodromous spawning runs (1978) from Lakes Ontario, Michigan, and Erie. The fish were placed on ice, transported to Guelph, and rinsed in water. The head and caudal fin were removed, and the eviscerated carcasses were frozen and stored at -25°C. The frozen carcasses were ground in a commercial meat grinder. Coho salmon obtained from the Fraser River, British Columbia (courtesy of Dr. E.M. Donaldson) were treated in a similar manner. Diets were formulated from the fish as follows: 33 kg fish (wet weight), 20 kg powdered Purina Rodent Chow, 100 g Vitamin Fortification Mix (Rx 900023) (Teklad Test Diets, Madison, WI), 300 g Mineral Mix (Williams-Briggs, Rx 811826) (Teklad Test Diets), and 450 g corn oil. The mixture was blended in a commercial mixer and stored frozen at -25°C.

Groups of 6 male and 6 female rats (Sprague-Dawley) were fed the Great Lakes salmon or Pacific Ocean salmon (40 g/day) for a 90day period. Other groups of males and females were fed pelleted Purina Roden Chow ad libitum. All groups were given tap water ad libitum. Blood was collected by cardiac puncture after light chloroform anaesthesia. Serum L-thyroxine (T4) and triiodo-L-thyronine (T3) were measured by radioimmunoassay as described previously (SONSTEGARD & LEATHERLAND 1979a). T3 uptake was measured by means of the Gammacoat assay (Clinical Assays, Cambridge, MA) and the free T4 index (FT4) was calculated according to the method of ANDERSON (1968). The rats were weighed and the thyroid glands removed and fixed overnight in 5% cacodylate-buffered (pH 7.4)

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glutaraldehyde. The fixed thyroid tissue was dissected free of connective tissue, blotted dry, and weighed. Pieces of tissue were embedded in Paraplast for routine histology; others were embedded in Epon. Epithelial cell height measurements were made by means of an ocular micrometer on each of ten randomly selected follicles at four points of the epithelium where the micrometer grid intersected the follicle in vertical and horizontal planes.

Data of thyroid weight, thyroid somatic index (TSI), thyroid epithelial cell height (TEH), serum T4 and T3 concentrations, and serum T4/T3 ratios were separately subjected to one-way analysis of variance. Where F values indicated significance, predetermined means were compared by the least significant difference test (STEEL & TORRIE 1960).

RESULTS

Thyroid weight (TW) and thyroid somatic index (TSI). There were no significant differences in either TW or TSI in female rats in any of the groups (Table 1). TW values in male rats fed the Lakes Ontario, Erie, or Michigan coho salmon diets were significantly higher than in male rats fed the Pacific Ocean coho salmon diet. However, when the TW data were converted to TSI, only in the males fed Lake Michigan coho salmon was the TSI significantly greater ($p < 0.05$) than in the Pacific Ocean salmon-fed controls (Table 1).

TW values in rats fed both the rat chow diet and Great Lakes salmon diets showed significant sex differences ($p < 0.05$), although those groups fed Pacific Ocean salmon diets displayed no such sex difference (Table 1). Sex differences in TSI were found in the groups fed rat chow ($p < 0.05$), Pacific Ocean salmon ($p < 0.01$), or Lake Ontario salmon diets ($p < 0.01$) (Table 1).

Thyroid epithelial cell height (TEH). There were significant sex differences ($p < 0.01$) in the TEH values of all groups fed fish diets, with values in males being greater than those in females; no such sex difference was evident in the rat chow-fed groups (Table 1). TEH values in female rats fed Lakes Ontario or Michigan salmon diets were significantly greater than in females fed either the Pacific Ocean salmon diet or the rat chow diet ($p < 0.01$ except for comparison of Lake Ontario salmon-fed group with rat chow-fed group where $p < 0.05$) (Table 1). There was no significant difference in TEH between females fed the Lake Erie salmon diet and either the Pacific Ocean salmon diet or the rat chow diet. Among the male group only in the Lake Erie salmon-fed group was the TEH significantly enlarged ($p < 0.05$) compared with the Pacific Ocean salmon-fed group (Table 1). The TEH in all the salmon-fed males was significantly larger ($p < 0.01$) than in the rat chow-fed males (Table 1).

Thyroid histology. Thyroid glands in male and female rats fed the chow or Pacific Ocean salmon diet were composed of a mixture of larger 'cold' follicles in the periphery of the gland and central 'hot' follicles with moderately vacuolated colloid. In male rats

Table 1. Thyroid Weight (TW), Thyroid Somatic Index (TSI), and Thyroid Epithelial Cell Height (TEH) in Rats Fed Diets of Coho Salmon from the Pacific Ocean or the Great Lakes Compared with Rat Chow-fed Rats.

Diet/Source of Salmon	Rat Sex	Body Weight (g)	Thyroid Weight (mg)	TSI	TEH (μm)
Rat Chow	Female	224 ± 10[5]	17 ± 1[4]	0.78 ± 0.06[4]	5.2 ± 0.3
	Male	387 ± 11	22 ± 1[1]	0.60 ± 0.03	5.8 ± 0.4
Pacific Ocean	Female	124 ± 6[5]	18 ± 3	0.84 ± 0.14[5]	4.3 ± 0.2
	Male	383 ± 9	17 ± 1	0.44 ± 0.03	8.2 ± 0.3
Lake Ontario	Female	211 ± 8[5]	16 ± 1[5]	0.77 ± 0.06[4]	6.2 ± 0.3[5,1,2]
	Male	387 ± 10	24 ± 2[1]	0.58 ± 0.08	8.1 ± 0.6
Lake Erie	Female	217 ± 8[5]	17 ± 1[5]	0.76 ± 0.06	4.6 ± 0.3[5]
	Male	376 ± 10	23 ± 1[1]	0.60 ± 0.02	9.3 ± 0.3[1,3]
Lake Michigan	Female	209 ± 5[5]	16 ± 1[5]	0.78 ± 0.06	6.7 ± 0.3[5,1,3]
	Male	375 ± 15	25 ± 2[1]	0.66 ± 0.03[1]	8.8 ± 0.4[3]

[1] significantly different ($p < 0.01$) from Pacific Ocean salmon fed group of the same sex;

[2,3] significantly different ($p < 0.05$, $p < 0.01$, respectively) from Rat Chow-fed group of the same sex;

[4,5] significantly different ($p < 0.05$, $p < 0.01$, respectively) from comparable male groups; the data are shown as mean ± s.e., n = 6.

Table 2. Serum L-Thyroxine (T4) and Triiodo-L-thyronine (T3) Concentrations, T3/T4 Ratios, T3 Uptake (T3U) Values, and Free T4 Index (FT4) in Rats Fed Diets of Coho Salmon from the Pacific Ocean or the Great Lakes Compared with Rat Chow-fed Rats.

Diet/Source of Salmon	Rat Sex	T4 (g/dL)	T3 (ng/dL)	T4/T3	T3U (%)	FT4
Rat Chow	Female	2.7 ± 0.1[6]	67 ± 11	48 ± 9	50.2 ± 2.0[6,1]	3.8 ± 0.2[6]
	Male	3.8 ± 0.4	72 ± 8	57 ± 7	56.7 ± 2.3	6.0 ± 0.1
Pacific Ocean	Female	2.7 ± 0.2[6]	28 ± 4	108 ± 18	54.9 ± 0.9[5]	4.3 ± 0.3[5]
	Male	3.3 ± 0.3	50 ± 7	73 ± 13	59.5 ± 1.1	5.7 ± 0.6
Lake Ontario	Female	1.8 ± 0.1[2,6,4]	61 ± 19	62 ± 28	49.9 ± 1.4[1,6]	2.5 ± 0.1[6,2,3]
	Male	3.1 ± 0.1[3]	65 ± 9	54 ± 9	56.7 ± 0.7	5.2 ± 0.2
Lake Erie	Female	3.2 ± 0.4	47 ± 16	96 ± 18	53.1 ± 1.0	4.9 ± 0.7
	Male	3.1 ± 0.1	56 ± 19	85 ± 32	56.7 ± 1.2	5.1 ± 0.2
Lake Michigan	Female	2.4 ± 0.2[6]	56 ± 21	61 ± 20	54.7 ± 2.2[3,5]	3.8 ± 0.2[6]
	Male	3.2 ± 0.3	45 ± 9	83 ± 14	59.1 ± 0.9	5.6 ± 0.5

[1,2] significantly different ($p < 0.05$, $p < 0.01$, respectively) from Pacific Ocean salmon-fed group of the same sex;

[3,4] significantly different ($p < 0.05$, $p < 0.01$, respectively) from Rat Chow-fed group of the same sex;

[5,6] significantly different ($p < 0.05$, $p < 0.01$, respectively) from comparable male group; the data are shown as mean ± s.e., n = 5 or 6.

fed either the Lake Ontario or Lake Michigan salon diets, the colloid was highly vacuolated, and much less so in males fed the Lake Erie salmon diet. In females fed the Great Lakes salmon diets there was little change in colloid vacuolation from the controls.

Serum thyroid hormone concentrations, T3U and FT4 indices. There were no significant differences in either serum T3 levels or serum T4/T3 ratios in any of the groups (Table 2). Also, there were no differences in serum T4 concentrations, T3 uptake (T3U), or free T4 index (FT4) in any of the groups of males fed salmon diets. Among the female groups, however, serum T4 concentration and T3U and FT4 indices in the rats fed the Lake Ontario salmon diet were significantly lower ($p < 0.05$) than in both the rat chow- and Pacific Ocean salmon-fed groups (Table 2).

There were significant ($p < 0.05$) sex differences in serum T4 concentrations and T3U and FT4 indices in all salmon-fed groups except those fed the Lake Erie salmon diet (Table 2).

DISCUSSION

The results reported here provide further support for the presence of bioaccumulated goitrogens in the flesh of Great Lakes coho salmon. The thyroid responses are less marked than in the earlier studies where the rats were fed only frozen fish without the rat chow supplementation used in these studies (SONSTEGARD & LEATHERLAND 1979a, LEATHERLAND & SONSTEGARD 1980), but the differential responses to salmon from different Great Lakes illustrate the complexity of interaction of the xenobiotics present in the fish. TSI enlargement was only evident in male rats fed the Lake Michigan salmon diet, and no such enlargement was evident in the females. Thyroid hyperplasia (as evidenced by increases in TEH) was present only in male rats fed the Lake Erie salmon diet, whereas in female rats it was only evident in groups fed the Lake Ontario or Lake Michigan salmon diet. Evidence of hypothyroidism (i.e. lowered serum T4 concentrations) was found only in the female rats fed the Lake Ontario salmon diet. In the same group T3U (an indirect measure of the unbound sites on serum thyroid hormone-building proteins (CAPLAN et al. 1979)) and the FT4 index (an estimate of unbound serum T4 concentration (ANDERSON 1968)) were also lower than in female rats fed the Pacific Ocean salmon diet. Thus in all of the groups of rats fed Great Lakes salmon diet there was evidence of a change in activity of the hypothalamic-pituitary-thyroid axis.

Moreover, although sex differences were noted in many of the parameters measured, with the exception of TW and TEH, they were conspicuously absent from the groups fed the Lake Erie salmon diet. This is further evidence that the factor(s) accumulated in the Lake Erie fish exerts a different effect to that of the salmon from the other Great Lakes.

In both these studies and the preliminary work reported earlier (SONSTEGARD & LEATHERLAND 1979a), there was marked liver enlarge-

ment and liver enzyme induction (e.g. 200% increase in cytochrome P450 and 37% increase in β-hydrolase activity in rats fed Pacific Ocean salmon diets) (unpublished data). These data similarly support the concept that Great Lakes salmon are contaminated with xenobiotics which have physiological consequence to organisms using them as a dietary source. As suggested previously (SONSTEGARD & LEATHERLAND 1979a) organochlorine residues, which are widespread and persistent pollutants in the Great Lakes, are suspect as regards their effects on mammalian thyroid physiology. Some organochlorine compounds are known to be goitrogenic in rats (BASTOMSKY 1977, COLLINS et al. 1977), and Great Lakes salmon are known to be contaminated with a diverse array of these compounds (SONSTEGARD & LEATHERLAND 1979b, MOCCIA et al. 1978). However, the possibility that other factors, for example some heavy metals (DER et al. 1977), are also involved cannot be rejected.

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