Bull. Environm. Contam. Toxicol. 22,779-784 (1979)

Hypothyroidism in Rats Fed Great Lakes Coho Salmon

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Since their introduction into the Great Lakes, coho salmon Oncorhynchus kisutch) have provided North American anglers with a spectacular fishery (CARTER 1968). The detection of persistent organochlorines and heavy metals in these fish has focused public conern on the health of the lake ecosystem and the possible health effects of consuming fish contaminated with a complex array of environmental pollutants. Earlier, we reported epizootics of thyroid hyperplasia in Great Lakes coho salmon which appear to have an environmental etiology (MOCCIA et al. 1977). In this paper, we summarize feeding trials in which coho salmon from the Great Lakes were fed to laboratory rats and found to induce hypothyroidism, thyroid hyperplasia and goiters suggesting the presence of tissue bound goitrogens in the flesh of these salmon.

MATERIALS AND METHODS

Sexually mature coho salmon were captured during their anadromous spawning runs (1977) from Lakes Ontario, Erie and Michigan. The fish were placed directly on ice and transported to the laboratory. They were rinsed with tap water, the head and caudal fin removed, eviscerated (apart from the kidney), ground in a commercial meat grinder, homogenized, and stored frozen at -20°C. Coho salmon from the Pacific Ocean (British Columbia) were received frozen, and processed as above. Male Wistar rats were fed the salmon diets in a series of experiments (Tables 1-3).

In the first experiment groups of 30 day old rats were fed either 30g of coho salmon per 100g body weight each day (based on the initial body weight) or rat chow ad libitum; all groups were given distilled water ad libitum. Five rats from each group were killed after one month and the remainder after two months. Diets of coho salmon from the Pacific Ocean and Lakes Ontario, Michigan and Erie were used (Table 1).

Blood was collected by cardiac puncture after light anaesthesia and serum thyroxine (T4) and triiodothyronine (T3) levels were measured by means of the Ames Tetralute and Seralute methods.

Thyroid tissue was fixed in Bouin's solution, embedded in paraffin, sectioned at 7μ and stained with hematoxylin and eosin. Four

0007-4861/79/0022-0779 $01.20

TABLE 1

SERUM THYROXINE (T4) AND TRIIODOTHYRONINE (T3) CONCENTRATIONS, SERUM T4/T3 RATIOS AND THYROID EPITHELIAL CELL HEIGHT IN RATS FED DIETS OF COHO SALMON FROM THE PACIFIC COAST, FROM LAKES ONTARIO, ERIE AND MICHIGAN AND A CONTROL PELLET DIET FOR 1 OR 2 MONTHS.

Diet/Source of Salmon	Tissue OC content[1]	1 month				2 months			
		T_4[2]	T_3[3]	T4/T3	Cell Height	T_4	T_3	T4/T3	Cell Height
Rat Chow		3.9±0.3	69.9± 5.6	55.9±4.3	9.6±0.4	2.9±0.2	57.9± 4.4	49.9±2.6	11.4±0.4
Pacific Ocean	0.13	3.1±0.6	84.0±10.2	38.0±7.8	10.1±0.2	2.2±0.4	47.9± 9.5	47.7±3.6	12.3±0.3
Lake Ontario	7.89	0.6±0.1$^{**}_{++}$	79.5± 5.0	7.2±1.2$^{**}_{++}$	13.8±0.5$^{**}_{++}$	0.4±0.1$^{**}_{++}$	79.8± 5.7	5.1±2.1$^{**}_{++}$	14.4±0.3$^{**}_{++}$
Lake Michigan	5.76	1.4±0.3$^{*}_{++}$	83.6±10.0	17.3±2.4$^{**}_{++}$	9.8±0.4	0.9±0.1$^{**}_{++}$	73.8±11.0	11.9±1.3$^{**}_{++}$	13.1±0.4^{++}
Lake Erie	1.56	2.7±0.4	77.8± 8.9	34.9±5.2	8.0±0.3$^{*}_{++}$	0.9±0.1$^{**}_{++}$	43.6± 7.5	22.6±3.7$^{**}_{++}$	12.4±0.4

[1] Organochlorines (OC's) in fish measured ($mg.kg^{-1}$): pp'DDE, pp'TDE, pp'DDT, op'DDT, Dieldrin, Hept. Epox., Chlordane (Oxy. & α, PCB's, photomirex and Mirex; analysis as described by HOLDRINET (1974); [2] $\mu g.100\ ml^{-1}$; [3] $ng.100\ ml^{-1}$; N = 5, all data are expressed as mean ± s.e.; *,** significantly different ($P < 0.05$ and $P < 0.01$ respectively) from rats fed Pacific Ocean coho; $^{++}$ significantly different ($P < 0.01$) from rats fed chow diet.

epithelial cell height measurements were made on each of 5 randomly selected follicles in each rat using an ocular micrometer grid.

In the second experiment groups of newly weaned rats were fed 40g of coho salmon per 100g body weight each day for one month; the amount of food given was adjusted for body weight changes at the beginning of each week; all groups were given distilled water ad libitum except for the iodide supplemented group which was given a 0.01% KI solution ad libitum (Table 2). Diets of coho salmon from the Pacific Ocean and Lake Ontario were used (Table 2).

TABLE 2

SERUM THYROXINE (T4) AND TRIIODOTHYRONINE (T3) CONCENTRATIONS AND SERUM T4/T3 RATIOS IN RATS FED COHO SALMON FROM LAKE ONTARIO (WITH AND WITHOUT IODIDE SUPPLEMENT) OR THE PACIFIC OCEAN.

Source of salmon	T_4 [1]	T_3 [2]	T_4/T_3
Pacific Ocean	1.72 ± 0.13	10.2 ± 0.2	170.4 ± 14.4**
Lake Ontario	0.74 ± 0.14++	21.1 ± 3.6+	43.7 ± 14.0
Lake Ontario + iodide supplement	0.99 ± 0.10++	13.2 ± 2.3	83.0 ± 13.4

[1] g.100 ml^{-1}; [2] ng.100 ml^{-1}; N = 5, all data are expressed as mean ± s.e.; ** significantly different ($P < 0.05$ and $P < 0.01$ respectively) from rats fed Pacific Ocean coho.

Blood was collected and serum T4 and T3 levels measured as described above.

In the third experiment newly weaned rats were fed 40g of coho salmon per 100g body weight each day for one month; the amount of food given was adjusted for body weight changes at the beginning of each week; all groups were given distilled water ad libitum. Diets of coho salmon from the Pacific Ocean and Lakes Ontario and Michigan were used (Table 3). The thyroid glands were dissected from each rat, preserved overnight in 50% cacodylate buffered (pH 7.4) glutaraldehyde, blotted dry and weighed.

RESULTS AND DISCUSSION

Serum T4 levels in rats fed coho salmon from Lakes Ontario, Michigan or Erie for two months were significantly lower than in rats fed Pacific coho or the rat chow diet ($P < 0.01$) (Table 1). Serum (T3) levels did not differ between the groups; differences in T4/T3 ratios paralleled those in T4 levels (Table 1). Thyroid epithelial cell height (TE) in rats fed Lake Ontario coho was signifi-

cantly higher than in rats fed Pacific salmon ($P < 0.01$) (Table 1) whereas TE in rats fed Lake Michigan or Lake Erie coho was not significantly different from rats fed Pacific coho salmon (Table 1).

Rats fed Lake Ontario coho salmon with and without iodide supplements had serum T4 levels which were significantly lower ($P < 0.01$) than similar animals fed Pacific Ocean coho (Table 2); there were no significant differences between the two groups fed Lake Ontario salmon.

Thyroid weights in rats fed coho salmon from Lakes Ontario or Michigan were significantly larger ($P < 0.01$) than in rats fed Pacific Ocean coho (Table 3).

TABLE 3

THYROID WEIGHT IN RATS FED COHO SALMON FROM LAKES MICHIGAN, ONTARIO AND THE PACIFIC OCEAN

Source of salmon	Thyroid weight[1]
Pacific Ocean	0.42 ± 0.04** (N = 10)
Lake Ontario	0.63 ± 0.04 (N = 10)
Lake Michigan	0.67 ± 0.04 (N = 6)

[1] expressed as a percent of body weight x 10^2; ** significantly different ($P < 0.01$) from other 2 groups; data are expressed as mean ± s.e.

The data from the three experiments (Tables 1 to 3) show that diets of Great Lakes salmon cause hypothyroidism, thyroid hyperplasia and hypertrophy (goiter) in rats. These effects do not appear to be induced by iodide deficiency since iodide supplements failed to significantly lessen the hypothyroidism (Table 2), and iodide levels in the Lake Ontario coho diets were found to approximate those in the Pacific Ocean coho (2.2 and 2.1 $mg.kg^{-1}$ wet weight respectively).

These observations suggest that the Great Lake fish contain goitrogenic materials which are not found in Pacific coho. Organochlorines are suspect since they are present in high levels in Great Lakes coho (SONSTEGARD and LEATHERLAND 1976, MOCCIA et al. 1978a,b) and are known to be goitrogenic in mammals (BASTOMSKY 1977, COLLINS et al. 1977) and birds (JEFFERIES and PARSLOW 1972). Total organochlorine levels in the coho diets had an inverse linear correlation with serum T4 in rats fed the diets for 1 month. The linear correlation was lost after 2 months on the diets, possibly indicating

bioaccumulation of contaminants (Table 1). Although there is a close correlation between organochlorine levels in the coho salmon diets and hypothyroidism in the rats fed the diets, no correlation was found between tissue organochlorine levels and goiter frequency in coho salmon from different Great Lakes. For example, feral Lake Erie coho salmon have the highest goiter frequency (79.5%) (MOCCIA et al. 1977) and the lowest organochlorine levels of Great Lakes coho assayed (Table 1) (MOCCIA et al. 1978a). The studies suggest that the goitrogen(s) effecting thyroid hypertrophy in Great Lakes coho salmon may not be tissue associated and remain unidentified.

Organochlorines are widespread, persistent pollutants in the biosphere. In light of the observations reported here, the consumption of Great Lakes salmon by sport fishermen (e.g. 14 million pounds of salmonids each year from Lake Michigan alone (HUMPHREY 1975)) is of considerable concern. As an example of the extent of the problem, serum T4 levels in rats fed Lake Ontario coho salmon decreased by 80.6% after one month on the diet which is comparable to the 75.2% decrease reported in rats fed chow diets contaminated with 500 mg. kg^{-1} of PCB for 3 months (COLLINS et al. 1977). Inasmuch as the total organochlorine burden in the coho diets (Table 1) were about 50 times lower than in the PCB-contaminated chow diets (COLLINS et al. 1977), similar response of the thyroid in the two situations may suggest a synergistic effect of mixtures of organochlorines, a phenomenon which has been found in fish (LEATHERLAND and SONSTEGARD 1978). The possibility of synergism of the several organochlorine compounds present in the Great Lakes coho flesh (Table 1) (MOCCIA et al. 1978a), either amongst themselves or with other etiological agents, raises questions as to "safe levels" of individual compounds as recommended by various national food and drug administrations. The regular consumption of a diverse group of chemicals as in a meal of Great Lakes coho salmon (e.g. an average of 24-25 pounds of Lake Michigan fish consumed by some sport fishermen each year for a two year period (CARTER 1968)) may elicit responses which constitute a serious health hazard.

ACKNOWLEDGEMENTS

This work was supported by grants from the National Research Council of Canada and the National Cancer Institute of Canada. We acknowledge the cooperation of the Ontario Ministry of Natural Resources, the Pennsylvania Fish Commission and the Michigan Department of Natural Resources.

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