Elevated thyroid hormone levels in the highly contaminated Baltic grey seals

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Introduction

During the last decades, exceptionally high levels of organochlorines (OCs) have been reported in the Baltic seals. Even if levels of polychlorinated biphenyls (PCB) and 1,1,1.trichloro-2,2-bis[p-chlorophenyl]ethane (DDT) have decreased in the Baltic seals since the early 1980s, levels are still high enough to threaten the well-being of the Baltic seals[1]. Furthermore, new organic contaminants are continuously reported in the Baltic fauna. Many pathological changes have been observed in the Baltic seals, such as uterine occlusions, intestinal ulcers, adrenocortical hyperplasia, renal failure, uterine tumours and skull lesions. The observed changes have been associated with high contaminant burdens[2,3,4], but mechanisms causing these pathological disorders are still unknown. Previous studies in the Baltic seals have reported altered physiological homeostasis i.e. disruption in the vitamin system and an induced xenobiotic metabolism[5].

Potential endocrine disruptors, such as environmental contaminants (including PCB and DDT), have increasingly received attention[6,7]. Several studies have shown that high levels of OCs may disturb the endocrine system in marine mammals, including the thyroid hormone system[5,6]. The aim of this study was to compare the levels of circulating thyroid hormones in the highly contaminated Baltic grey seals to relatively clean grey seals from Eastern Canada, and to discuss a possible linkage between the contaminant burden and the disruption of the thyroid hormone system.

Materials and methods

Baltic grey seals and reference samples were sampled in 1996-1998. Seals from in the Baltic Sea were collected from the Bothnian Bay and the reference grey seals from Sable Island, Canada. Detailed descriptions of the sampling procedure and the condition of the Baltic and reference seals are reported elsewhere[1]. Seal samples were obtained in spring during their moulting season. All the seals were adults (Baltic mean age 15±9 years, Canada mean age 19±8 years) and approximately at the same condition (condition index Baltic 2.1±0.5, Canada 1.7±0.6).

Analyses of plasma concentrations of total thyroxine (TT4), total tri-iodothyronine (TT3), free thyroxine (FT4) and free tri-iodothyronine (FT3) were conducted using radioimmunoassay (Coat-A-Count, Diagnostic Products, Los Angeles, CA, USA).

All the statistical analyses were performed using SPSS 12.0 (SPSS Inc., Chicago, IL, USA). Univariate analysis of variance (ANOVA) was used to compare the means of thyroid hormones between populations. Normality of the data was verified using normal probability plots of standardised residuals. To compare the means of thyroid hormones between males and females within populations, the Mann-Whitney U-test was performed.

Results and discussion

Levels of thyroid hormones are presented in Table 1. Plasma free T4, and total T3 and T4 were comparable with earlier studies in grey seals pups from the Baltic and United Kingdom [8,9], while the levels of the free form of T3

were lower in the Baltic grey seals[7,8]. Thyroid hormones are important during the developmental phase, and the main active form is T3[10]. As the level of free T3 mainly describes the availability of active thyroid hormone[9], it would be expected that the level of free T3 is higher in young animals than in adults.

Table 1. Levels of plasma thyroid hormones TT4 (nmol/L), TT3 (nmol/L), FT4 (pmol/L), FT3 (pmol/L) in Baltic and reference seals.

	Baltic (n=12)		**Canada** (n=18)		
	Mean	SD	Mean	SD	*p*-value
TT4	**40**	9,1	17	6,1	**0,000**
FT4	**8,5**	2,2	3,55	1,5	**0,000**
TT3	**1,6**	0,27	0,66	0,21	**0,000**
FT3	**0,87**	0,27	0,12	0,19	**0,000**
FT3:TT3	**0,53**	0,11	0,15	0,21	**0,000**
FT4:TT4	0,21	0,02	0,20	0,04	0,376
FT3:FT4	**0,10**	0,02	0,03	0,03	**0,000**
TT3:TT4	0,04	0,01	0,04	0,01	0,567

Thryoid hormone levels were approximately similar between males and females (Table 2). Only a weak difference was observed between Canadian grey seal males and females in some thyroid hormone levels and ratios.

Table 2. Thyroid hormone levels and relationships in the grey seal males and females from the Baltic and Canada. * $p < 0.05$, ** $p < 0.01$.

	Baltic				**Canada**			
	Males (n=4)		**Females (n=8)**		**Males (n=8)**		**Females (n=10)**	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
TT4	38,21	14,00	40,66	6,66	19,73	7,11	15,58	4,85
TT3	1,55	0,28	1,64	0,28	**0,78***	0,25	0,56	0,09
FT4	7,98	3,20	8,73	1,75	4,20	1,88	3,03	0,93
FT3	0,87	0,34	0,87	0,25	**0,22***	0,25	0,04	0,03
FT3TT3	0,55	0,12	0,53	0,12	**0,25***	0,29	0,06	0,06
FT4TT4	0,21	0,02	0,22	0,03	0,21	0,04	0,20	0,04
FT3:FT4	0,11	0,02	0,10	0,02	**0,04****	0,04	0,01	0,01
TT3:TT4	0,04	0,01	0,04	0,01	0,04	0,01	0,04	0,01

In the Baltic grey seals, the levels of all thyroid hormones (free and total T3 and T4) were more than twice as high compared to the reference seals from Canada ($p < 0.001$). There are several explanations which might explain the high thyroid hormone levels in the Baltic seals.
(1) It might be caused by a genetic difference between the two populations.
(2) The difference in the sampling conditions between the two populations could be another explaining factor. Sampling was conducted on ice in the Bothnian Bay, while seals were sampled on sand on Sable Island in warmer conditions. As thyroid hormones are known to play a key role in thermogenesis and in maintenance of constant body temperature[9], colder conditions in the Bothnian Bay could increase the thyroid hormone levels. In harbour seals, higher levels of TT4, TT3 and FT3 have been observed during colder seasons[11].
(3) The thyroid hormone levels could also be influenced by the reproductive phase, as the reproduction period is slightly earlier in Eastern Canada as in the Baltic.

(4) Another explanation for the elevated levels of the thyroid hormones in the Baltic grey seals could be their high level of contaminants which may influence thyroid hormones by various mechanisms[12]. Previous studies in the same grey seal material have shown that the expression of contaminant metabolising enzymes (cytochrome P450) is much higher in the Baltic seals than in the Canadian seals[13]. It could be thus supposed that also the general metabolic rate is higher in the Baltic seals and it could be thus increase also the thyroid hormone levels. The high ratio FT3:FT4 also supports the increased need of thyroid hormones in the Baltic grey seals, because the level of free triiodothyronine (FT3) mainly describes the availability of active thyroid hormone[9].

Most of total T4 and T3 in blood are associated with binding proteins[9]. One of the main TH-binding proteins is transthyretin (TTR)[9]. This protein is also involved in the transport of retinol, the circulating form of vitamin A, by forming a carrier protein complex (TTR-RBP) with retinol binding protein (RBP) (Goodman 1984). Several marine mammal studies have reported negative relationship between circulating thyroid hormone levels and OCs[7,14,15] and in two of those studies levels of circulating retinol levels have also had a negative relationship with OCs. However previous findings in the present grey seal material show that circulating retinol levels are significantly higher in the Baltic grey seals compared to seals from Sable Island[5]. A possible explanation for the increased THs and retinol levels in plasma in the Baltic grey seals could be that the amount of TTR-RBP is upregulated due to the contaminant exposure. As the vitamin A reserves in the liver and blubber are decreased in these Baltic seals[5] it could be supposed that mobilisation of vitamin A from the storing tissues could increase the amount of carrier proteins. Also Hall et al.[8] found positive correlations between thyroid hormone levels and contaminants. In addition they reported a positive relationship between contaminants and albumin levels which is also a thyroid carrier protein. In this study the FT3:TT3 ratio was several times higher in the Baltic than in the reference seals. A competitive binding of OCs to the TTR[16] may influence the ratio of FT3:TT3. FT3:FT4 ratio was also higher in the Baltic grey seals compared to reference seals (Table 1).

In conclusion, several reasons could be suggested to explain the high levels of thyroid hormones in the contaminated Baltic seals including genetic difference, environmental conditions, reproduction status and contaminants acting by various mechanisms. Further research should be directed to statistical analysis on relationship between individual contaminants and thyroid hormones, and also on possible mechanism between organochlorines and thryoid hormones.

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