BONE TISSUE IS ALTERED IN TRANSGENIC MICE WITH A CONSTITUTIVELY ACTIVE ARYL HYDROCARBON RECEPTOR

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Abstract
Recent studies have revealed that bone is a target tissue for dioxins. Transgenic mice expressing a constitutively active AhR (CA-AhR) were used to study the effects of an activated AhR in bone tissue. The long bones were investigated with quantitative computed tomography (pQCT) and the strength of the bones was tested with biomechanical testing. Serum levels of carboxyterminal telopeptide (CTX) and diaphyseal mRNA expression of CYP1A1, osteopontin (OPN) and alkaline phosphatase (ALP) were analyzed. In female CA-AhR mice, pQCT analysis of the tibia revealed a reduced cortical bone mineral density (BMD) in the diaphysis. Biomechanical testing showed an increased displacement and a decreased max stiffness of the bones from transgenic females. In the diaphysis, gene expression of CYP1A1 was induced. Osteopontin were, however, down-regulated. The serum level of CTX was slightly increased in male CA-AhR mice, which might imply a higher bone resorption. In conclusion, a constitutively active AhR affects bone tissue in both sexes of CA-AhR-mice.

Introduction
Most, if not all, toxic effects of dioxins are mediated trough the aryl hydrocarbon receptor (AhR), also known as the dioxin receptor. Dioxins cause a wide range of toxic effects, including immunosuppression, reproductive and developmental effects. Recent studies have revealed that also bone is a target tissue for dioxins and dioxin-like compounds[1-4]. Dioxins and dioxin-like compounds exert their effects by binding to the aryl hydrocarbon receptor (AhR) with high affinity and modulate the transcription of various genes[5].
Transgenic mice with a constitutively active AhR (CA-AhR) have been developed and may function as a model for studying the effects of dioxins and the mechanisms behind these. The effects in the CA-AhR mice resemble the effects of mice exposed to low doses of dioxin[6,7]. A bone associated protein, osteopontin, was significantly down-regulated in stomach tumours of these CA-AhR mice[8]. Osteopontin is a protein suggested to be involved in bone tissue remodelling and promotes attachment of the bone cell to the matrix, thus, changes in the expression of osteopontin may be involved in an altered bone remodelling. *In vitro* studies with an osteoblastic cell line, UMR-106, have shown that osteopontin is rapidly down-regulated in these cells when exposed to TCDD[9].

Aim
The aim of the present study was to investigate if bone tissue of male and female CA-AhR mice is affected by the active AhR. In addition, the effect of CA-AhR on biomarkers of bone formation and bone resorption was examined in male CA-AhR mice. We also further explored the possibility that these mice can be used as a model to study dioxin toxicity in mice.

Methods
Young adult transgenic mice expressing a constitutively active AhR (CA-AhR) were used to study the effects of an activated AhR in bone tissue. The long bones were investigated with three dimensional peripheral quantitative computed tomography (pQCT) and the strength of the bones was tested with three point bending test. Serum levels of carboxyterminal telopeptide (CTX) and mRNA expression of CYP1A1, osteopontin (OPN) and alkaline phosphatase (ALP) were analyzed.

Statistics. The pQCT and biomechanical data were evaluated by ANOVA (one-way analysis of variance) and adjusted for body weight. Two-tailed Student's t-test was used to compare the means of qRT-PCR parameters. A p-value ≤ 0.05 was considered to be statistically significant.

Results and discussion

Several effects on bone tissue were observed both in female and in male transgenic mice.
In female CA-AhR mice pQCT analysis revealed a reduced cortical BMD in the diaphysis **(Table 1)**.
Biomechanical testing showed an increased displacement and a decreased max stiffness of the bones, findings in accordance with the results from the pQCT scans of the diaphysis **(Table 2).**
In males, a decrease in total BMD in the diaphysis was observed, but not statistically significant. This is further reflected by the biomechanical testing, that did not reveal any differences in strength between transgenic and wild-type male mice **(Table 2)**.
In the diaphysis, gene expression of CYP1A1 was induced, while OPN were down-regulated **(Table 3)**. The serum level of CTX was slightly increased in male CA-AhR mice (7.5 ± 0.1 ng/ml) compared with for the wt-mice (6.9 ± 0.14 ng/ml; $p<0.01$), which might imply a higher bone resorption.
In conclusion, a constitutively active AhR affect bone tissue in both male and female mice. Furthermore, the results might imply that these mice can be used as a model to study dioxin toxicity in mice.

Table 1. Results from the peripheral quantitative computer tomography (pQCT). Values are given in mean ± SD. P-values ≤ 0.05 * were considered as significant.

	Female		**Male**	
	WT (n=9)	**CA-AhR** (n=8)	**WT** (n=12)	**CA-AhR** (n=12)
Final body weight (g)	20,7 ± 1,0	20,3 ± 1,1	27,7 ± 2,2	26,4 ± 2,3
Length of tibia (mm)	16,67 ± 0,23	16,53 ± 0,23	17,01± 017	16,92 ± 0,33
DIAPHYSIS				
Total cross-sectional area (mm^2)	0,96 ± 0,06	1,00 ± 0,05	1,23 ± 0,09	1,23 ± 0,15
Cortical area (mm^2)	0,56 ± 0,02	0,57 ± 0,04	0,72 ± 0,06	0,70 ± 0,10
Cortical bone mineral content (mg/mm)	0,65 ± 0,02	0,66 ± 0,04	0,86 ± 0,07	0,83 ± 0,13
Cortical bone mineral density (mg/cm^3)	1166± 15	1149 ± 9, *****	1201 ± 15	1177 ± 37
Total bone mineral density (mg/cm3)	830 ± 25	808 ± 13	845 ± 25	818 ± 36

Table 2. Results from the three point bending test Values are given in mean ± SD. P-values ≤ 0.05 * were considered as significant

	Females		**Males**	
	WT (n=7)	**CA-AhR** (n=8)	**WT** (n=11)	**CA-AhR** (n=12)
Load (N)	11,42 ± 1,18	11,47 ± 1,05	15,73 ± 1,42	15,24 ± 2,72
Displacement (mm)	0,72 ± 0,05	0,83 ± 0,08 *	0,70 ± 0,15	0,68 ± 0,15
Energy at failure (N*mm)	4,45 ± 1,60	5,02 ± 0,73	6,14 ± 1,83	6,38 ± 1,89
Stiffness (N/mm)	15,94 ± 2,10	13,98 ± 1,53	23,01 ± 4,15	23,35 ± 6,17
Max stiffness (N/mm)	22,07 ± 4,01	18,00 ± 3,15	28,58 ± 6,66	44,58 ± 37,68

Table 3. Quantitative RT-PCR investigated the mRNA expression of genes from marrow-flushed diaphysis of the humerus of male CA-AhR and wild-type mice. The results are expressed as mean fold change ± SD. P-values ≤ 0.05 * were considered as significant

	WT (n=6)	**CA**-AhR (n=5)
CYP1A1	1 ± 0,74	54 ± 12*
ALP (alkaline phosphatase)	1 ± 0,65	0,62 ± 0,16
OPN (osteopontin)	1 ± 0,42	0,66 ± 0,17 *

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