# TCDD INDUCES TRABECULAR BONE LOSS AND BONE FRAGILITY IN A TCDD-SENSITIVE BUT NOT IN A TCDD-RESISTANT RAT STRAIN

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## Introduction

Bone development and maintenance are strictly controlled by hormonal interactions. Dioxins have been reported to affect several hormonal systems[1]. Because bone development and maintenance are controlled by a complex interaction of several endocrine factors, dioxins could interact also with bone remodelling. Therefore dioxin exposure may cause trabecular bone loss and decrease of bone strength at sites containing both trabecular and cortical bone such as long bone metaphysis. Sensitivity to dioxin-induced toxicity is highly variable among animal species and strains of the same species. An animal model based on different sensitivity to various endpoints of dioxin toxicity has previously been developed. Long-Evans (L-E) is the most TCDD-sensitive rat strain, Han/Wistar (H/W) is the most resistant[2]. Molecular biology studies showed that H/W rats exhibit a structurally aberrant aryl hydrocarbon receptor (AhR) with altered transactivation domain[3]. In a recent study L-E rats demonstrated higher sensitivity than H/W rats to TCDD-induced changes in diaphyseal geometry and strength of tibia[4]. In this study we used L-E and H/W rats to examine the effects of TCDD on cortical and trabecular bone of femur metaphysis.

## Material and Methods

Ten weeks old female L-E and H/W rats were assigned into treatment groups of ten animals and given a total dose of 0, 0.17, 1.7 and 170 (H/W only) μg/kg TCDD s.c. weekly during 20 weeks. The right femur was dissected and the length was measured using an electronic sliding caliper. The femoral metaphysis was scanned at a point distanced 20% of the bone length from the distal end of femur with a peripheral quantitative cumputed tomography (pQCT) system (Stratec XCT 960A, Birkenfeld, Germany) with a voxel size of 0.148–$mm^3$. A 0.400 $cm^{-1}$ attenuation threshold was set as a lower limit to define the trabecular bone region. The cross-sectional area (CSA), trabecular bone mineral density (BMD) and area, as well as cortical/subcortical BMD and area were analysed. Three-point bending (electromechanical material testing machine, Avalon technologies, MN, USA) with a span length of 10 mm and a loading speed of 0.5 mm/sec was used to measure the femoral strength. The load was applied to the anterior surface of the femur where the pQCT scan had been done. Maximal load to failure, deformation at failure, stiffness and energy to failure were defined. Each group was tested against the respective control group using the Mann-Whitney Rank Sum test. A significance level of $p<0.05$ was chosen.

**Results**

Exposure to TCDD resulted in significantly decreased femur length in L-E rats at the doses of 1.7 and 17 µg/kg and in H/W rats at 170 µg/kg dose levels (Table 1). CSA was lowered in L-E rats at 1.7 and 17 µg/kg and in H/W rats at 17 and 170 µg/kg due to a decrease in cortical/subcortical area (Table 1). Trabecular bone mineral density was decreased in L-E rats at 17 µg/kg (Fig. 1a), but not in H/W rats, meanwhile cortical BMD was not affected in either of the strains.

The breaking force of femur methaphysis after TCDD treatment was significantly reduced in L-E rats at the doses of 1.7 and 17 µg/kg, and bending stiffness was significantly lower in all L-E rats, exposed to TCDD (Fig. 1b). H/W rats were not affected.

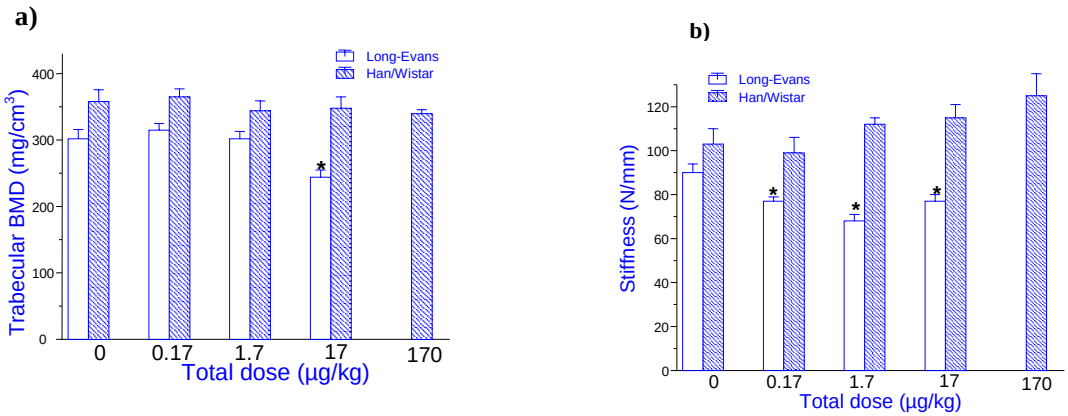


*Figure 1 a) trabecular bone mineral density (BMD; mg/cm$^3$) and b) stiffness (N/mm) in the femoral metaphysis of H/W and L-E rats treated with different doses of TCDD (µg/kg). Values represent the mean ± SEM*

**p<0.05 compared to corresponding controls.*

| | Dose (µg/kg) | Length (mm) | CSA (mm$^2$) | Trab area (mm$^2$) | C/s area (mm$^2$) | Breaking force (N) |
|---|---|---|---|---|---|---|
| **L-E** | control | 34.3 ± 0.5 | 13.6 ± 0.7 | 2.5 ± 0.9 | 11 ± 1.3 | 163 ± 18 |
| | 0.17 | 34.6 ± 0.5 | 13 ± 0.6 | 2.5 ± 0.6 | 10.4 ± 0.9 | 153 ± 19 |
| | 1.7 | 33.4 ± 0.9* | 12.3 ± 0.4* | 2.7 ± 0.7 | 9.6 ± 0.8* | 135 ± 14* |
| | 17 | 31.8 ± 0.5* | 10.9 ± 1* | 3 ± 0.2 | 7.9 ± 1.1* | 137 ± 7* |
| **H/W** | control | 33.6 ± 0.8 | 13.2 ± 1.2 | 1.7 ± 0.8 | 11.5 ± 1.4 | 166 ± 18 |
| | 0.17 | 33.6 ± 0.9 | 13.3 ± 0. | 1.8 ± 0.9 | 11.5 ± 1.3 | 167 ± 9 |
| | 1.7 | 32.8 ± 1 | 11.8 ± 1.4 | 2 ± 0.5 | 9.8 ± 1.4 | 159 ± 19 |
| | 17 | 33.4 ± 0.7 | 11 ± 1.2* | 1.4 ± 0.8 | 9.6 ± 0.9* | 165 ± 22 |
| | 170 | 32.6 ± 0.7* | 11.2 ± 0.9* | 1.8 ± 0.6 | 9.5 ± 0.9* | 176 ± 10 |

*Table 1. Femoral length, cross-sectional area (CSA), trabecular, cortical/subcortical(C/s) area and breaking force in the femoral metaphysis of H/W and L-E rats treated with different doses of TCDD (µg/kg). Values represent the mean ± SD*

**Discussion**

When effects of TCDD on bone geometry, volumetric density and strength of tibial midshaft were investigated in a previous study, a significant decrease in mechanical strength and cross-sectional area, but not in density were found[4]. Exposure to dioxinlike PCB-congener 3,3,4,4,5-pentachlorobiphenyl (PCB126) has been associated with a decreased torsional strength and collagen concentration of the rat humerus[5].

In the present study we chose to perform densitometric analyses at the metaphyseal part of the bone and investigate biomechanical parameters at the same site as pQCT measurements to better appreciate bone strength in relation to architecture modifications. TCDD treatment resulted in shorter and smaller bones in both strains. L-E rats demonstrated decreased trabecular BMD, correlating with decreased strength at femur metaphysis, meanwhile neither trabecular BMD nor bone strength were affected in TCDD-resistant H/W rats with altered AhR. This may indicate that the observed effects are AhR-mediated and that AhR transactivation domain plays a role in determining sensitivity to TCDD-induced bone effects. AhR, mediating most of the toxic effects of dioxins, have been shown to modify several important regulators of bone turnover and homeostasis, such as the estrogen receptor (ER) and the retinoid systems. TCDD-induced inhibitory AhR-ERα cross talk was observed in rodent and human cells[6]. Trabecular osteopenia, observed in the present study, is a manifest effect of estrogen deficiency[7,8]. An approximate 10-fold difference in sensitivity for retinoid effect in L-E and H/W rats have been shown in a previous study[9], which suggests an involvement of AhR in TCDD-altered retinoid homeostasis. Retinoids have been shown to have profound effect on bone metabolism[10].

Bearing in mind the differences in previously observed effects of TCDD-treatment on long bone midshaft and the present observations on long bone metaphysis, additional studies of loading-bearing skeletal sites are needed to pinpoint the most sensitive endpoints for dioxin-induced AhR-mediated bone toxicity.

**References**

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