# EFFECTS OF SUBCHRONIC EXPOSURE TO FRACTIONS OF BALTIC HERRING OIL ON METAPHYSEAL BONE OF RAT FEMUR.

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## Introduction

The Baltic Sea is one of the most contaminated water bodies in Europe. Recently, the European Commission introduced limit values for dioxins and dioxin-like compounds in Baltic fish [1]. Concern of health effects of dioxin-like as well as additional fish-derived organochlorine pollutants prompted the performance of a subchronic toxicity study in the rat [2-4].

Different fractions of Baltic Sea herring (*Clupea harengus*) oil were isolated and chemically characterised. A subchronic toxicity study was performed with rats given fish oil or its fractions at different doses in the diet to examine the contribution to toxicity and biological effects of different halogenated organic pollutants. The selected low, medium and high dose levels corresponded to a human fish intake of 1.6, 8.2 and 34.4 kg per week respectively. The fat concentrations of the diets were standardised at each dose level with soy oil. The lodda oil diet was used in order to provide a nutritionally equivalent control group. The levels of contaminants in herring oil, its fractions and liver tissues from exposed rats as well as results from the subchronic toxicity study have been reported elsewhere [3]. Briefly, the fractionation procedure resulted in a substantial reduction of most of the pollutants in the triacylglycerol fraction (F1), and a pronounced enrichment of most of the pollutants into the two other fractions (F2 and F3).

The toxicological examination showed that the major effects could be related to pollutants such as chlorinated biphenyls, dioxins and furans, despite the fact that they contribute to a minor part of the extractable organically bound chlorine [4]. Interactions between contaminants, with regard to these effects, were additive, and consistent with the TEQ concept, i.e. the expected responses based on the chemically determined doses were obtained.

A significant decrease in total bone density was observed in several diet groups as compared to corresponding controls. This effect, however, was not directly related to organochlorine exposure. Bone development and maintenance are controlled by complex interactions between several hormones and vitamins. Several groups of persistent organohalogen pollutants have been shown to disturb the balance of many hormonal systems, interacting also with bone modelling and remodelling. The aim of the present study was to follow up the preliminary observations on decreased bone density using the more sophisticated method of peripheral quantitative computed tomography (pQCT).

## Material and Methods

The right femur was dissected, cleaned, wrapped in plastic film and stored at –20 °C until analysis. The total bone length was measured using an electronic sliding caliper. The metaphyseal part of the left femur were scanned with a pQCT system (Stratec XCT 960A with software version 5.20 Norland Stratec Medizintechnik, GmbH, Birkenfeld, Germany) at a point distanced 20 % of the femur length from the distal part of the bone. A 0.148–$mm^3$ voxel size and a 0.700 $cm^{-1}$ attenuation threshold were selected in order to establish a tomographic limit between cortical and trabecular bone regions. A 0.400

$cm^{-1}$ attenuation threshold was set as a lower limit to define the trabecular bone region. The trabecular bone mineral density and trabecular bone area were analysed. 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**

The femur length was significantly lower in rats fed F1 diet at high-dose level and in rats fed F2 and F3 diets at low and high dose levels as compared to the corresponding control groups (Figure 1).

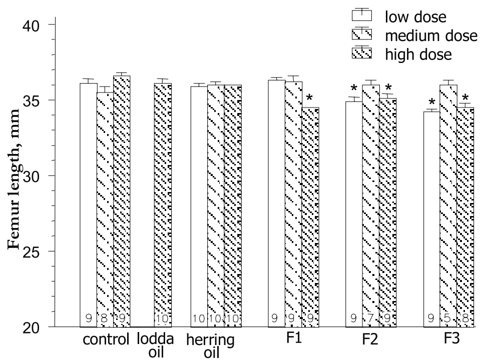


**Figure 1.** Femur length (mm) for female Sprague-Dawley rats fed diets containing soy oil, lodda oil, baltic herring oil and its fractions for 13 weeks. Values represent the mean ± SEM, the number of animals are given within the bars.
*p<0.05 compared to corresponding controls.

The trabecular bone mineral density was increased in rats fed F1 medium and high-dose diets, F2 low-dose and F3 low and high dose diets (Figure 2).

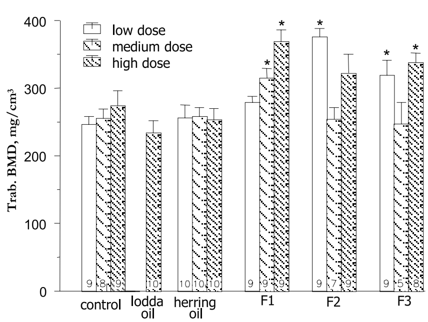


**Figure 2.** Trabecular bone mineral density density (BMD) ($mg/cm^3$) in femur metaphysis for female Sprague-Dawley rats fed diets containing soy oil, lodda oil, baltic herring oil and its fractions for 13 weeks. Values represent the mean ± SEM, the number of animals are given within the bars.
*p<0.05 compared to corresponding controls.

The trabecular area was decreased in rats fed F1 diet at high-dose level and F2 diet at low-dose level (Figure 3).

Generally, the inhibited bone growth correlated with tomographic observations of increased trabecular bone mineral density and decreased trabecular bone area.

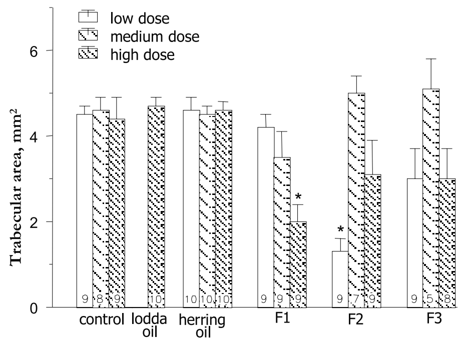


**Figure 3.** Trabecular area (mm²) in femur metaphyses for female Sprague-Dawley rats fed diets containing soy oil, lodda oil, baltic herring oil and its fractions for 13 weeks. Values represent the mean ± SEM, the number of animals are given within the bars.
*p<0.05 compared to corresponding controls

**Discussion**

Bone modelling and remodelling is regulated by complex interactions between an individual's genetic profile, environmental influences, and nutrition. In this study rats were exposed to diets, containing both vegetable and fish oils and fish oil-derived organohalogen pollutants in different combinations. Organohalogen pollutants have been reported to affect several biological regulators that also control local bone metabolism, such as estrogen, thyroid hormone and vitamin A [5]. In addition, specific dietary fatty acids has been found to improve bone formation rates in animal models [6]. It has been shown that lowering the dietary ratio of vegetable/fish derived fatty acids resulted in increased bone marrow cellularity [7] and bone strength [8].

Histological and biomechanical studies of the bones as well as further chemical analyses of fish oils and fractions will be needed to allow any conclusions about whether the combined effects of environmental pollutants and different dietary fat components can be the cause of the observed effects on the metaphyseal part of the long bones.

**Acknowledgments**

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