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Oestrogen receptor beta mediates decreased occlusal loading induced inhibition of chondrocyte maturation in female mice

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ABSTRACT

Objective: Temporomandibular joint (TMJ) disorders predominantly afflict women, suggesting that estrogen may play a role in the disease process. Defects in mechanical loading-induced TMJ remodelling are believed to be a major etiological factor in TMJ degenerative disease. Previously, we found that, decreased occlusal loading caused a significant decrease in early chondrocyte maturation markers (Sox9 and Col 2) in female, but not male, C57BL/6 wild type mice (1). The goal of this study was to examine the role of Estrogen Receptor (ER) beta in mediating these effects.

Design: 21-day-old male ($n = 24$) and female ($n = 25$) ER beta KO mice were exposed to decreased occlusal loading (soft diet administration and incisor trimming) for 4 weeks. At 49 days of age the mice were sacrificed. Proliferation, gene expression, Col 2 immunohistochemistry and micro-CT analysis were performed on the mandibular condyles.

Results: Decreased occlusal loading triggered similar effects in male and female ER beta KO mice; specifically, significant decreases in Col 10 expression, subchondral total volume, bone volume, and trabecular number.

Conclusion: Decreased occlusal loading induced inhibition of chondrocyte maturation markers (Sox9 and Col 2) did not occur in female ER beta deficient mice.

1. Introduction

Approximately 35 million people in the United States suffer from TMJ problems at any given time. Thirty to fifty percent of individuals diagnosed with a temporomandibular joint disorder have temporomandibular joint degenerative disease (TMJ-DD).[2] TMJ-DD predominantly afflicts women, with a mean age range of 44–55 years.[3,4] The cause of this sex predilection remains largely unknown. Current theory suggests that TMJ

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degeneration is the result of excessive altered joint loading surpassing the mechanical loading-induced remodelling capacity of the joint.[5,6]

We have developed a murine model of decreased occlusal loading TMJ remodelling, enabling us to study mechanical loading-induced TMJ remodelling. In this decreased occlusal loading model, the incisors are trimmed at regular intervals (1 mm every other day) and the normal hard pellet diet is replaced with a soft-dough diet. We previously found that decreased occlusal loading consistently led to decreased subchondral bone volume and decreased mandibular condylar cartilage thickness.[7] In addition, decreased occlusal loading was found to cause sex differences in chondrocyte maturation expression in the temporomandibular joints of C57BL/6 and CD-1 wild type (WT) mice. When compared with normal loading controls, 21-day-old female, but not male, WT mice exposed to 4 weeks of decreased occlusal loading exhibited significant decreases in Sox9 and Collagen type II expression.[1] This gender-related disparity suggests that estrogen may play a role in TMJ mechanical loading-induced remodelling.

There are two classic estrogen receptors: alpha and beta. Male and female mice deficient in estrogen receptor alpha express a characteristic skeletal phenotype.[8] In contrast, only female mice deficient in estrogen receptor beta display a unique skeletal phenotype.[9,10] It is believed that high estrogen levels are required to activate estrogen receptor beta; therefore, the skeletal phenotype is not expressed in male estrogen receptor beta knockout mice due to insufficient estrogen levels. Characterization of the TMJ from estrogen receptor beta deficient mice, compared with age- and sex-matched WT mice, revealed increased mandibular condylar cartilage thickness in female deficient (KO) mice, due to decreased cartilage turnover.[11] The purpose of this study is to examine the role of estrogen receptor beta in mediating the effects of decreased occlusal loading induced TMJ remodelling. Our hypothesis is that ER beta deficiency in C57BL/6 WT mice will prevent sex differences in mandibular chondrocyte maturation that normally occur with decreased occlusal loading. In order to examine this effect, we evaluated male and female ER beta KO mice exposed to decreased occlusal loading.

2. Materials and methods

2.1. Animals

ER beta KO mice (homozygous male, heterozygous female, Cat# 004745) were purchased from the Jackson Laboratory (Bar Harbor, ME, USA). For the experiments requiring ER beta KO mice, only homozygous females and males were used. 21-day-old male ($n = 24$) and female ER beta KO mice ($n = 25$) were used in this study.

2.2. Experimental design

2.2.1. Loading model

At 21 days of age, male and female ER beta KO mice, in a C57BL/6 background, were each divided into two groups: (1) normal loading for 4 weeks, and (2) decreased occlusal loading for 4 weeks (Table 1). The decreased loading groups were fed a soft-dough diet (Transgenic Dough Diet, with the same nutritional composition as the normal-pellet diet; BioServ, Frenchtown, NJ) and their mandibular incisors were trimmed, using an orthodontic light wire clipper, by approximately 1 mm every other day for 4 weeks (the unimpeded eruption rate of mandibular incisors in mice is approximately 0.4 mm per day[12]; incisor trimming maintains an anterior open bite of 0.5–1.0 mm). After 4 weeks of decreased loading (49 day old), the mice were sacrificed. Three hours prior to euthanasia, the mice were injected, intraperitoneally, with 0.1 mg bromodeoxyuridine (BrdU) per gram body weight. The body weight was monitored twice per week. Mice lost more than 20% of body weight were eliminated from the experiment. All experiments were performed in accordance with animal welfare based on an approved IACUC protocol #AAAD0950 from the Columbia University animal care committee.

2.2.2. Histomorphometry

Whole mouse heads were sectioned into two halves, fixed in 10% formalin for 4 days at room temperature and decalcified in 14% EDTA (pH 7.1) (Sigma, St. Louis, MO, USA) for 10 days. Subsequently, the samples were processed through progressive concentrations of ethanol, cleared in xylene and embedded in paraffin. The TMJ was serially sectioned along the sagittal plane at 5-μm thickness, by Microm HM 355s microtome (Thermo Fisher Scientific, Waltham, MA, USA); every fifth section was stained with haematoxylin and eosin (H&E). Mandibular condylar cartilage thickness was measured in a blinded, nonbiased manner using the BioQuant computerized image analysis system (BioQuant, Nashville, TN, USA). Mandibular condylar cartilage thickness was performed on H&E sagittal sections corresponding to the mid-coronal portion of the mandibular condylar head. The mandibular condylar cartilage area was divided into two layers, non-hypertrophic (cells <8 μm in height, includes superficial, polymorphic and flattened zones) and hypertrophic (cells >8 μm in height) (Fig. 1A). The anterior–posterior region of the mandibular condylar cartilage was restricted to the region that

Table 1 – Number of mice.

Sex	Total (n)	Treatment	Real time PCR (n)	Histology/μCT (n)
Male	24	Normal loading	6	6
		Decreased occlusal loading	6	6
Female	25	Normal Loading	6	7
		Decreased occlusal loading	6	6

μCT: micro-computed tomography; n = number of mice.