SCIENTIFIC ARCHIVES OF DENTAL SCIENCES (ISSN: 2642-1623)

Volume 4 Issue 8 August 2021

Research Article

Insulin-Like Growth Factor Receptor Expression in the Mandibular Cartilage is Modified by the Physical Consistency of the Diet

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Received: May 26, 2021; **Published:** July 31, 2021

Abstract

Background and Aim: The authors' research has demonstrated that the mandibular condyle's biology is affected by the oral functions, among them the physical consistency of the diet. In order to better understand how that phenomenon may occur at the cellular level, this study aimed to quantify the numbers of cells expressing unbound IGF-1R alpha in the chondroblasts of the rat mandibular condylar cartilage, when the animals were fed with either a liquid, soft or hard diet.

Methods: Thirty-six Wistar rats were randomly assigned to three groups. Twelve animals composed each group and they were fed with either a liquid, soft or hard diet. Six animals were sacrificed from each group at days 20, and 40 respectively. Their mandibles were removed, the mandibular condyle was harvested, and tissues were processed and immuno-stained against IGF-1R alpha.

Results: Animals fed with a harder diet over a period of 40-days reported a significantly lower number of IGF-1R alpha immune-positive cells ($p < 0.05$) at the postero-superior area of the mandibular condylar cartilage.

Conclusion: The current study reported that the physical consistency of the diet directly affect the expression of the IGF-1R alpha in the mandibular condylar cartilage of rats. That result suggests the IGF-1R/IGF-1 axis may be modified in the cartilage cells by the physical consistency of the diet.

Keywords: *Diet; Physical Consistency; Mandibular Condylar Cartilage; Immunohistochemistry; IGF-1 Receptor*

Introduction

The mandibular condyle is regarded as a significant growth center in the mandible. Mastication induces mechanical stress which markedly impacts the biology of the mandibular condyle, as the repeated load delivered at the temporomandibular joint may change the patterns of the endochondral ossification occurring at the mandibular condyle [1,2].

In a recent study, the authors reported that animals fed with a harder diet had an improved bone quality in their mandibles, with a higher mineral content at the end of the experimental period, when compared with those from animals fed with either a soft or a liquid diet [3]. However, that study did not elucidate how the physical consistency of the diet could affect the biological activity of the condylar cartilage, positively switching the bone turnover and its biology at the mandibular condyle.

The Insulin-like growth factor 1 (IGF-1) is expressed in the mandibular condylar cartilage of rats, where it regulates cell growth and proliferation [4,5]. Its effect is induced when the IGF-1 binds to its receptor in the cartilage cells, the IGF-1 Receptor (IGF-1R). That receptor is composed of two tetramers, alpha and beta, with the alpha tetramer being the one containing the binding site for the IGF-1 [6]. The expression of the IGF-1R has been reported to appear mainly at the superior and postero-superior regions of the mandibular condylar cartilage of rats [7].

In order to better understand the effect of the diet's physical consistency on the biology of the mandibular condylar cartilage, this study aimed to quantify the numbers of cells expressing unbound IGF-1R alpha in the chondroblasts of the rat mandibular condylar cartilage, when the animals were fed with either a liquid, soft or hard diet.

Materials and Methods

The Board for Animal Ethics of the University of Manitoba approved this animal study (Ref. 10-021). It followed the guidelines regarding the care and use of animals for experimental procedures established in Canada. This study involved thirty-six, 20 days of age, male Wistar rats. Twelve animals were randomly allocated at each experimental group and, each group was exposed to a diet with different physical consistency: The first group of animals were fed with a hard diet (HD), being fed with regular rat pellets. Another group of rats were served with a softer diet (SD), so they were fed with a powder from the same rat pellets mixed in a 1:1 ratio with water. The last group were served with a liquid diet (LD). That group was fed with the same pellet powder but mixed in a 1:4 ratio with water. Maintaining the same food source for the three groups allowed the physical consistency of the diet to be varied while maintaining a constant nutritional value for the three experimental groups.

The experimental period was split into two periods, with half of the animals from each group being sacrificed at day 20 and, the other half being sacrificed at day 40. The animals in the three groups were kept with no entertainment objects in the cage, water and food *ad libitum* and a 12 hour light/dark cycles during the whole experimental period. Also, the animals in the three groups were closely monitored for changes in behavior and weight gain.

All animals sacrificed at the end of both experimental periods were prior anesthetized with Ketamine/Xylazine in a dose of 80 mg/kg and 5 mg/kg respectively. Under general anesthesia, all tissues were perfused with 4% paraformaldehyde in 0.1M phosphate buffered saline (PBS). The animals were sacrificed by exsanguination. The mandibles from all animals were removed and split in half. The right half mandibles were used for this study. Samples were immersed in 4% paraformaldehyde in 0.1M PBS for 4 hours, and then, washed in PBS overnight. Demineralization of the tissues in the hemi-mandibles included in this study was attained with 4.13% EDTA at a 7:47 pH, over approximately four weeks. Once demineralization was confirmed with x-rays, every hemi-mandible was embedded in paraffin, then sagittal sectioned (5µ thick) and mounted. Two slides from the tissues harvested from each animal in the three experimental groups were obtained and processed for immune-histochemical staining.

Immunohistochemistry

Immunohistochemistry proceeded as reported previously.[8] Paraffin was removed from the tissues and they were rehydrated in a series of xylene/alcohol dilutions. Then, endogenous peroxidase activity was reduced by exposing the tissues at room temperature to 3% hydrogen peroxide in PBS over 15 minutes. Protein binding in the tissues was eluded by incubating the slides one hour in 1:10 goat serum in 3% BSA. All histological sections remained overnight at 4ºC incubating with anti-IGF-1R alpha (Santa Cruz, sc-712, CA, USA), as the primary antibody. The next day, all sections were immersed for 10 minutes in PBS and then, exposed for 30 minutes to rabbit IgG (Vectastain, ABC kit, PK-6101, CA, USA), as secondary antibody. Tissues were washed again in PBS for 10 minutes and then, exposed to DAB (Fisher Scientific, substrate kit, ON, Canada). As a final step, tissues were stained for 20 seconds with Hematoxylin, washed in alcohol, dehydrated in xylene and cover slipped.

Cell counting

All immuno-stained histological sections were digitally photographed focusing on the mandibular condylar cartilage at a magnification of 10X, via a digital camera mounted on the microscope (AxioCam HRc Carl Zeiss, Jena, Germany). The immuno-positive cells in the proliferative layer at the postero-superior region of the mandibular condylar cartilage were counted on the digital photographs from each sample. For determining the proliferative layer, computer software (Adobe Photoshop version 7.01, USA) was used to demarcate an area of 250,000 μm^2 by means of a scalar of 100 µm. All immuno-positive cells contained in that determined area were counted on each sample of the slides processed for this study. The clearest photograph from the two immune-stained slides processed from each sample was used for the cell counting.

Statistics

Each counting was performed twice on different days (five days apart) by the same operator. An average from both counts was used

as final data for each sample. One-way ANOVA plus Dunns post-test statistical analyses were computed (GraphPad Prism 4.0 Software Inc., San Diego, CA, USA), to determine significant differences between the various groups. Significance was determined at the 95 per cent level of confidence.

Results

The numbers of immune-positive cells at the mandibular condylar cartilage for the IGF-1R alpha in the animals sacrificed after 20 days reported a higher number of inmuno-positive cells in the liquid diet group, followed by the hard diet group and with the lower number in the soft diet group (Figure 1). However, the differences in the numbers of inmuno-positive cells did not reach a significant statistical difference.

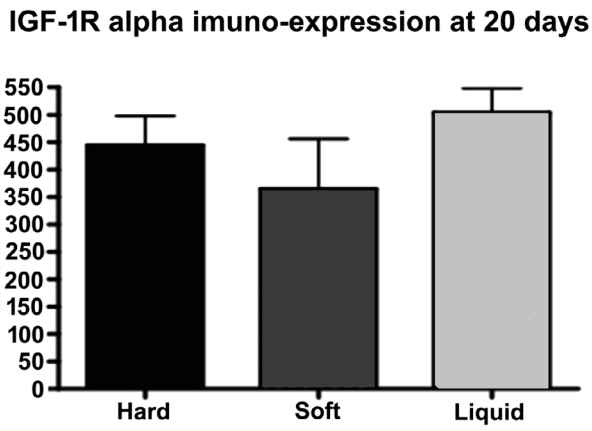

Figure 1: Graphics showing the mean and SD of the immuno-positive cells counted at the postero-superior area of the mandibular condylar cartilage of those rats fed with three different physical consistencies of the diet over 20 days. No statistical significant differences were computed at the 20 days-experimental period.

The numbers of immune-positive cells expressing the IGF-1R alpha at the mandibular condylar cartilage in the animals sacrificed after 40 days reported a significantly lower number of immuno-positive cells in the hard diet group ($p < 0.05$), when it was contrasted against both, the liquid and soft diets groups (Figure 2). Photographs of stained tissues of the mandibular condylar cartilage for the three groups included in this study, liquid, soft and hard diet, are presented in figure 3.

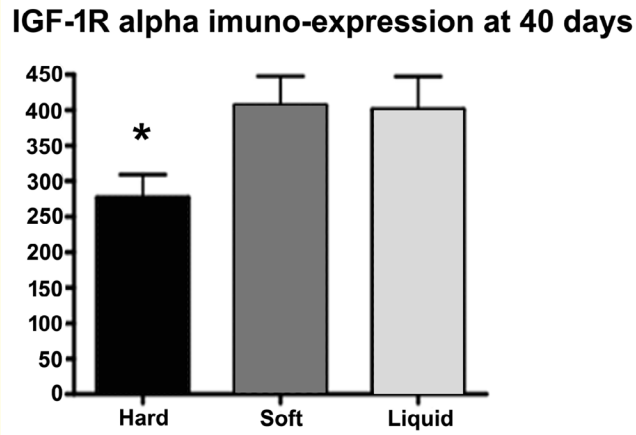

Figure 2: Graphics showing the mean and SD of the immuno-positive cells counted at the postero-superior area of the mandibular condylar cartilage of those rats fed with three different physical consistencies of the diet over 40 days. A statistical significant lower number of cells immuno-stained against IGF-R alpha was computed for the group of rats fed with a hard diet at the 40 days-experimental period. * $p < 0.05$.

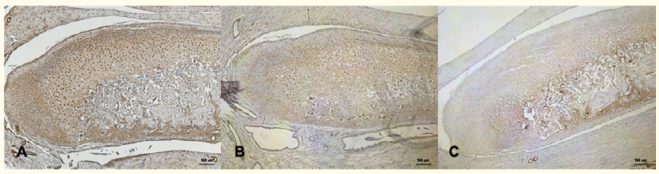

Figure 3: Photographs showing the IGF-1 R alpha immuno-positive cells in the mandibular condylar cartilage of rats fed with a liquid (A); soft (B); and hard (C) diet during a 40 days-experimental period. Photographs shown at 10X magnification.

Discussion

The result from this study showed that after feeding the animals for a 20-days period, the expression of the unbound IGF-1R alpha was higher in the liquid diet group but it did not reach statistical significant difference when comparing with the other two groups. On the other hand, after feeding the animals continuously for 40 days with either a liquid, soft or hard diet, the expression of the unbound IGF-1R alpha was statistically significantly lower in the hard diet group when compared with the other two groups fed with a different physical consistency diet.

Mastication involves a displacement of the mandibular condyle in the mandibular fossa when the mandible displaces, allowing the teeth to process the food before swallowing. A harder diet requires more masticatory cycles [9] which is associated with a higher number of loads on the temporomandibular joint [10,11]. Furthermore, a higher number of loads on the temporomandibular joint directly affects the biological response of the cartilage cells in the mandibular condylar cartilage of the mandible [12]. In that context, the results from this study agreed with other studies reporting that the physical consistency of the diet directly affects the biology of the mandibular cartilage [13,14]. Based on the presented results, it may be inferred that such a phenomenon could be due to the numbers of the IGF-1 alpha receptors and, probably the amount IGF-1 expressed in the mandibular condylar cartilage being directly modified by the diet's physical consistency. In that context, the presented results agree with other studies showing that the IGF-1 pathway plays an important role on cartilage's growth and development, as well as in cartilage's repair by promoting the novo synthesis of macromolecules and cartilage matrix deposition by chondroblasts [15,16].

Insulin growth factor-1 has been reported to be required for the survival of the cartilage cells, as well as a promoter of endochondral ossification in the mandibular condylar cartilage [17,18]. Concomitantly, the IGF-1R alpha has been reported to stimulate cell proliferation and matrix protein synthesis in the mandibular condyle of rats [5,19]. The effects produced by IGF-1R alpha are due to a cascade of intracellular events occurring after it has been bound by IGF-1. In that context, it can be argued that the significantly lower expression of the IGF-1R alpha counted in this study in the harder diet group was due to the fact that more receptors were bound to the IGF-1, leaving a lesser number of receptors able to bind to the IGF-1R antibody when the current tissues were immunostained against it. Such a biological response to the physical consistency of the diet would further explain the results previously reported by the authors [3], where the bone mineral density and the bone mineral content was significantly higher in the mandibular condyles of those animals fed with a harder diet. Based on the results from this and that previous study, it can be proposed that a harder diet modifies the biology of the mandibular condylar cartilage positively stimulating the endochondral ossification. It can be proposed that more cartilage matrix is produced by the cartilage cells that are stimulated by a higher binding of the IGF-1 to its receptor, leaving less unbound IGF-1R alpha in those animals fed with a harder diet.

Therefore, the results from the present study confirmed the physical consistency of the diet affects the biology of the mandibular condylar cartilage. Such an effect appears to occur in part through the IGF-1/IGF-1R alpha axis. However, this study did not quantify the expression of IGF-1 in the mandibular condylar cartilage of the rats when exposed to various physical consistencies of the diet and thus, further studies are necessary to better understand the effect of the diet on the IGF-1 expression. Future studies may also aim to elucidate other pathways that could produce a positive response in the bone of the mandibular condyle when loaded by a harder physical consistency in the diet.

The results from this study and others published by these authors suggest that maintaining a diet with harder physical consistency may help to produce better results in patients whose treatments aim to stimulate mandibular growth. A harder physical consistency of the diet may be recommended in those patients, as it has been demonstrated to stimulate more mandibular bone apposition at the mandibular condylar cartilage, as well as switching the mandibular condylar cartilage's biology into a more favorable stage for cartilage matrix deposition by the chondrocytes, as demonstrated in this study.

Conclusion

The current study reported that the physical consistency of the diet directly affect the expression of the IGF-1R alpha in the mandibular condylar cartilage of rats. That result suggests the IGF-1R/IGF-1 axis may be modified in the cartilage cells by the physical consistency of the diet.

Declaration of Competing Interest

The authors have none to declare.

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