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The effect of the physical consistency of the diet on the bone quality of the mandibular condyle in rats

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ABSTRACT

Objective: This study aimed to analyze how the physical consistency of the diet affects the bone quality at the mandibular condyle.
Design: Sixty-three Wistar rats were randomly assigned to three groups. Twenty-two animals composed each group and they were fed with either a liquid, soft or hard diet. Seven animals were sacrificed from each group at days 7, 20, and 40 respectively. Their mandibles were removed and scanned at the postero-superior area of the condyle with a micro-CT scan.
Results: showed a statistically significant difference for the bone mineral density ($p < 0.01$) and total mineral density ($p < 0.01$), when comparing the hard against the liquid group after seven days. After 20 days both, the soft and the liquid diet groups, computed a statistically significant difference demonstrating a significant decrease in the measured values for bone mineral density, bone mineral content, total mineral density, and total mineral content. At day 40, the values stayed lower for the soft and liquid diets, even though they did not reach a significant difference.
Conclusions: This study supports the idea that a soft or liquid diet has a negative impact on the bone quality of the mandible, particularly during the periods of more active growing.

1. Introduction

Mastication is the process of chewing, which involves a group of muscles capable of moving the mandible through the temporo-mandibular joint in order to grind and break up food between the occlusal surfaces of the maxillary and mandibular teeth. (Lund, 1991) Depending on the physical consistency of the diet, muscular activity in those masticatory muscles, as well as the loads delivered to the maxillary bones can vary. Those loads can stimulate changes in bone's quality, as well as stimulate either apposition or resorption of bone in some specific areas, primarily at the attachment sites (Dias, Cook, & Mirhosseini, 2011; Renaud, Auffray, & de la Porte, 2010; Tsai, Yang, Chen, & Chiu, 2010). So, the physical consistency of the diet can affect the strength of the loadings delivered on the maxillaries and the mandibular condyle, and so, affecting the bone biology (Enomoto et al., 2014; Sasaguri, Jiang, & Chen, 1998).

The mandibular condyle is considered today an important growth center in the mandible, depositing bone through endochondral ossification (Ingervall, Carlsson, & Thilander, 1976). The mechanical stress induced by mastication markedly affects the mandibular condylar cartilage, as those loads may change the pattern of growth and development of the mandible (Enomoto et al., 2010). It has been reported that mandibles from animals fed with a hard diet had significantly greater condyles, longer mandibular lengths and greater ramus heights when comparing with mandibles from other animals fed with a softer diet (Enomoto et al., 2010; Yonemitsu, Muramoto, & Soma, 2007).

Current knowledge supports the idea that an increase in muscular activity at the masticatory muscles caused by harder consistency of the diet leads to better bone quality (Mavropoulos, Ammann, Bresin, & Kiliaridis, 2005; Mavropoulos, Odman, Ammann, & Kiliaridis, 2010; Tsai et al., 2010). Bone volume is a given volume of interest that is occupied by mineralized bone, which has been shown to be reduced in animals fed with a soft diet (Enomoto et al., 2010). Other studies have also demonstrated that a hard diet produces a significantly higher degree of mineralization of the trabecular bone in the condyle, when comparing with animals fed with a soft diet (Tanaka et al., 2007). Although there has been some research focusing on how the consistency of the diet may impact the mandibular condyle, there is still a lack of knowledge on the effect of the physical consistency of the diet on the mineralization process during endochondral ossification in the mandibular condyle. Thus, in order to evaluate the accumulative

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effect of different masticatory loadings on the mandibular condyle, this study aimed to investigate how the quality of the diet affects the bone density and the mineral content at the mandibular condyle in growing rats.

2. Materials and methods

This study was approved by the Bannatyne Campus Protocol Management and Review Committee at the University of Manitoba, Canada (Ref 10-021; July 2, 2010).

Sixty-three male Wistar rats, 20 days of age, were included in the study. All animals were kept in the same conditions with a 12 h cycles of light and dark. They had food and water ad libitum and were weighed every second day. The study ran for 40 days and at the end of the study all animals were sacrificed, as described below.

2.1. Diet modification

The animals were randomly divided into 3 groups of 21 animals each. Thus, one group was placed into a hard diet (HD group), and fed with regular rat food pellets. Another group of animals were placed into a soft diet (SD group), which was fed with a slurry of pellets softened in water with a ratio of 1:1. The last group was fed with a blended mixture of pellets and water with a ratio of 1:4, and composed the liquid diet group (LD group). In that way, the physical consistency of the diet was modified without affecting its nutritional value.

2.2. Experimental period

As stated above, all animals entered the study at the age of weaning (20 days old). During the course of the study, seven animals from each group were sacrificed at three different ages as explained below. For that, the animals were perfused with 4% paraformaldehyde in 0.1 M PBS under general anesthesia (1 mL per kilogram of 1:10 Xylazine: Ketamine), and then, sacrificed by exsanguination. Then, their mandibles were harvested and microCT-scanned.

After removing the mandibles from the animals, they were split in half and stored in 2% paraformaldehyde solution for 24 h. Then, they were stored in phosphate buffer saline solution. The left hemi-mandibles were used for micro CT scans to analyze any differences in the bone quality between the three groups at the different growing periods. The right sides of the mandibles were used for other studies.

The methodology used in this study permitted to measure the bone quality of the mandibular tissues at three different intervals during the growing period. So, a third of the animals from each experimental group were sacrificed at day 40, at the age of maturity (60 days), while the others were similarly sacrificed at days 7 and 20, during a rapidly growing age.

2.3. Micro CT scan

The hemi-mandibles were scanned using an Explore Locus micro-computarized tomogram (mCT, GE systems, London, ON) with a voxel size of 14 μm. Each mandible was scanned with the same mCT scanner using the GE Healthcare explore MicroView v. 2.1 Software Guide. Three mandibles were scanned together at a time along with a bone standard in which 700 x-rays were taken and then reconstructed to give a 3D representation. Each mandible was cropped from the original scan of three and reconstructed at a 30 μm resolution.

The area of interest, postero-superior area of the mandibular condyle (Fig. 1), was traced on the 2D x-ray and with 30–40 tracings approximately 5–10 frames apart. These 2D tracings were then interpolated to include all of the 2D x-rays between the first and last tracing and a 3D volume was created and analyzed. The specimens maintained a similar position for the x and y axis during the scanning process by means of using the flat cut surface of the condyles as a reference mark to similarly placing the specimens into the scanner.

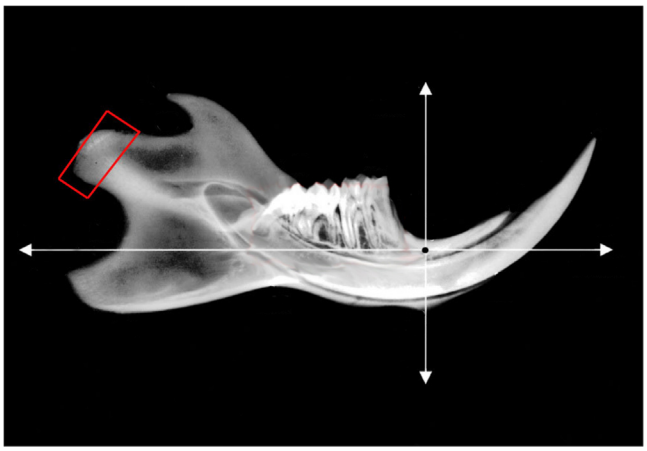

Fig. 1. Photograph showing a mCT scan of one of the rats' mandible included in the study. The red box indicates the area of the mandibular condyle where bone quality assessment was performed. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

The bone quality was analyzed by looking at the micro CT scan values of the bone mineral density (BMD), Bone mineral content (BMC), Total mineral density (TMD), and, Total mineral content (TMC) at the mandibular condyle. BMD reports the strength of the bone based on the amount of calcium, expressed as mg/cc; BMC measures the amount of calcium and other minerals in the volumetric area of the bone, expressed as mg; TMD reports the amount of bone tissue in the volumetric measured area, expressed in mg/cc; and, TMC reports the total amount of minerals in the measured area, expressed in mg.

2.4. Statistical analysis

All measurements were performed 3 times by the same operator. A final average from the three different measurements was statistically analyzed by one-way ANOVA, using a statistical computer package (Graph-Prism 4.0, GraphPad Software Inc., San Diego CA, USA). A Dunn's post-test was performed when a significant difference at the 95 percent level of confidence was observed. $P < 0.05$ was considered significant.

3. Results

All animals grew within normal limits and there were no significant differences in weights between the three experimental groups. (Table 1) Measurements of bone quality (BMD, BMC, TMD and TMC) at the postero-superior area of the mandibular condyle reported statistically significant differences between the hard and

Table 1
Weight of the animals involved in the study at the various experimental periods designed for this study, when exposed to a different physical consistency of the diet: hard, soft and liquid diets.

Age	28 days		40 days		60 days	
Diet	Mean	SEM	Mean	SEM	Mean	SEM
Hard	33.1	1.8	37.2	1.4	114.2	3.2
Soft	32.9	1.6	36.5	1.5	116.6	4.2
Liquid	32.5	1.7	35.5	1.0	114.7	2.1

Table 2
Mean and SEM for the various groups of animals included in the study. The Bone Mineral Density (BMD) and the Total Mineral Density (TMD) were significantly lower at 7 and 20 days of the experimental period in those animals exposed to soft and liquid diet when comparing with those receiving a hard diet. The Bone Mineral Content (BMC) and the Total Mineral Content (TMC) were also significantly lower in the animals exposed to soft and liquid diets, but only at day 20 of the experimental period.

	Days	7			20			40		
	Diet	Mean	SEM	Sig	Mean	SEM	Sig	Mean	SEM	Sig
BMD (mg/cc)	Hard	347.18	2.507		373.75	2.807		431.9	6.210	
	Soft	314.64	1.658	**	337.13	1.442	*	409.14	7.185	
	Liquid	303.42	4.075	**	325.86	0.942	***	420.02	2.122	
BMC (mg)	Hard	0.49	0.021		1.57	0.036		1.71	0.057	
	Soft	0.64	0.026		0.66	0.011	***	1.35	0.053	
	Liquid	0.63	0.032		0.79	0.021	*	1.29	0.038	
TMD (mg/cc)	Hard	445.51	2.990		509.89	4.041		585.05	5.260	
	Soft	406.51	1.405	**	439.35	2.567	**	567.13	6.943	
	Liquid	393.23	4.507	**	441.58	2.575	**	566.21	2.556	
TMC (mg)	Hard	0.37	0.015		1.18	0.031		1.35	0.046	
	Soft	0.52	0.020		0.54	0.009	***	1.05	0.043	
	Liquid	0.51	0.025		0.59	0.014	*	1.02	0.030	

* $p < 0.05$.
** $p < 0.01$.
*** $p < 0.001$.

soft diet groups, as well as between the hard and liquid diet groups, within all three age groups. The mean values from those results are represented in Table 2.

The tissues from those animals sacrificed at day seven during the experimental period showed a significant reduction of the BMD and TMD when comparing the hard vs. liquid diets. ($p < 0.01$) ANOVA analysis reported a significant decrease of the values for the softer diets on day 20th of the experimental period, when contrasting the hard against the soft and, the hard against the liquid diet groups for BMD ($p < 0.05$ and $p < 0.01$ respectively), BMC ($p < 0.001$ and $p < 0.05$ respectively), TMD ($p < 0.01$ for both), and TMC ($p < 0.001$ and $p < 0.05$ respectively).

After 40 days of being fed with the respective diets, values seem to level off, but a significant difference in the TMC persisted in the hard vs. liquid diet groups. All other values remained lower in both the soft and the liquid diet groups when comparing with those values recorded for the hard diet group, even though they did not reach significance.

4. Discussion

The results from this study showed that the physical consistency of the diet did not interfere with the normal growth and development of the mandible in the studied animals. Also, their weights did not report any statistically significant difference when comparing the various groups at the various experimental periods designed for this study.

Each diet group requires a different amount of force delivered by the muscles in order to destroy and make the food consumable. So, the different muscular activities associated with the physical consistency of the diet load the bones differently (Tsai et al., 2010). In that context, the hard diet group requires the most muscle force, so producing the highest loading on the bony structures, while the liquid diet the least.

Considering that a reduction in the force exerted during mastication results in reduced bone density (Mavropoulos et al., 2005), and that the muscle contraction exerts a force on the periosteum stimulating, bone deposition (Frost, 2004), it is valid to argue that the current results demonstrated that there is a reduced functional force delivered during the muscular contraction when the diet is softened, which causes a lower functional strain on the mandibular condyle, diminishing the quality of the bone on the mandibular condyle. Although it was not the aim of this study, these results may suggest that a decreased bone quality can result in an induced morphological change at the mandible (Kiliaridis, Thilander, Kjellberg, Topouzelis, & Zafiriadis, 1999).

The bone density at the mandibular condyle showed the greatest variation during the rapidly growing phase in the rats (7 and 20 days of the experimental periods), with the soft and liquid diet groups having significantly lower values in the BMD, BMC, TMD, and TMC comparing with the hard diet group. At the age of maturity of the animals, the soft and liquid diet groups still had a tendency to have lower values for all the measurements, even though they did not reach significance. In that context, these results confirmed that softening the diet significantly affects the bone quality, more significantly during the growing period, then staying in a reduced condition after the adulthood is reached in the animals.

These findings are similar to those reported by other investigators. Tanaka and coworkers found that trabecular bone of the mandibular condyle in the hard diet group expresses a higher degree of mineralization when comparing with a soft diet group (Tanaka et al., 2007). Similar to the current results, another study reported that animals fed with a soft diet have a significantly lower BMD when comparing with a hard diet fed group (Mavropoulos et al., 2005).

Based on the results from other studies on bone remodelling (Hernandez, Majeska, & Schaffler, 2004; Sugiyama et al., 2012), we should have expected to see that bone from the hard diet fed animals should have a high remodelling rate, which should result in a less mineralized bone, as it would be immature and have no time to fully mineralize. However, these results suggest that the soft and liquid diets fed groups with a decreased mechanical loading during mastication may have a rapid remodelling rate because of the decreased stimulus, and therefore, presented with the less mineralized bone. Therefore, the current results along with those reported by Tanaka et al. and Mavropoulos et al., (Mavropoulos et al., 2005; Mavropoulos et al., 2010; Tanaka et al., 2007) support the idea that, mechanical loading associated with the force delivered by the muscles during mastication affect the remodelling rate at the mandibular condylar cartilage, so affecting the endochondral ossification and the deposited bone density. Further studies involving the molecular biology of the mandibular condylar cartilage cells are required to elucidate how it happens.

Another report found opposite results. Grunheid and coworkers found that there were no significant changes in the degree of mineralized tissue between the animals fed with different diet consistencies (Grünheid, Langenbach, Brugman, Everts, & Zentner, 2011). One reason for the difference in findings between that and this study is that he analyzed the anterior portion of the mandibular condyle. Different forces may be applied to these different areas of the condyle therefore having different outcomes. As stated earlier, this study looked at the postero-superior area of the mandibular condyle, where endochondral ossification and bone deposition occurs more actively in the mandibular condyle (Fuentes et al., 2003a, 2003b; Ramirez-Yanez, Daley, Symons, & Young, 2004; Ramirez-Yanez, Smid, Young, & Waters, 2005).

A limitation in this study is the numbers of animals per group. Further research should include higher numbers, as well as other species of animals to fully elucidate this issue. Also, these results are limited when applied to humans. Although it may infer what may occur in humans as bone biology is similar in both species, there are different forces and movements in humans as compared with rats. Rats have no lateral movements with their mandibles,

which may affect differently, regarding the quality of the bone, at the mandibular condyle when the physical consistency of the diet is modified in humans.

5. Conclusions

The results from this study demonstrated that rats fed with a soft diet significantly decrease the bone mineral density and content in the postero-superior area of the mandibular condyle. Such an effect is highly observed during the growing periods of the mandible. Therefore, this study supports the idea that softening the diet negatively impact on the bone quality of the mandible.

Conflict of interest

Authors declare that there is no conflict of interest.

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