

Effects of a Liquid Diet on the Temporomandibular Joint of Growing Rats

Tsuyoshi Kato^{a, b} Shigeru Takahashi^a Takanori Domon^a

Division of Oral Functional Science, Departments of ^aOral Functional Anatomy and ^bOral Rehabilitation, Hokkaido University Graduate School of Dental Medicine, Sapporo, Japan

Key Words

Liquid diet · Temporomandibular joint · Growth · Histomorphometry · BrdU

Abstract

Objective: The aim of the present study was to clarify the effects of a liquid diet on the temporomandibular joint (TMJ) in growing rats. **Materials and Methods:** Twenty-four male Wistar rats were weaned at 21 days and divided into control and experimental groups (12 in each group). Control rats were fed a solid diet and experimental rats were fed a liquid diet from 1 to 8 weeks. After injection with 5-bromo-2'-deoxyuridine (BrdU), the animals were perfused and the heads were removed. Serial coronal sections of the TMJ were stained with hematoxylin and eosin, or BrdU immunohistochemistry was done (12 rats in each group). Three dimensions and the thicknesses of the cartilage layers of the TMJ were measured, and cell proliferation in the TMJ was examined. **Results:** After 4 weeks, the height and width of the mandibular fossa and the width and length of the mandibular condyle were smaller in the experimental groups than in the control groups. The cartilage layer in these areas was also thinner at 4 weeks. The BrdU levels in the intermediate zone of the mandibular fossa (at 4 weeks) and the mandibular condyle (at 1 and 4 weeks) were lower in the experimental groups than in the controls. **Conclusion:** These findings sug-

gest that the growth of the mandibular fossa and mandibular condyle of rats was inhibited by the low proliferative activity of intermediate zone cells induced by liquid feeding.

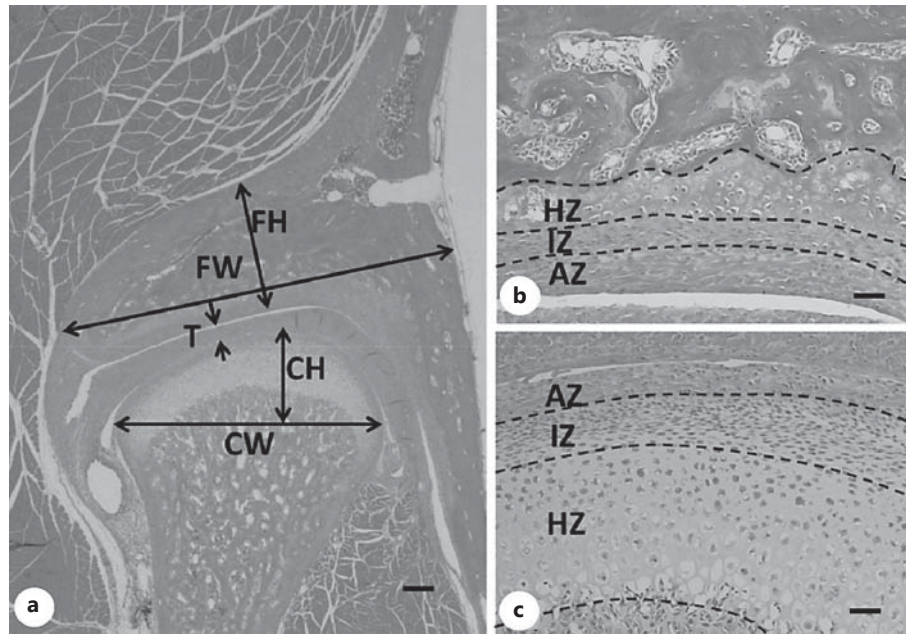
© 2015 S. Karger AG, Basel

Introduction

Experimental evidence has confirmed that a liquid or powdered diet has an unfavorable effect on the maxillofacial growth [1–6]. In mice or rats, the mandible and maxilla tend to be smaller than average [1, 2], with low mineral apposition in the mandibular ramus [3]. The masticatory muscles of experimental animals fed a soft diet are lighter [4], and histological observation reveals changes in the composition of the muscle fiber types [4–6] and a decrease in the diameters of the type I fibers [5].

As the temporomandibular joint (TMJ) plays a significant role in oral function, it is important to establish how the growth of the TMJ, and especially the mandibular condyle, is affected by a liquid diet. In earlier reports using macroscopic measurements, the mandibular condyles of growing rats fed a liquid diet were found to be smaller than those fed a solid diet [7–9], and this has been recently confirmed by Chen et al. [10] using micro-computed tomography; however, in their study the experimental animals were mice. While some researchers reported that the car-

Fig. 1. Histomorphometric measurements of the right TMJ. **a** Three-dimensional measurements of the TMJ. FW = Width of the mandibular fossa; FH = height of the mandibular fossa; CW = width of the mandibular condyle; CH = height of the mandibular condyle; T = thickness of the articular disk. Bar: 250 μ m. **b, c** Cartilage layers of the mandibular fossa (**b**) and the mandibular condyle (**c**). Bar: 50 μ m.



tilage layers of the mandibular condyle of liquid-diet animals were thinner than those of solid-diet animals [3, 7, 9, 10], others observed that they were thicker [8, 11]. Pirtti-niemi et al. [12] and Chen et al. [10] observed that the proliferative activity of cells in the intermediate zone in the mandibular condyle was low in liquid- or powdered-diet animals; however, Sato et al.'s [13] findings contradicted this observation. These conflicting findings indicate that the influence of a liquid or powdered diet on the growth of the mandibular condyle remains a controversial topic. In addition, the effects of a liquid diet on the growth of the mandibular fossa and articular disk, both important components of the TMJ, have not been investigated.

The aim of the present study was to clarify how the growth of the TMJ is affected by a liquid diet by examining the mandibular fossa, mandibular condyle, and articular disk of growing rats fed a liquid diet using histomorphometric analysis and immunohistochemistry with 5-bromo-2'-deoxyuridine (BrdU) as a marker of cell proliferation.

Materials and Methods

Animal Experiment

Twenty-four male Wistar rats were weaned at 21 days and divided into control and experimental groups (12 in each group). Control rats were fed a solid diet consisting of protein, fat, dietary fiber, and minerals. Experimental rats were fed a liquid diet (in a bowl) made by mixing one part of a powdered form of the solid

diet with two parts of water. Drinking water was available ad libitum in both groups. During the experimental period, the rats were weighed daily. Animals of both groups were euthanized after 1, 4, or 8 weeks, four control rats and four experimental rats at each time point. All rats were intraperitoneally injected with BrdU (2.5 mg/100 g body weight) and perfused with 4% paraformaldehyde under general anesthesia with pentobarbital 1 h after BrdU injection. Whole heads including the TMJ were removed, decalcified in 10% EDTA (pH 7.4) and embedded in paraffin. The frontal paraffin sections of the TMJ were cut serially at a thickness of 4 μ m.

The experimental protocol was approved by the Laboratory Animal Committee of Hokkaido University and complied with the Guidelines for the Care and Use of Laboratory Animals of Hokkaido University.

Histomorphometrical Analysis

Serial sections were stained with hematoxylin and eosin and used for histomorphometrical analysis of the TMJ. The following measurements of the right TMJ were taken under low magnification (fig. 1a): width of the mandibular fossa (FW: length from the most lateral point of the mandibular fossa to the medial point of the temporal bone); height of the mandibular fossa (FH: thickness of the mandibular fossa at the middle point of the line FW); width of the mandibular condyle (CW: maximal mediolateral length of the mandibular condyle); height of the mandibular condyle (CH: length from the top of the mandibular condyle to the line CW); thickness of the articular disk (T: thickness at the thinnest articular disk). The length of the mandibular condyle was calculated by multiplying 4 μ m by the number of sections of the mandibular condyle. Under medium magnification, the thickness of the cartilage layers of the mandibular fossa (fig. 1b) and the mandibular condyle (fig. 1c) were measured. The cartilage layers were classified into three zones: the articular zone (AZ), the intermediate zone (IZ), and the hypertrophic zone (HZ) according to Blackwood's classification [14].

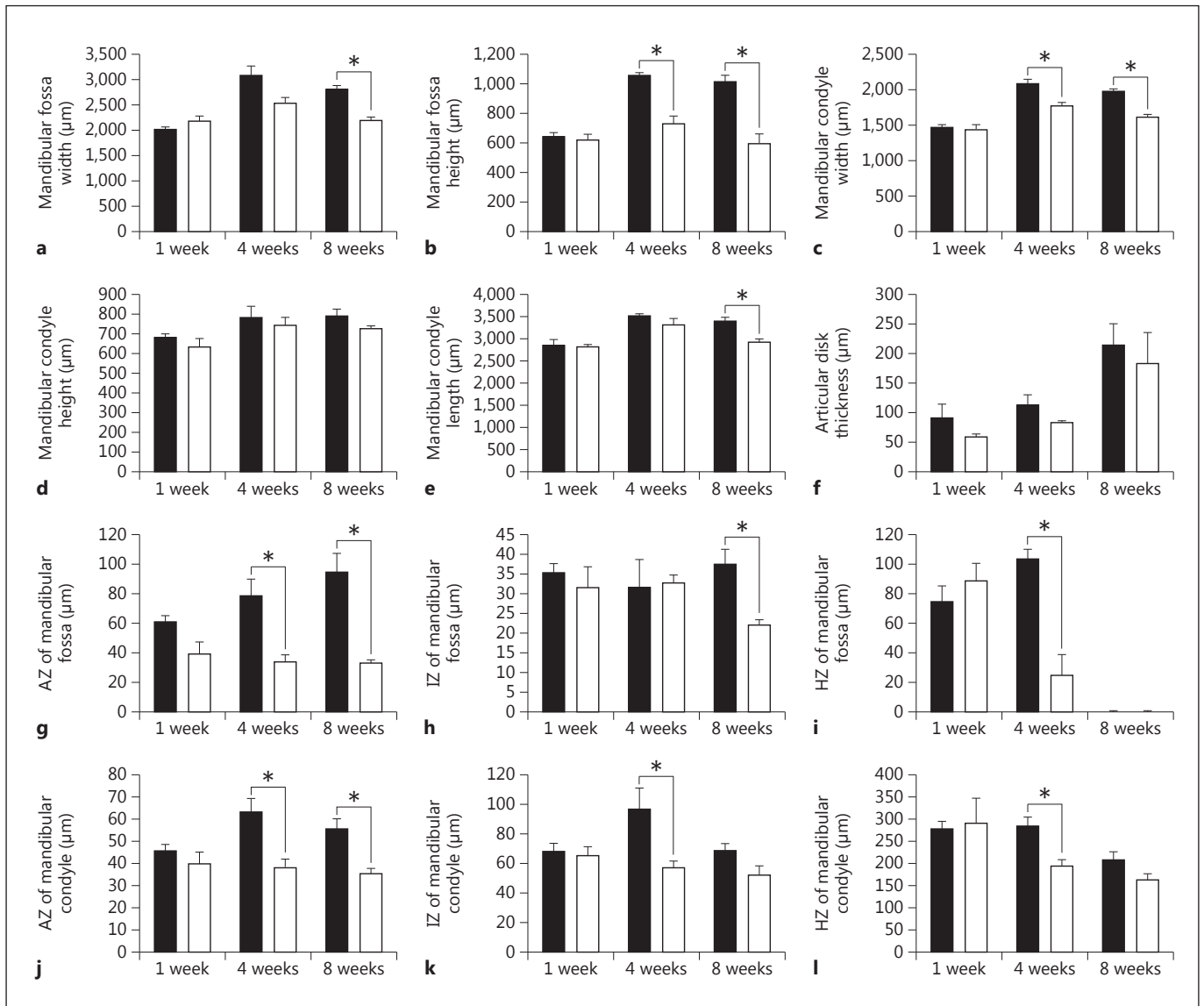


Fig. 2. a–l Histomorphometry of the TMJ. Black bars = Control groups; white bars = experimental groups (significant difference at * $p < 0.05$).

BrdU Immunohistochemistry

Three sections were chosen from the anterior, central, and posterior regions of the TMJ for immunohistochemical examination. Deparaffinized sections were immersed in 0.4% hydrogen peroxide/methanol for endogenous peroxidase blocking. The sections were then incubated with 0.1% trypsin for 20 min at 37°C and with 3 N HCl for 10 min as pretreatment. The pretreated sections were reacted with an anti-BrdU mouse monoclonal antibody (Bu20a; DakoCytomation, Copenhagen, Denmark) for 120 min, an anti-mouse-rabbit polyclonal antibody (DakoCytomation) for 60 min, and streptavidin-biotin horseradish peroxidase complex (DakoCytomation) for 30 min in turn. The immunoreaction was visualized using 3,3'-diaminobenzidine and the sections were lightly stained with hematoxylin. Normal mouse serum

was substituted for the primary antibody in the negative control sections.

The BrdU-positive cells were counted in the IZ of the mandibular fossa, mandibular condyle, and articular disk between both ends of each structure in three immunostained sections from each animal at a magnification of $\times 200$ under microscope. The labeling index was calculated for four control animals and four experimental animals at each of the three time points.

Statistical Analysis

The data for body weight, histomorphometry, and BrdU labeling index were statistically analyzed with the Mann-Whitney U test for comparisons between control and experimental groups. p values < 0.05 were considered statistically significant.

Results

General Condition of Rats

The body weights of all rats increased throughout the experimental period, and all of them remained in good condition with no symptoms of diarrhea. There was no significant difference in body weight between the solid-diet (control) groups and the liquid-diet (experimental) groups during the entire experimental period (not shown).

Histomorphometry

Histomorphometry revealed that the mandibular fossa and the mandibular condyle in the experimental groups were smaller than in the control groups. There were significant differences in the width at 8 weeks and in the height at 4 and 8 weeks of the mandibular fossa, and in the width at 4 and 8 weeks and in the length at 8 weeks of the mandibular condyle (fig. 2a–e). No significant difference was identified in the thickness of the articular disk between the two groups at any time point (fig. 2f).

Although the cartilage layers of the mandibular fossa and the mandibular condyle in the liquid-diet rats were histologically similar to those in the solid-diet rats at 1 week, several cartilage layers in the liquid-diet rats were thinner than in the solid-diet rats at 4 and 8 weeks (fig. 3a–d). Significant differences between the two groups were observed in the AZ at 4 and 8 weeks, in the IZ at 8 weeks, and in the HZ at 4 weeks in the mandibular fossa, and in the AZ at 4 and 8 weeks, in the IZ and in the HZ at 4 weeks in the mandibular condyle (fig. 2g–l).

BrdU Immunohistochemistry

The BrdU-labeled cells were observed mainly in the IZ of the mandibular fossa and the mandibular condyle, and sparsely in the AZ and HZ (fig. 3e–h). There were more BrdU-positive cells in the control groups than in the experimental groups (fig. 3e–h). In the negative control sections, no immune-positive cells were observed. Statistical analysis showed that the labeling index of BrdU of IZ in the mandibular fossa in the experimental groups was significantly different ($p = 0.025$) from that in the control groups at 4 weeks (fig. 4a). In the mandibular condyle, there were significant differences ($p = 0.025$) between the experimental and control groups at 1 and 4 weeks (fig. 4b). In the articular disk, the labeling indices of BrdU were low and no significant differences were identified between the experimental and control groups at any time point (fig. 4c).

Discussion

In these studies, the body weights of rats increased throughout the experimental period (not shown), but the size of the TMJ did not increase in the last 4 weeks similar to the report of Kiliaridis et al. [15]. The authors examined the craniofacial morphology of growing rats, thereby indicating that the growth of the TMJ was accomplished earlier than the growth of the whole body.

In this study, cell proliferation decreased in the IZ of both the mandibular fossa and the mandibular condyle of liquid-diet rats at 4 weeks. Mechanical stress has been shown to increase the proliferative activity of cells in the IZ in vitro [16–18]; therefore, we hypothesize that no or extremely low masticatory stimulus provided by a liquid diet caused the decrease in cell proliferation in the IZ in this study. Our data are consistent with those of Pirttiniemi et al. [12], who observed the TMJ of rats fed a powdered diet for 48 h only after weaning, but not with those of Sato et al. [13], who used a powdered diet for 4 weeks after weaning. The difference between the study of Sato et al. and our study could be the different counting methods used. In our study, the number of immune-positive cells and total number of cells were counted to calculate the labeling indices, while Sato et al. counted only immune-positive cells. As another possibility, the difference of biomarkers used in two studies could not be completely ruled out because the phase of the cell cycle identified by BrdU is different from proliferating cell nuclear antigen used by Muskhelishvili et al. [19].

In this study, the AZ, IZ, and HZ of both the mandibular fossa and the mandibular condyle of liquid-diet rats were thinner than those of the solid-diet rats at 4 and 8 weeks. The AZ consists of fibrous connective tissue covering the cartilage surface of the TMJ, and is considered to have a protective function [14, 20]. This protective function would not be necessary because of the weak masticatory stress caused by long-term feeding with a liquid diet in this study. The cells in the IZ proliferate mitotically and then differentiate into chondroblasts and chondrocytes in turn [14], and the growth of the IZ and HZ depends on the proliferative activity of cells in the IZ [14]. Thus, the thin IZ and HZ in the liquid-diet rats in this study could be explained by the decrease in cell proliferation in the IZ. This supposition is consistent with the finding that the intermediate and hypertrophic zones became thinner following the decrease in cell proliferation in the intermediate zone.

The present study confirmed previous reports that a liquid diet is linked with a smaller mandibular condyle not only in rats or mice [7–10, 21, 22] but also in rabbits

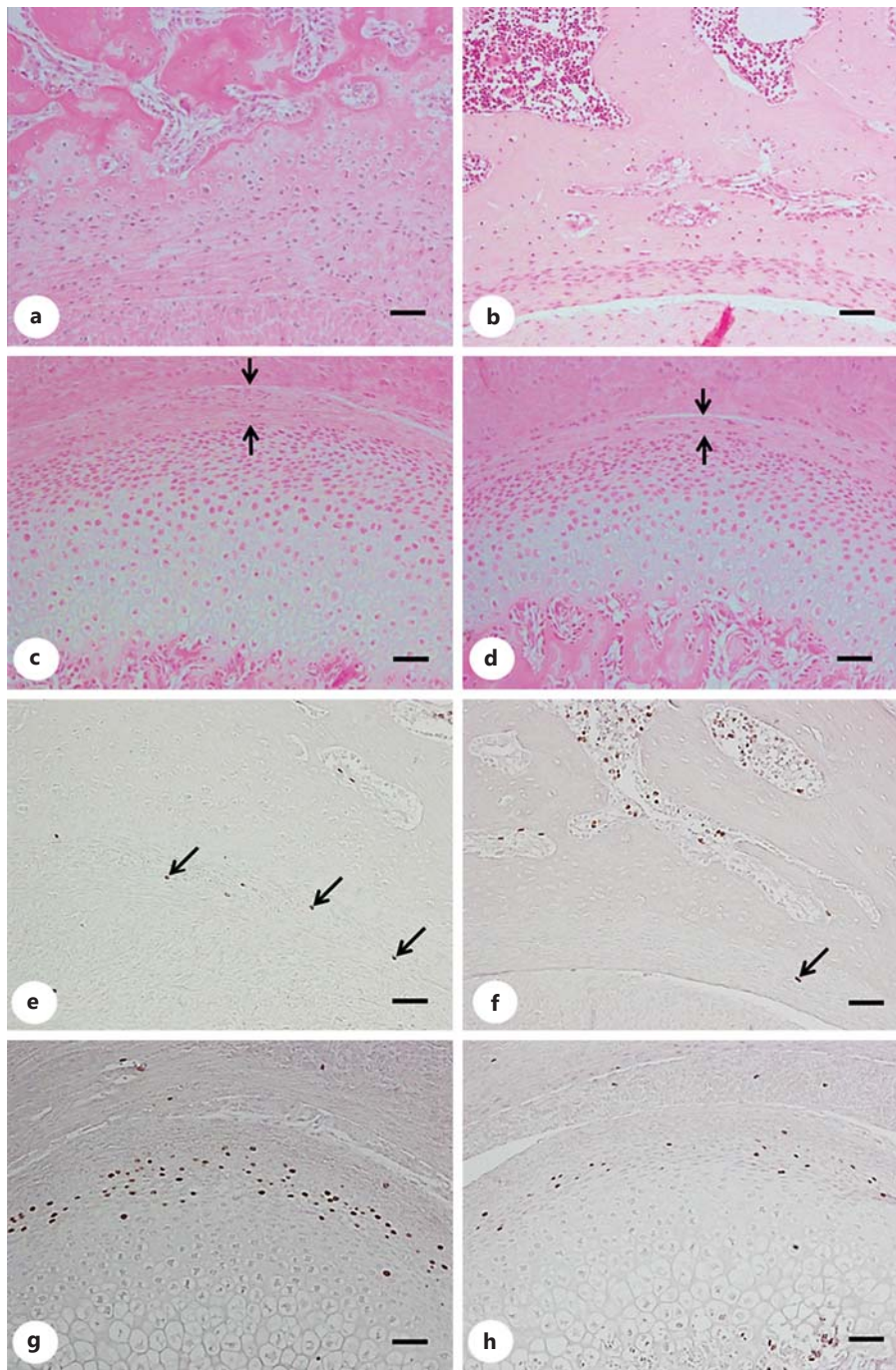
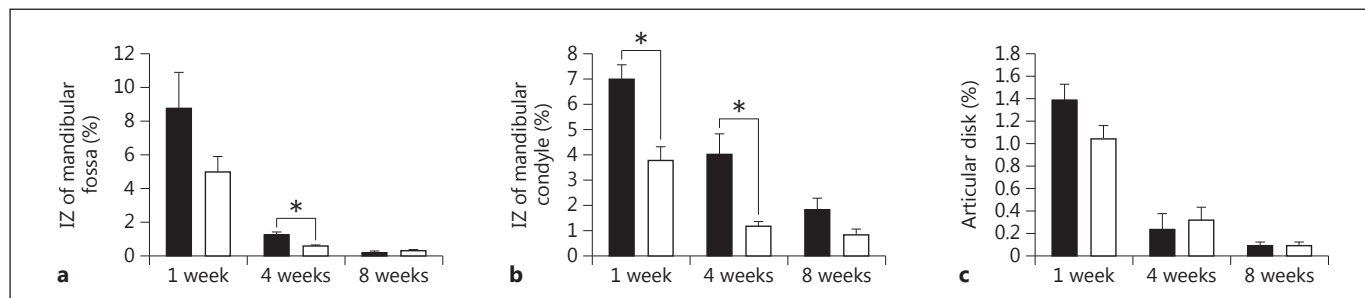


Fig. 3. Histology (a–d) and BrdU immunohistochemistry (e–h). **a** Mandibular fossa, control groups at 4 weeks. **b** Mandibular fossa, experimental groups at 4 weeks. **c** Mandibular condyle, control groups at 8 weeks. **d** Mandibular condyle, experimental groups at 8 weeks. HZ of the mandibular fossa is observed clearly in the control sample (a), but is barely visible in the experimental sample (b) at 4 weeks. AZ (arrows), IZ, and HZ of the mandibular condyle in the control sample (c) are thicker than in the experimental sample (d) at 8 weeks. **e** Mandibular fossa, control groups at 4 weeks. **f** Mandibular fossa, experimental groups at 4 weeks. **g** Mandibular condyle, control groups at 1 week. **h** Mandibular condyle, experimental groups at 1 week. More BrdU-labeled cells (arrows) are identified in the control (e, g) than in the experimental sample (f, h). Bars = 50 μm.

Fig. 4. BrdU-labeling indices. **a** IZ of the mandibular fossa. **b** IZ of the mandibular condyle. **c** Articular disk. Black bars = Control groups; white bars = experimental groups (significant difference at * $p < 0.05$).



[23]. The HZ in the mandibular fossa and the mandibular condyle is known to be replaced by bone [14]. This suggests that the inhibition of cartilage formation results in a small TMJ in liquid-diet rats. Taking these findings and the immunohistochemical findings of the present study into account, we suggest that liquid-diet feeding inhibits cell proliferation in the IZ, which in turn leads to inhibited cartilage growth and underdevelopment of the TMJ. However, this finding may not be applicable to humans because rats and mice have specialized TMJ and masticatory mechanics [24].

There was no difference in the thickness or cell proliferation of the articular disk between liquid-diet rats and solid-diet rats in this study, demonstrating that liquid-diet feeding does not affect the growth of the articular

disk. However, Magara et al. [25] reported that in the TMJ of rats wearing an appliance exerting continuous compressive force on the TMJ, collagen fibers decrease in number and then recover after removal of the appliance. This suggests that the articular disk could be affected by strong masticatory loads, but not by weak loads.

Conclusion

The present study showed that growth of the mandibular fossa and mandibular condyle of rats was inhibited by the reduced proliferative activity of cells in the IZ induced by liquid-diet feeding, but no effects were observed in the articular disk.

References

- 1 Watt DG, Williams CHM: The effects of the physical consistency of food on the growth and development of the mandible and the maxilla of the rat. *Am J Orthod* 1951;37:895–928.
- 2 Ito G, Mitani S, Kim J: Effect of soft diets on craniofacial growth in mice. *Anat Anz* 1988; 165:151–166.
- 3 Yamada K, Kimmel D: The effect of dietary consistency on bone mass and turnover in the growing rat mandible. *Arch Oral Biol* 1991; 36:129–138.
- 4 Mische B, Fanghänel J, Kubein-Meesenburg D, et al: Masticatory musculature under altered occlusal relationships – a model study with experimental animals. *Ann Anat* 1999; 181:37–40.
- 5 Kiliaridis S, Engstrom C, Thilander B: Histochemical analysis of masticatory muscle in the growing rat after prolonged alteration in the consistency of the diet. *Arch Oral Bio* 1988; 33:187–193.
- 6 Kitagawa Y, Mitera K, Ogasawara T, et al: Alterations in enzyme histochemical characteristics of the masseter muscle caused by long-term soft diet in growing rabbits. *Oral Dis* 2004;10:271–276.
- 7 Bouvier M, Hylander W: The effect of dietary consistency on gross and histologic morphology in the craniofacial region of young rats. *Am J Anat* 1984;170:117–126.
- 8 Kiliaridis S, Thilander B, Kjellberg H, et al: Effect of low masticatory function on condylar growth: a morphometric study in the rat. *Am J Orthod Dentofacial Orthop* 1999;116:121–125.
- 9 Vaid L, Pradhan P, Chakrabarti S: Effect of dietary consistency on the growth of the condylar cartilage of the mandible in rats. *J Anat Soc India* 2002;51:229–231.
- 10 Chen J, Sobue T, Utreja A, et al: Sex differences in chondrocyte maturation in the mandibular condyle from a decreased occlusal loading model. *Calcif Tissue Int* 2011;89:123–129.
- 11 Kantomaa T, Tuominen M, Pirttiniemi P: Effect of mechanical forces on chondrocyte maturation and differentiation in the mandibular condyle of the rat. *J Dent Res* 1994;73: 1150–1156.
- 12 Pirttiniemi P, Kantomaa T, Sorsa T: Effect of decreased loading on the metabolic activity of the mandibular condylar cartilage in the rat. *Eur J Orthod* 2004;26:1–5.
- 13 Sato I, Uneno R, Miwa Y, et al: Distribution of tenascin-C and tenascin-X, apoptotic and proliferating cells in postnatal soft-diet rat temporomandibular joint (TMJ). *Ann Anat* 2006;188:127–136.
- 14 Blackwood H: Growth of the mandibular condyle of the rat studied with tritiated thymidine. *Arch Oral Biol* 1966;11:493–496.
- 15 Kiliaridis S, Engstrom C, Thilander B: The relationship between masticatory function and craniofacial morphology. I. A cephalometric longitudinal analysis in the growing rat fed a soft diet. *Eur J Orthod* 1985;7:273–283.
- 16 Horizono H, Owan I, Kudoh H, et al: Mechanical stress regulates chondrocyte proliferation and differentiation during endochondral bone formation. *Ryukyu Med J* 2007;26: 57–67.
- 17 Wang P-Y: Dynamic compression modulates chondrocyte proliferation and matrix biosynthesis in chitosan/gelatin scaffolds. *J Biomed Mater Res B Appl Biomater* 2009;91B:143–152.
- 18 Ryan J, Eisner E, DuRaine G, et al: Mechanical compression of articular cartilage induces chondrocyte proliferation and inhibits proteoglycan synthesis by activation of the ERK pathway: implications for tissue engineering and regenerative medicine. *J Tissue Eng Regen Med* 2009;3:107–116.
- 19 Muskelishvili L, Latendresse JR, Kodell RL, et al: Evaluation of cell proliferation in rat tissues with BrdU, PCNA, Ki-67(MIB-5) immunohistochemistry and in situ hybridization for histone mRNA. *J Histochem Cytochem* 2003;51:1681–1688.
- 20 Blackwood H: Vascularization of the condylar cartilage of the human mandible. *J Anat* 1965;99:551.
- 21 Chen J, Sorensen K, Gupta T, et al: Altered functional loading causes differential effects in the subchondral bone and condylar cartilage in the temporomandibular joint from young mice. *Osteoarthritis Cartilage* 2009;17: 354–361.
- 22 Enomoto A, Watahiki J, Yamaguchi T, et al: Effects of mastication on mandibular growth evaluated by microcomputed tomography. *Eur J Orthod* 2010;32:66–70.
- 23 Ravosa MJ, Kunwar R, Stock SR, et al: Pushing the limit: masticatory stress and adaptive plasticity in mammalian craniomandibular joints. *J Exp Biol* 2007;210:628–641.
- 24 Herring SW: TMJ anatomy and animal models. *J Musculoskelet Neuronal Interact* 2003;3: 391–394, discussion 406–407.
- 25 Magara J, Nozawa-Inoue K, Suzuki A, et al: Alterations in intermediate filaments expression in disc cells from the rat temporomandibular joint following exposure to continuous compressive force. *J Anat* 2012;220:612–621.