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Research Article

Changes in collagens and chondrocytes in the temporomandibular joint cartilage in growing rats fed a liquid diet

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ABSTRACT

The temporomandibular joint (TMJ) of growing rats fed a soft diet is reported to be smaller in size and to have thinner condyle and glenoid fossa cartilage than rats fed a solid diet. The aim of this study was to determine the effect of a soft diet on the collagens and chondrocytes in the growing TMJ cartilage. Forty-eight male Wistar rats were divided into a control group fed a solid diet and an experimental group fed a liquid diet for 1–8 weeks. After the experimental period, the TMJs were harvested and examined histologically, immunohistochemically for collagen types I, II, and X, and with transmission electron microscopy. The condylar cartilage in the experimental rats showed weak immunoreactions for three types of collagens compared with the controls. The ultrastructure had fewer fine collagen fibrils in the experimental rats compared with that of the controls. The glenoid fossa cartilage in the experimental rats showed narrower Alcian blue-positive areas than the control staining. The immunoreactions for three types of collagen in the experimental rats were also weaker than those of the controls. The chondrocytes in the experimental rats appeared dark, had extended thin cytoplasmic processes, and had formed gap junctions, as assessed by transmission electron microscopy. Fewer fine collagen fibrils, but thick bands of collagen fibrils were observed in the glenoid fossa of the experimental cartilage. The results of the present study showed that a liquid diet had deleterious effects on the quality and quantity of collagens and chondrocytes in the TMJ cartilage in growing rats.

1. Introduction

The relationship between mastication and the growth of the temporomandibular joint (TMJ) has proved an interesting area in the field of dental science (Abed et al., 2007; Bouvier and Hylander, 1984; Enomoto et al., 2010; He and Kiliaridis, 2003; Ishida et al., 2009; Lindsten et al., 2004; Sato et al., 2006) because modern childhood diets tend to be soft, rather than hard. The experimental studies listed above examined the TMJ of growing animals fed a soft diet consisting of a powder or a liquid. In the condyle of growing animals fed a soft diet, the size is smaller and the thickness of cartilage layer is thinner than that of animals fed a hard diet (Bouvier, 1988; Bouvier and Hylander, 1984; Chen et al., 2009; Enomoto et al., 2010; Hinton and Carlson, 1986; Kato et al., 2015; Kiliaridis et al., 1999; Pirttiniemi et al., 1996). A reduction in the number of chondrocytes and their proliferation in the condyle have also been reported (Kato et al., 2015; Pirttiniemi et al., 1996, 2004). Kato et al. (2015) and Tuominen et al. (1996) observed similar phenomena in the glenoid fossa, which is the other bone of the TMJ.

The different types of collagen secreted by chondrocytes are important components of the cartilage extracellular matrix in the TMJ. The inhibition of cartilage growth in animals on a soft diet suggests that a soft diet affects the chondrocytes and their collagen secretion. In fact, a reduced immunoreaction for type II collagen in condylar cartilage (Chen et al., 2009; Pirttiniemi et al., 1996) and in glenoid fossa cartilage (Tuominen et al., 1996) of animals fed a soft diet has already been reported, but the effects of a soft diet on the other types of collagens in the TMJ cartilage of growing animals remain unclear. In addition, no studies have reported the effects of a soft diet on the chondrocytes themselves.

The aim of this study was to determine the effects of a soft diet on the collagens and chondrocytes in the growing TMJ cartilage. For this purpose, we examined the condylar and glenoid fossa cartilage

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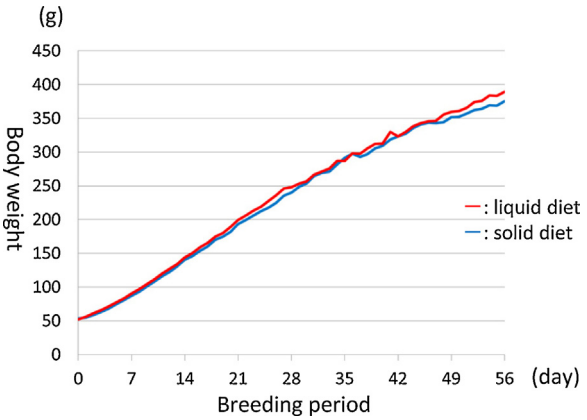

Fig. 1. There were no significant differences in body weights between the control and experimental rats throughout the experiment.

of rats fed a liquid diet by histology, immunohistochemistry with anti-types I, II, and X collagen antibodies, and transmission electron microscopy (TEM).

2. Materials and methods

2.1. Experimental animals

Forty-eight male Wistar rats were used in this study. The animal protocols used in this study were approved by the Hokkaido University Animal Care and Use Committee.

After being weaned at 21 days old, they were divided into two groups: control rats that were fed an ordinary solid diet and experimental rats that were fed a liquid diet prepared by mixing water with a powdered diet consisting of protein, fat, dietary fiber, and minerals, containing the same nutrition as the solid diet, at a ratio of 2:1 by weight. Drinking water was available to all animals ad libitum. The animals were maintained on these diets for 1, 2, 4, or 8 weeks, and each group at each time point consisted of six rats. During the experiment, all animals were weighed every day. Mann–Whitney *U*-tests were used to compare the body weights of the control and experimental rats, and *p*-values less than 0.05 were considered statistically significant.

2.2. Histology

Four rats from each group at each time point were perfused with 4% paraformaldehyde. The entire head was dissected and fixed overnight with the same fixative. After decalcification with 10% EDTA (pH 7.4) at room temperature, the specimens were embedded in paraffin and serial frontal sections were cut to a thickness of 4 μm. The sections were stained with hematoxylin–eosin, Alcian blue, or Azan stain.

2.3. Immunohistochemistry

Deparaffinized sections were treated with 0.3% H_2O_2 in methanol for 10 min at room temperature to block endogenous peroxidase activity. Next, the sections were digested with 2.5% hyaluronidase (Sigma–Aldrich, St. Louis, MO, USA) for 60 min at 37 °C and then incubated with a rabbit anti-rat type I collagen

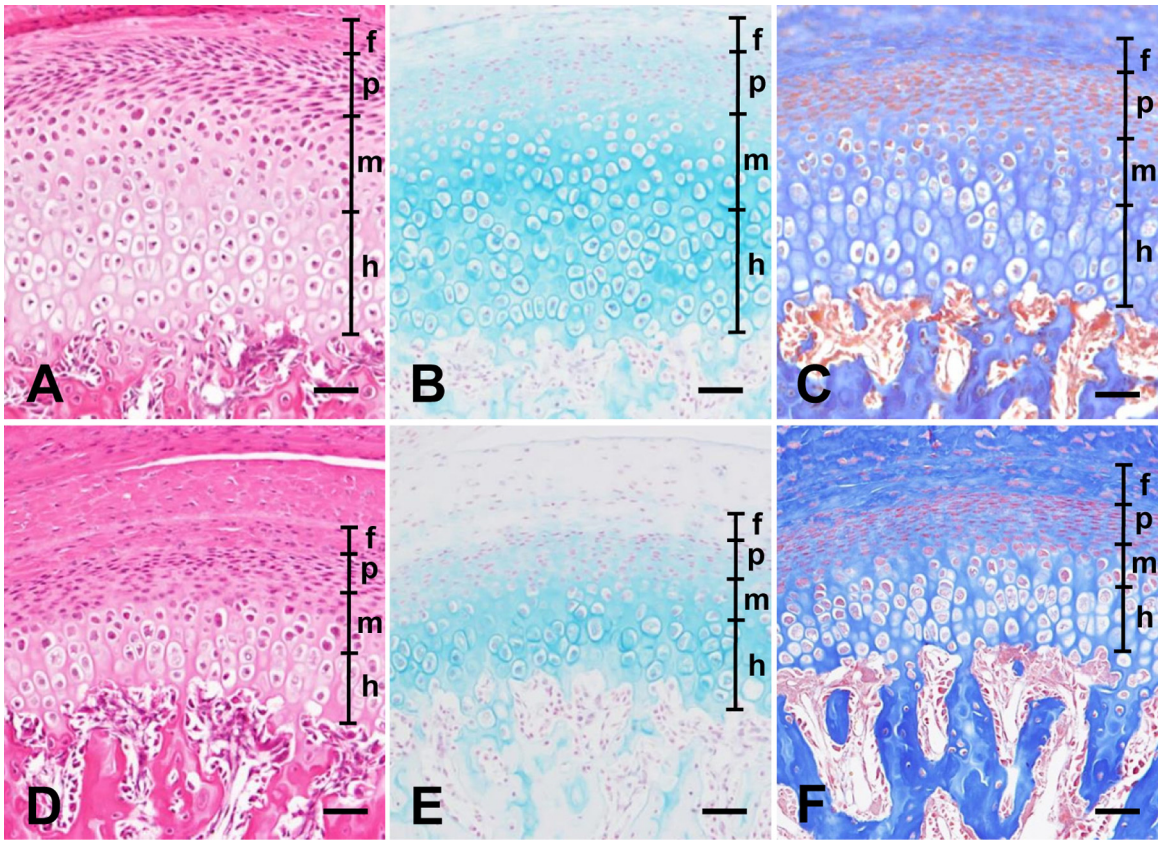

Fig. 2. Histology of the condylar cartilage in control (A–C) and experimental (D–F) groups at 8 weeks. HE (A and D), Alcian blue (B and E),and Azan (C and F) staining. The thickness of the cartilage layer in the experimental group (D) was thinner than that in the control group (A). The Alcian blue-positive staining in the maturative cell layer in the experimental group (E) was weaker than that in the control group (B), but no difference was found in Azan staining (C and F). Bars: 50 μm. f: fibrous layer; p: proliferative cell layer; m: maturative cell layer; h: hypertrophic cell layer.