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Reducing dietary loading decreases mouse temporomandibular joint degradation induced by anterior crossbite prosthesis

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Abstract

Objective—Dietary loading has been reported to have an effect on temporomandibular joint (TMJ) remodeling via periodontal-muscular reflex. We therefore examined whether reducing dietary loading decreased TMJ degradation induced by the unilateral anterior crossbite prosthesis as we recently reported.

Methods—Forty 6-week-old female C57BL/6J mice were randomly divided into two experimental and two control groups. One experimental and one control group received small-size diet and the other two groups received large-size diet. Unilateral anterior crossbite prosthesis was created in the two experimental groups. The TMJ samples were collected 3 weeks after experimental operation. Histological changes in condylar cartilage and subchondral bone were assessed by Hematoxylin & Eosin, toluidine blue, Safranin O and tartrate-resistant acid phosphatase staining. Real-time polymerase chain reaction (PCR) and/or immunohistochemistry were performed to evaluate the expression levels of Collagen II, Aggrecan, a disintegrin and metalloproteinase with thrombospondin motifs 5 (ADAMTS-5) and RANKL/RANK/OPG in TMJ condylar cartilage and/or subchondral bone.

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Author contributions

Yun-Dong Liu, Di Chen and Mei-Qing Wang contributed to the conception and design of the study. Yun-Dong Liu, Li-Fan Liao and Hong-Yun Zhang contributed to the acquisition, collection and assembly of data. Yun-Dong Liu and Li-Fan Liao contributed to the statistical analysis. Yun-Dong Liu, Lei Lu, Di Chen and Mei-Qing Wang contributed to the analysis and interpretation of the data. All authors contributed in revising the manuscript critically. All authors approved the final version to be submitted.

Conflict of interest

The authors declare no potential conflicts of interest with respect to the authorship and/or publication of this article.

Supplementary data

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Results—Thinner and degraded cartilage, reduced cartilage cellular density, decreased expression levels of Collagen II and Aggrecan, loss of subchondral bone and enhanced osteoclast activity were observed in TMJs of both experimental groups. However, the cartilage degradation phenotype was less severe and cartilage ADAMTS-5 mRNA was lower while OPG/RANKL ratio in cartilage and subchondral bone was higher in the small-size than large-size diet experimental group. No differences of histomorphology and the tested molecules were found between the two control groups.

Conclusions—The current findings suggest that a lower level of functional loading by providing small-size diet could reduce TMJ degradation induced by the biomechanical stimulation from abnormal occlusion.

Keywords

Temporomandibular joint; Dental occlusion; Dietary loading; Osteoarthritis; Cartilage; Subchondral bone

Introduction

The loading on temporomandibular joint (TMJ) originates from the contraction of jaw-closing muscles. Variations in food properties, such as volume or hardness, will initiate different levels of masticatory muscle contraction, leading to differences in levels of occlusal force and TMJ loading[1–3]. Normal loading is believed to be essential for the development and metabolic activity of TMJ condyle cartilage and subchondral bone[4,5]. Growing rats on soft diet showed thinning cartilage, enhanced cartilage catabolic activity and decreased subchondral bone volume fraction in condyles[6–8]. 3-week-old female mice with trimmed incisors and soft diet for 4 weeks showed reductions in not only the cartilage thickness, but also the subchondral bone volume fraction and trabecular thickness in TMJs[9,10].

TMJ osteoarthritis (OA) is a wide-spread problem that is characterized by chondrocyte death, cartilage matrix loss and sub-chondral bone resorption in early stage followed by abnormal reparative bone turnover[11,12]. Aberrant biomechanical stimulation from abnormal dental occlusion plays an important role in TMJ OA development[13,14]. We previously reported that people who had lost posterior-teeth distributed in more dental quadrants were more susceptible to temporomandibular disorders (TMD)[15], the severe form of which is TMJ OA. We also reported experimental posterior-teeth occlusal disorder induced TMJ OA-like lesions in rats[12,16]. Recently, we developed a unilateral anterior crossbite prosthesis in rats and mice and found more significant TMJ degradation lesions than those induced by experimental posterior-teeth occlusal disorder[17–19]. It is conceivable to test whether this TMJ degradation could be decreased with a lower level of functional stimulation by feeding the experimental mice with a small-size diet.

Several studies have found the important role of the receptor activator of NF-κB (RANK), receptor activator of NF-κB ligand (RANKL) and osteoprotegerin (OPG) in OA onset and progression. In early stage OA, the decreased OPG/RANKL ratio favors the RANKL binding to RANK in osteoclasts, leading to increased osteoclast activity and subchondral

bone resorption[20]. OPG and RANKL are also produced by cells in cartilage[21–23] and regulate both cartilage remodeling and subchondral bone turnover. Increased OPG/RANKL ratio is considered protective for the cartilage and subchondral bone[12,24–27], while decreased OPG/RANKL ratio was found in the synovia of TMD patients[28] and rat or mouse TMJ cartilage with osteoarthritic lesions[16,29].

Incisors of rodent animals function more than other teeth because when a rodent catches or cuts foods they used to move the mandible in the sagittal direction following the incisal guidance. Experimentally created unilateral anterior crossbite prosthesis will heavily interfere with this function because the totally opposite guidances will be provided by the normal incisor pair and the prosthetic crossbite incisor pair [Fig. 1(A)] while the TMJ can support only one pattern of them at the same time. A small-size diet may diminish the requirement of the cutting function of incisors, and thus may reduce the harmful biomechanical stimulation from the anterior crossbite prosthesis. In the present study, we tested this hypothesis by exposing the mice that received the unilateral anterior crossbite prosthesis to a small-size vs a large-size diet. The histomorphological analysis for TMJs was performed, and real-time polymerase chain reaction (PCR) and/or immunohistochemistry were carried out to evaluate the expression levels of Collagen II, Aggrecan, a disintegrin and metalloproteinase with thrombospondin motifs 5 (ADAMTS-5) and RANKL/RANK/OPG in condyle cartilage and/or subchondral bone. The hypothesis is that the small-size diet could decrease the degradation lesions in mouse TMJs induced by the unilateral anterior crossbite prosthesis.

Material and methods

Mice and sample preparation

Forty 6-week-old C57BL/6J female mice (weight 17–19 g), and mouse food, were provided by the Animal Center of Fourth Military Medical University. All procedures and animal care were approved by the University Ethics Committee and performed according to institutional guidelines. The mice were randomly divided into four groups (n = 10): small-size diet control and experimental groups, and large-size diet control and experimental groups. For experimental groups, metal tubes were bonded onto the mouse left maxillary and mandibular incisors to create unilateral anterior crossbite relationship. The metal tubes were made of a pinhead (Shinva Ande, Shandong, China; inner diameter = 0.61 mm, thickness = 0.3 mm). The maxillary tubes were 1.5 mm long to fit the maxillary incisors. The mandibular tubes were curved to form a 135° labially inclined occlusal plate. The tubes were carefully bonded with zinc phosphate cement (Shanghai Dental Instrument Factory, Shanghai, China) under anesthesia using intraperitoneal injection of 40 mg/kg pentobarbital and were checked every other day. No prosthesis fell off during the experimental period. For the control groups, the mice underwent the similar procedures but no metal tubes were bonded [Fig. 1(A)] showed the anterior teeth occlusal relationship 3 weeks post-operation. The mice in the small-size diet groups were fed with the food particles ground into less than 3 mm thick, and the mice in the large-size diet groups were fed with cylindrically shaped pressed food pellets, about 12.5 mm in diameter and 15–20 mm in length [Fig. 1(B)]. The body weight was recorded every other day.

The mice were sacrificed at the end of the third week after operation. Because no differences in degrading changes were found between the left side and right side TMJs in the experimental mice in our previous report[19], left side TMJ tissue blocks from six mice of each group were fixed, decalcified and embedded in paraffin. Fifteen central and para-central five-μm thick sagittal sections were prepared consecutively by a professional technician for all the embedded TMJ blocks. To exclude selection bias, sections were randomly selected for Hematoxylin & Eosin (H–E) staining, toluidine blue staining, Safranin O staining, tartrate-resistant acid phosphatase (TRAP) staining and immunohistochemical staining of Collagen II, OPG and RANKL.

For each group, the condylar cartilages and subchondral bones of the other 14 TMJs (including four left side and ten right side TMJs) were respectively separated and preserved at −80°C. Four to five condyles from three or four different mice were pooled to create a single sample of the cartilage or subchondral bone respectively for RNA extraction and three independent samples were formed without the condyles from the same mouse in different samples.

Histochemical staining and histomorphometric measurements

H–E was used to assess the condylar histochemical changes. Toluidine blue and Safranin O staining were performed to determine proteoglycan changes. Condyle cartilage thickness, condylar cartilage cellular density, the percentages of degraded cartilage areas and subchondral bone histomorphometric parameters were measured as reported previously[16,19]. The measurements were carried out from three H–E sections per joint and the averaged value was used to represent this joint for statistical analysis ($n = 6$). Briefly, the stained sections were imaged by the Leica DFC490 system (Leica, Wetzlar, Germany). As shown in [Fig. 1(C)], the four bold lines divide the condyle cartilage into anterior, central and posterior thirds and three slender lines further divide the central and posterior thirds into four smaller portions, respectively. For each H–E section, the cartilage thickness in the central or posterior third was measured as the average length of the three slender lines in the corresponding thirds[19]. The condylar cartilage cellular density and the percentages of degraded cartilage areas were calculated by the value of the total cell number or the value of the total degraded cartilage areas in condylar cartilage central and posterior thirds divided by the value of the total area of condylar cartilage central and posterior thirds, respectively. For subchondral bone histomorphometry, two squares (0.3 mm × 0.3 mm) located beneath the osteochondral interface were chosen [Fig. 1(C)] and bone volume fraction (BV/TV), trabecular thickness (Tb.Th), trabecular number (Tb.N) and trabecular separation (Tb.Sp) were measured as described previously[19].

TRAP staining was performed to examine osteoclast activity of the condylar subchondral bone following the manufacturer's instructions (Sigma 387-A, MO, USA). TRAP-positive osteoclasts were counted in five randomly selected high-power (400×) fields under a microscope (Leica DM 2500, Wetzlar, Germany) and the averaged value was used as the value for this section. The number of TRAP-positive osteoclasts was averaged from three sections of each animal to represent this joint for statistical analysis ($n = 6$).

RNA extraction and real-time PCR

Total RNA was extracted by using Trizol (Invitrogen Life Technologies, CA, USA). The primers for target genes were listed in Table I. Gene expression was analyzed with the Applied Biosystems 7500 Real-time PCR machine (Applied Biosystems, CA, USA) with glycer-aldehyde-3-phosphate dehydrogenase (GAPDH) as the internal control. The results were calculated as the relative quantification compared to the small-size diet control group, which was set at 1. Data were collected from three independent pooled samples ($n = 3$).

Immunohistochemical staining

A standard, three-step, avidin–biotin complex 3,3′-diaminobenzidine (DAB) immunohistochemical staining protocol was performed[16,17]. The primary antibodies were goat anti-Collagen II (sc-7763, 1:50, Santa Cruz Biotechnology, CA, USA), rabbit anti-OPG (sc-11383, 1:50, Santa Cruz Biotechnology, CA, USA), and goat anti-RANKL (sc-7628, 1:50, Santa Cruz Biotechnology, CA, USA). For negative control slides, the primary antibody was replaced by phosphate buffered saline (PBS). Collagen II-positive area was measured from the condylar cartilage central and posterior thirds and the percentages of Collagen II-positive areas were calculated by the value of Collagen II-positive area divided by the value of total area of condylar cartilage central and posterior thirds. The protein expression level of OPG or RANKL in condylar cartilage was represented by the percentage of OPG-positive or RANKL-positive chondrocytes from the six square frames (each 0.08 mm × 0.08 mm) [Fig. 1(C)]. The averaged value of the percentages from three sections of each joint was calculated to represent the sample for further statistical analysis ($n = 3$) as reported previously[16].

Statistical analysis

The measurement procedures for stained images were performed in a blinded fashion by two independent observers (YDL and LFL) by using Photoshop CS3.0 software (Adobe Systems Ltd, USA). The inter-observer reliability was analyzed by calculating the Intraclass Correlation Coefficient (ICC) for the measurements[30]. A two-way random model (“single measures”) based on “absolute agreement” [ICC (2, 1) or ICC (agreement)] was used. There was a high level of agreement between the two observers (Table SI, ICC> 0.9) and the average value of the two measurements from the same sample was used for further statistical analysis. Data in the figures are expressed as means and 95% confidence intervals (CIs), and “*n*” represents the number of independent observations from different mice per group[31]. Normality of data distribution was tested by Shapiro–Wilk test with 95% confidence and Levene’s test was used to assess homogeneity of variance. The percentages of degraded cartilage areas were compared by using the non-parametric Kruskal–Wallis test and Mann–Whitney *U* test. For the other data, the assumptions of parametric tests were fulfilled and the statistical significance among groups was evaluated by one-way analysis of variance (ANOVA) with *post hoc* comparison between groups by Tukey test. SPSS 16.0 (SPSS Inc, IL, USA) was used for the statistical analysis and *P*-values less than 0.05 were considered statistically significant for all statistical tests.

Results

No significant body weight difference was noticed among the four groups at sampling time [Fig. 2(A)].

Histomorphological observations and expression of Collagen II, Aggrecan and ADAMTS-5 in cartilage

The small-size and large-size diet control groups showed no histomorphological differences in the mouse TMJ condylar cartilage and subchondral bone and displayed similar expression levels of Collagen II, Aggrecan and ADAMTS-5 in cartilage [Figs. 2(B)–(C), 3 and 4]. The cartilage thickness was similar between the two control groups [central third thickness: $P = 0.985$; posterior third thickness: $P = 0.970$, [Fig. 2(B)]]. Although the proliferative and hypertrophic zones were not as distinguished as those of rats[17,18], the cells were arranged in a regular pattern. Proteoglycans, revealed by toluidine blue and Safranin O staining, were rich in the deep layer of the cartilage [Fig. 2(C)].

The fibrocartilage on the surface of the condylar cartilage was complete and not broken in all samples. While in large-size diet experimental group, pronounced proteoglycan loss was observed and the cartilage central and posterior third thickness reduced to about half of the large-size diet control group [both $P < 0.001$, [Fig. 2(B)–(C)]]. However, in small-size diet experimental group, the degradation changes in TMJ cartilage were less severe and the loss of proteoglycan was less obvious compared to large-size diet experimental group [Fig. 2(C)]. Although cartilage thickness decreased in small-size diet experimental group (central third thickness: $P = 0.002$; posterior third thickness: $P = 0.006$), the decreased cartilage thickness in the posterior third was smaller in small-size than large-size diet experimental group [$P < 0.001$, [Fig. 2(B)]]. In the experimental groups, the arrangement of chondrocytes was irregular [Fig. 2(C)]. Cell density was found to be much lower in the large-size diet experimental group than in the corresponding control group ($P < 0.001$) and thus the scattered cell-free areas resulting from cell loss were pronounced in the cartilage of the large-size diet experimental group, while the observed cell density reduction in small-size diet experimental group ($P < 0.001$ vs corresponding control) was not as obvious as that in large-size diet experimental group [$P < 0.001$, [Figs. 2(C) and 3(A)]]. The degraded cartilage areas which take the form of a homogeneous eosinophilic mass and local loss of proteoglycans were found in the two experimental groups [small-size diet: $P = 0.022$; large-size diet: $P = 0.001$, [Figs. 2(C) and 3(B)]] as we previously reported in rat TMJs[14,17,18]. However, the percentages of degraded cartilage areas in small-size diet experimental group were significantly lower than those in large-size diet experimental group [$P = 0.036$, [Figs. 2(C) and 3(B)]]. In addition, decreased Collagen II-positive areas were observed in both experimental groups (small-size diet: $P = 0.001$ and large-size diet: $P < 0.001$ vs corresponding control groups) while the decrease was more obvious in large-size than small-size diet experimental group ($P = 0.007$) [Figs. 2(C) and 3(C)]. The percentage of Collagen II-positive areas was also significantly lower in large-size diet experimental group compared with corresponding control group ($P < 0.001$). However, in small-size diet experimental group, the percentages of Collagen II-positive areas were higher than those in large-size diet experimental group ($P < 0.001$) and were similar with small-size diet control group ($P =$

0.646) [Figs. 2(C) and 3(D)]. Furthermore, in the condylar cartilage, the two experimental groups had similar decreased mRNA levels of Collagen II (small-size diet: $P = 0.036$; large-size diet: $P = 0.007$; small-size diet vs large-size diet experimental group: $P = 0.959$) and Aggrecan (small-size diet: $P = 0.003$; large-size diet: $P = 0.001$; small-size diet vs large-size diet experimental group: $P = 0.885$) while the aggrecanase ADAMTS-5 mRNA was found increased only in large-size diet experimental group ($P < 0.001$) and was unchanged in small-size diet experimental group ($P = 0.946$) [Fig. 3(E)–(G)].

In large-size diet experimental mice, subchondral bone loss and large marrow cavities were noticed and there were decreased values of BV/TV and Tb.Th and increased value of Tb.Sp compared to large-size diet control group [all $P < 0.001$, [Figs. 2(C) and 4(A)–(C)]]. However, compared to large-size diet experimental group, small-size diet experimental mice showed less subchondral bone loss, as indicated by values of BV/TV ($P = 0.001$ vs corresponding control; $P = 0.036$ vs large-size diet experimental group), Tb.Th ($P = 0.025$ vs corresponding control; $P = 0.034$ vs large-size diet experimental group) and Tb.Sp ($P = 0.004$ vs corresponding control; $P = 0.025$ vs large-size diet experimental group) [Fig. 4(A)–(C)]. The value of Tb.N showed no difference among the four groups [Fig. 4(D)].

Osteoclast activity in condyle subchondral bone

In condyle subchondral bone, TRAP-positive osteoclast counting demonstrated that osteoclast activity was similar in the two control groups but was significantly enhanced in both experimental groups (both $P < 0.001$), however, at a lower level in small-size diet than large-size diet experimental group [$P < 0.001$, [Fig. 5(A)–(B)]]. The RANK mRNA levels in subchondral bone displayed the same trend [small-size diet: $P = 0.002$; large-size diet: $P < 0.001$; small-size diet vs large-size diet experimental group: $P < 0.001$, [Fig. 5(C)]].

Expression of OPG/RANKL in condyle cartilage and subchondral bone

The mRNA expression levels of OPG and RANKL showed no differences in the two control groups in both the condyle cartilage and subchondral bone [Fig. 6(A)]. In large-size diet experimental group, expression levels of OPG decreased (cartilage: $P = 0.005$; subchondral bone: $P = 0.002$) but those of RANKL increased (cartilage: $P = 0.001$; subchondral bone: $P < 0.001$), which led to decreased levels of OPG/RANKL ratio (cartilage: $P < 0.001$; subchondral bone: $P < 0.001$) in both the condylar cartilage and subchondral bone compared to large-size diet control group [Fig. 6(A)]. In small-size diet experimental group, however, there was an increase in OPG mRNA level ($P = 0.018$), but a decrease in RANKL mRNA level ($P = 0.041$) in cartilage, resulting in an increased OPG/RANKL ratio vs small-size diet control group [$P < 0.001$, [Fig. 6(A)]]. The mRNA levels of OPG and RANKL in subchondral bone showed no differences between the small-size diet control and experimental group (OPG: $P = 0.175$; RANKL: $P = 0.735$). Compared to large-size diet experimental group, the RANKL mRNA levels were lower (cartilage: $P < 0.001$; subchondral bone: $P < 0.001$), but the OPG levels (cartilage: $P < 0.001$; subchondral bone: $P = 0.023$) and the values of OPG/RANKL ratio were higher (cartilage: $P < 0.001$; subchondral bone: $P = 0.003$) in small-size diet experimental group both in cartilage and subchondral bone [Fig. 6(A)]. Further immunohistochemical analysis revealed similar trends [Figs. 6(B) and 7].

Discussion

In this study, we tested the alleviating effects of the small-size diet on the progression of TMJ degradation induced by the unilateral anterior crossbite prosthesis. Consistent with our hypothesis the mice in the small-size diet experimental group showed mild degradation compared to the large-size diet experimental group as evaluated by histological analysis, osteoclast activity and the expression levels of Collagen II and ADAMTS-5 or RANK/OPG/RANKL. The present data indicated that a lower level of functional stimulation appeared to reduce the biomechanically induced TMJ degradation.

The observed degradation changes in both small-size and large-size diet experimental groups, although mild in small-size diet experimental group, were similar with previously reported early TMJ OA[11,12,14,16,29,32,33] and early knee OA[34]. As previously reported[17–19], the large-size diet experimental mice displayed loss of proteoglycan and Collagen II, reduced cartilage cellular density and increased degraded cartilage areas and also showed decreased cartilage thickness which was found in rabbits receiving the repetitive steady mouth opening[35]. This implies that the thinning of the present condylar cartilage is attributed either to the decreased matrix synthesis (Collagen II and Aggrecan) and enhanced activity of aggrecanase ADAMTS-5 or matrix metalloproteinases (MMPs)[17] or to the enhanced chondrocyte apoptosis[11,36] which resulted in decrease of cell density and increase of cell-free areas that were also found in the TMJ cartilage caused by experimental posterior-teeth occlusal disorder[36], joint cavity injection of iodoacetate[11], surgical discectomy[33] or genetic modification[32]. Subchondral bone resorption is also considered as one of the characteristics of early stage knee or TMJ OA[11,12,34]. Further, the present subchondral bone loss and enhanced osteoclast activity in large-size diet experimental group are in agreement with the findings of occlusion-induced or iodoacetate-induced TMJ OA[11,12,16,19], *bgn*$^{-/0}$*fmod*$^{-/-}$mouse TMJs[29] and early knee OA[34].

Biomechanical factors are critical to joint health and thus are most often referred to in OA pathogenesis[14,37,38]. Dental occlusion is most important in the modulation of biomechanical loading on TMJs[39] and dietary loading is often considered as an important functional stimulation to TMJ remodeling[4,5,9,10]. Unilateral removal of teeth led to mandibular condylar cartilage thickening and sulfated glycosaminoglycans elevation[13]. When they were on powdered diet, the mice with experimental posterior crossbite via shifting the mandible laterally using an occlusal guidance appliance showed significant reductions in condylar width and mandibular bone mineral density compared with mice with normal occlusion[40]. The hard diet is thought to initiate a higher level of masticating activity[3,5,41] and to positively stimulate condylar development[4,5]. Therefore it is reasonable to assume that the large-size diet might be associated with a higher level of loading on TMJs due to the higher level of contraction of jaw-elevator muscles in comparison to the small-size diet. The higher level of joint loading from the large-size diet should be within the physiological range for the control mice. However, when the mice were fitted with the present unilateral anterior crossbite prosthesis, extra or alternative muscular activity pattern might be evoked via periodontal feedback and the TMJs might bear extra loadings during incising and chewing. When these aberrant loadings persist, the bearing ability of TMJs would then be decreased,

so that an originally physiological simulation, like the large-size diet, became harmful to TMJs.

The previous report had shown that when the rats were given small-size pellets, there was no incision stage but only chewing stage in the masticatory sequence[42]. That means the present small-size diet lead to a lower level of incising function, decreasing the harmful stimulation from the designed abnormal occlusion. The fact that the small-size diet experimental group showed a lower level of decreasing condyle cartilage thickness, lower degraded cartilage areas, lower expression of ADAMTS-5 and less loss of the cartilage matrix and subchondral bone resorption, agrees with this assumption. It has been reported that although the soft diet plays a negative role in condyle development[5,9,10], rabbits or rats that received TMJ discectomy had less degenerative cartilage changes[43,44] and higher condylar cartilage sulfate uptake[45] when fed with soft diet. Besides catabolic changes, the chondrocytes in mechanically induced degraded articular cartilage displayed the reparative capability, including the activities of phagocytizing dead cells[46] and proliferation[26,33,36,37]. Hence, there might be a stronger repair response in TMJ cartilage in the small-size diet than the large-size diet group while the mice received the same kind of abnormal prosthesis. The degradation in small-size diet experimental mice seemed to be suppressed by the reparative activity of TMJs via altering the expression of some molecules, such as ADAMTS-5 or OPG/RANKL. In large-size diet experimental group, RANKL was found to be increased, but OPG decreased, which led to decreased OPG/RANKL ratio in both cartilage and subchondral bone. Increased chondrocyte-originating RANKL may diffuse through osteochondral interface in the mandibular condyle where there is no visible osteoplate in 6- to 9-week-aged mouse TMJ, and thus mice may experience an enhanced osteoclastic activity in subchondral bone[16,22,23,47]. However, this scenario was not found in small-size diet experimental mice. There were no significant changes in OPG/RANKL expression in the subchondral bone of small-size diet experimental mice, even though they also displayed a significant subchondral bone loss, which was less obvious than that observed in large-size diet experimental group. The chondrocyte-produced RANKL may not be the main contributor of their subchondral bone loss, because the RANKL level was decreased, and the OPG level and OPG/RANKL ratio were increased in cartilage. Other mechanisms may be involved in the promotion of osteoclastogenesis in small-size diet experimental mice, for example proinflammatory factor mediated osteoclastic activity[48], which requires further investigation. The increased OPG and OPG/RANKL ratio in small-size diet experimental condyle cartilage may support the reparative response, as intra-articular administration of OPG partially rescued the decreased cartilage thickness caused by surgically induced mouse knee OA[25].

Another group reported that at 2 or 4 weeks, there was no difference of mRNA levels of OPG/RANKL in TMJ condyles between the mice with reduced dietary loading (soft diet with incisor trimming) and the mice with normal dietary loading, although after 6 weeks, reduced dietary loading resulted in a significant decrease in RANKL mRNA and little change in OPG mRNA in TMJ condyles[9]. In addition, soft diet alone (without incisor trimming) seldom causes changes of the morphology and the expression of Collagen II or OPG/RANKL in mouse TMJs compared to hard diet[9]. Yamada *et al*.[8] used 3-week-old rats

and reported no significant differences of cartilage thickness and subchondral bone volume fraction between the group treated with 4-week hard diet but switched to 4-week soft diet and the group that took a complete 8-week hard diet. The present data of the two control groups agreed with these reports[8,9]. Altogether, these results indicated that in normal biomechanical environment, the physiological stimulation of small-size or large-size diet showed no obvious different effects on TMJ remodeling within the 3-week period. But in an aberrant biomechanical environment, for example, when there was an abused prosthesis, large-size diet would induce more obvious degradation lesions in mouse TMJs than small-size diet.

In summary, functional reduction of occlusal loading, for example, by taking small-size diet, displayed decreased TMJ degradation when the occlusion is harmful, although the animals and human beings are not totally equal. Although soft diets are often recommended to TMD patients[49], clinical trials may be needed to explore the effects of reducing dietary loading on TMJ degradation.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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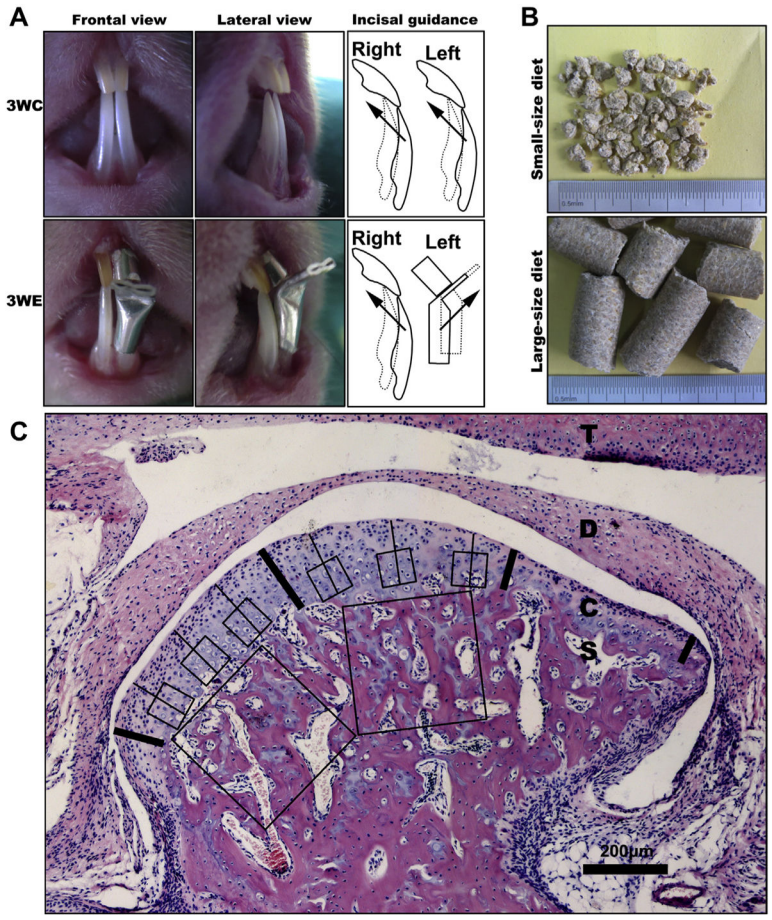

Fig. 1.

Illustration of the mouse anterior teeth occlusal relationship, mouse diets and methods of quantitation scheme. **(A)** The representative frontal and lateral view of anterior teeth occlusal relationship in the control and experimental mice 3 weeks after operation, and the schematic diagram of the normal incisal guidances in the control mice and the totally opposite guidances in the experimental mice when the mandibles move in the sagittal direction during incising. Arrows indicate the direction of incisal guidances. The bilateral incisors in control mice provide incisal guidances in the same directions while those in experimental mice provide incisal guidances in the opposite directions. **(B)** The photos showed the small-size and large-size diets. Minimal scale length = 0.5 mm. **(C)** The representative central sagittal H–E section of TMJ from the 3-week small-size control mice. T: temporal bone; D: articular disc; C: cartilage; S: subchondral bone. The central or posterior third cartilage thickness was measured as the average length of the three slender lines in the corresponding thirds. Two squares (0.3 mm × 0.3 mm) located beneath the osteochondral interface were chosen for subchondral bone histomorphometry. The percentage of immunopositive cells in cartilage was measured from the six square frames (each 0.08 mm × 0.08 mm). Bar = 200 μm 3WC = 3-week control group; 3WE = 3-week experimental group (The same group designations are also used in the other figures).

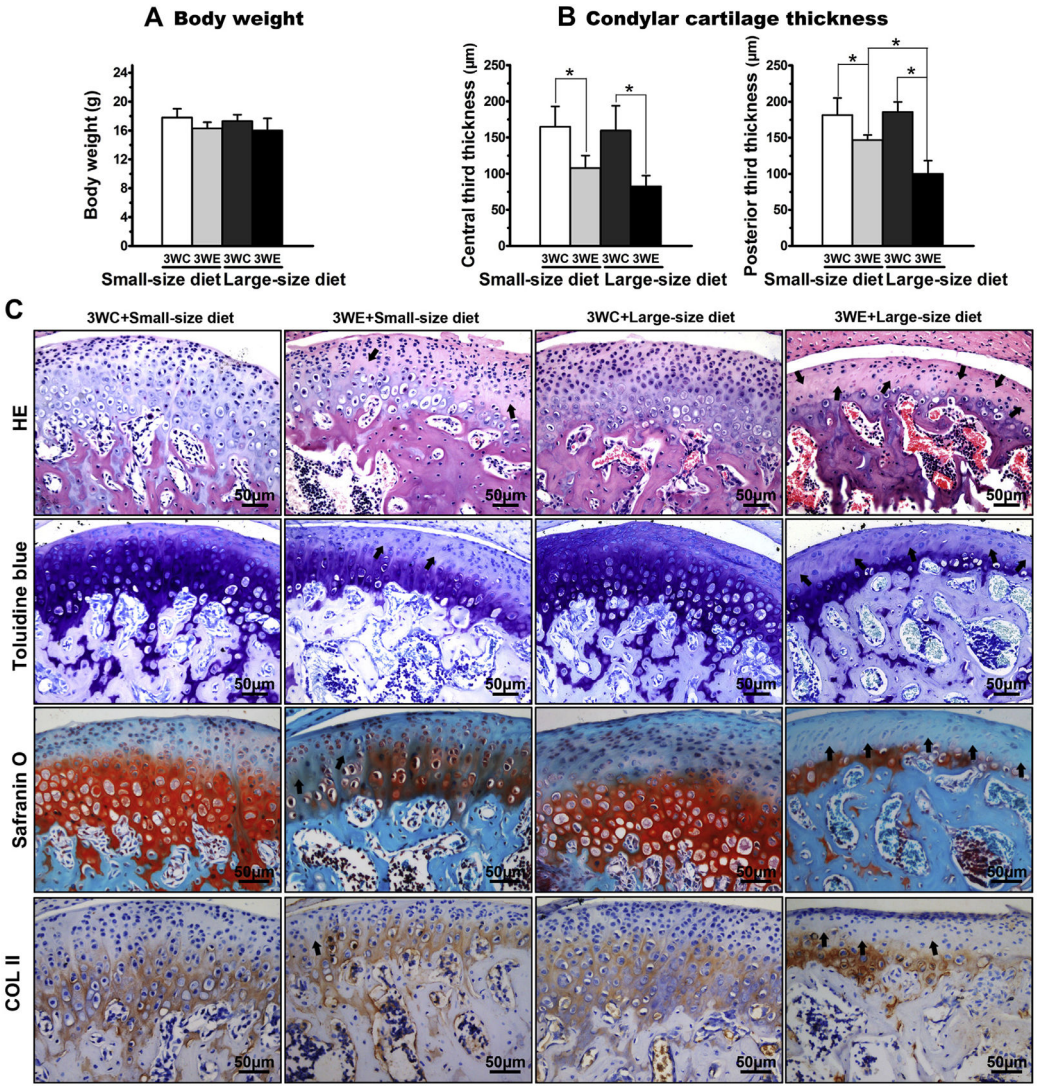

Fig. 2.

Histomorphological observations on TMJ condyles from the small-size diet control and experimental mice and the large-size diet control and experimental mice. **(A)** Body weight of the control and experimental mice 3 weeks after operation (n = 10). **(B)** Changes of mouse condylar cartilage central and posterior third thickness (n = 6). **(C)** Representative sections of Hematoxylin & Eosin (H–E) staining, toluidine blue staining, Safranin O staining and Collagen II immunohistochemical staining were shown. Arrows point the cartilage degraded areas, which take the form of local loss of proteoglycans or Collagen II and cell reduction or cell loss. Bars = 50 μm. Data are expressed as means and 95% CIs; * indicates statistically significant differences between groups.

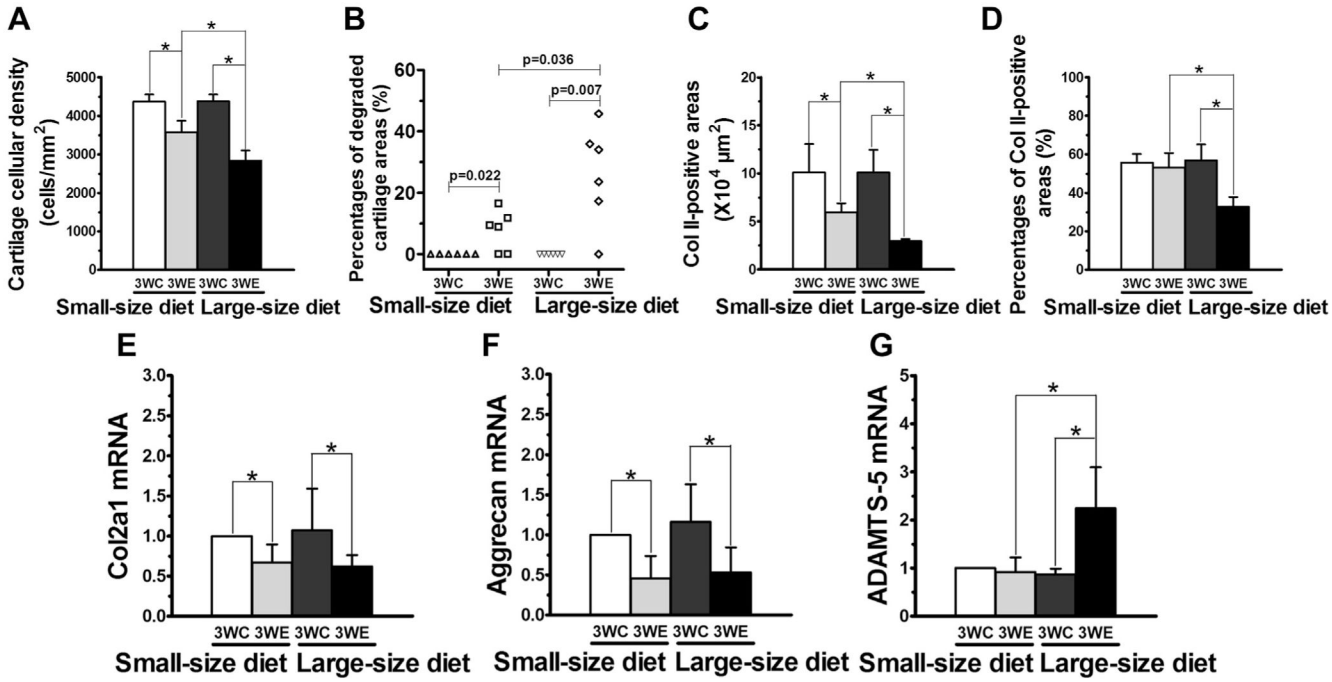

Fig. 3.

Changes of condylar cartilage cellular density **(A)**, percentages of degraded cartilage areas **(B)**, Collagen II-positive areas **(C)**, percentages of Collagen II-positive areas **(D)** and cartilage mRNA expressing levels of Collagen II **(E)**, Aggrecan **(F)** and ADAMTS-5 **(G)** in the TMJs from the small-size diet control and experimental mice and the large-size diet control and experimental mice. $n = 6$ in **(A**–**B)** and $n = 3$ in **(C**–**F)**. For the percentages of degraded cartilage areas in **(B)**, every point represents the measurement outcome of an independent sample while the other data are expressed as means and 95% CIs and * indicates statistically significant differences between groups.

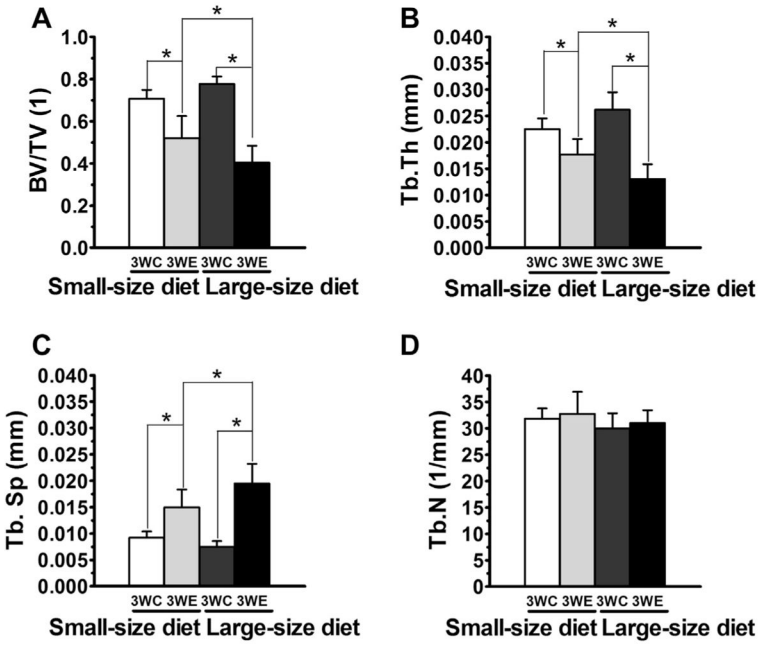

Fig. 4.

TMJ condyle subchondral bone histomorphometry in the small-size diet control and experimental mice and the large-size diet control and experimental mice. Bone histomorphometric results of mouse condyle subchondral bone were shown. **(A)** Bone volume fraction (BV/TV), **(B)** trabecular thickness (Tb.Th), **(C)** trabecular separation (Tb.Sp) and **(D)** trabecular number (Tb.N) were calculated from the selected subchondral squares. $n = 6$; data are expressed as means and 95% CIs; * indicates statistically significant differences between groups.

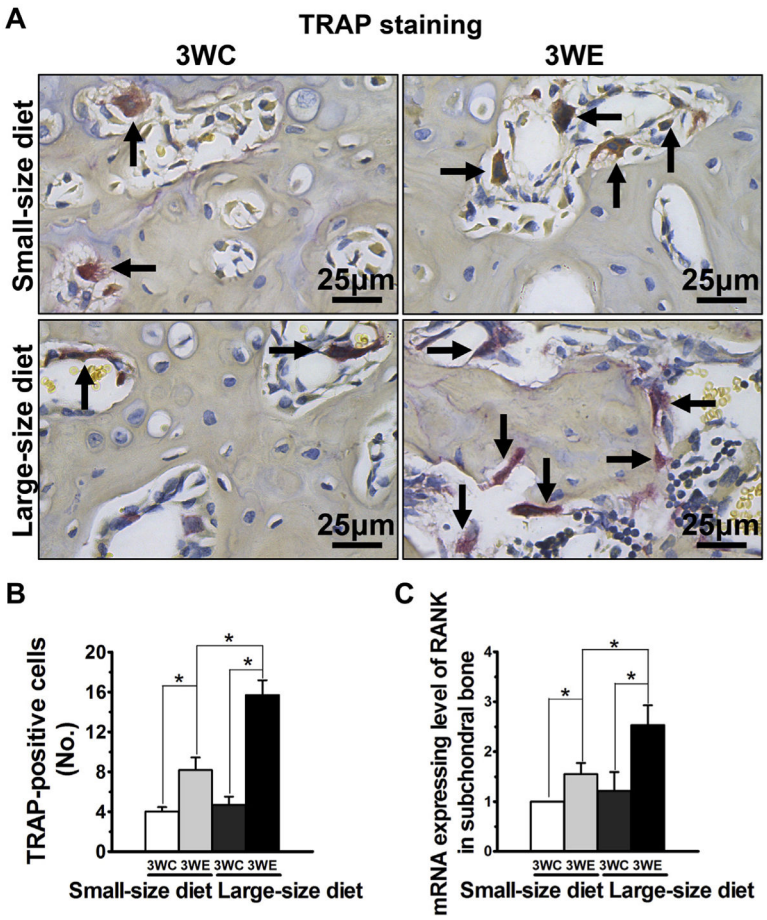

Fig. 5.

TMJ condyle subchondral bone osteoclast activity in the small-size diet control and experimental mice and the large-size diet control and experimental mice. **(A)** TRAP staining in the mouse TMJ condyle subchondral bone. Arrows indicate TRAP-positive osteoclasts. Bars = 25 μm. **(B)** Quantitative analysis of TRAP-positive osteoclasts (n = 6) and **(C)** mRNA expressing levels of RANK in condyle subchondral bone in the four groups were shown (n = 3). Data are expressed as means and 95% CIs; * indicates statistically significant differences between groups.

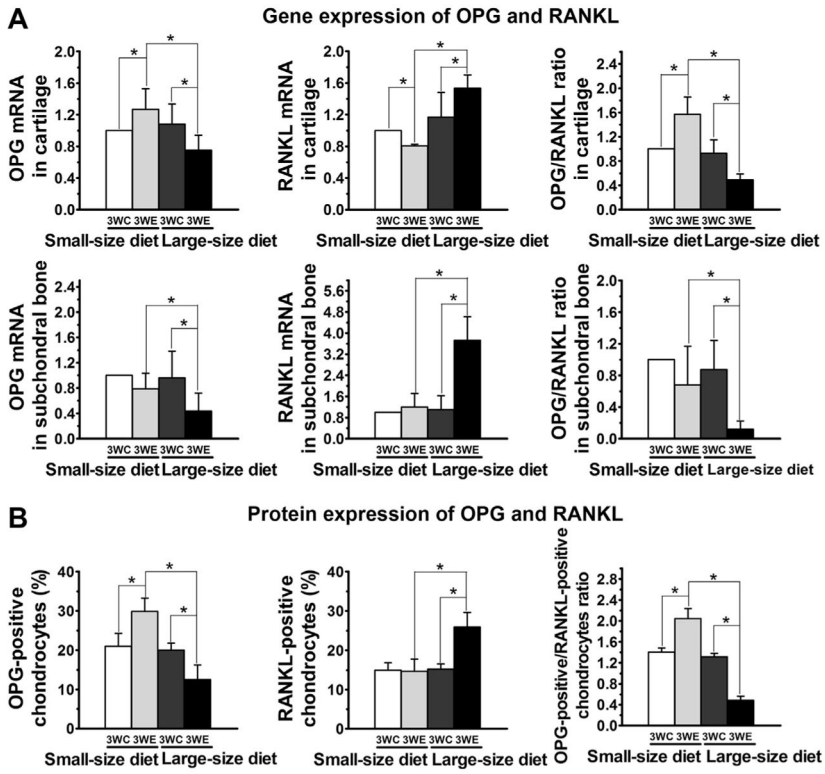

Fig. 6.

Expression changes of OPG and RANKL in TMJ condylar cartilage and subchondral bone of the small-size diet control and experimental mice and the large-size diet control and experimental mice. **(A)** Real-time PCR results of OPG and RANKL and OPG/RANKL mRNA ratio in the mouse condylar cartilage and subchondral were shown (n = 3). Detailed P-values are shown in the text. **(B)** Comparison of the percentages of OPG-positive and RANKL-positive chondrocytes and OPG-positive/RANKL-positive chondrocytes ratio in condylar cartilage (n = 3). The percentage of immunopositive chondrocytes was calculated from the selected six squares in condylar cartilage and P-values between groups with * are less than 0.001. Data are expressed as means and 95% CIs; * indicates statistically significant differences between groups.

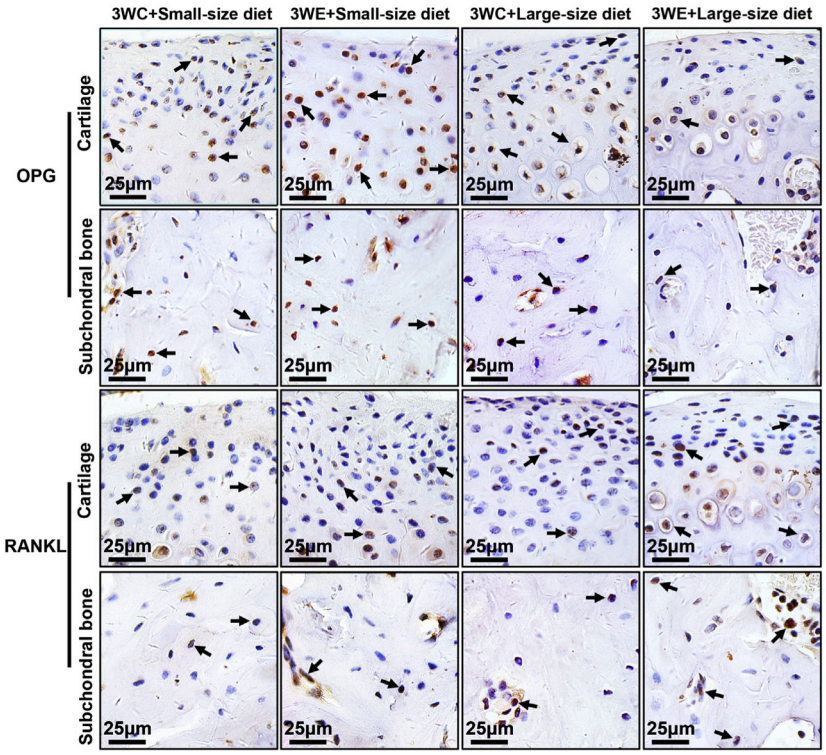

Fig. 7.

Immunohistochemical analysis of OPG and RANKL in TMJ condylar cartilage and subchondral bone of the small-size diet control and experimental mice and the large-size diet control and experimental mice. Representative sections of immunohistochemical staining of OPG and RANKL in condylar cartilage and subchondral bone were shown. Arrows indicate immunopositive chondrocytes and subchondral osteocytes or osteoblasts. Bars = 25 μm.

Table I

Gene primers

Genes	Forward primer	Reverse primer
Col2a1	CATCCAGGGCTCCAATGATGTA	ATGTCCATGGGTGCGATGTC
Aggrecan	TTCCACCAGTGCGATGCAG	TGGTGTCCCGGATTCCGTA
ADAMTS-5	AAGGGCACAGGCTACTATGTGGTC	CAATAATGCCGTCACATCCAGTTC
RANK	GGCTTACCTGCCCAGTCTCATC	AAGCATCATTGACCCAATTCCAC
OPG	TTACCTGGAGATCGAATTCTGCTTG	GTGCTTTCGATGAAGTCTCACCTG
RANKL	GCAGCATCGCTCTGTTCCTGTA	CCTGCAGGAGTCAGGTAGTGTGTC
GAPDH	TGTGTCCGTCGTGGATCTGA	TTGCTGTTGAAGTCGCAGGAG