Do combined glucosamine sulfate and chondroitin sulfate supplements affect condylar remodelling during functional appliance therapy?

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Objectives: The purpose of this study was to qualitatively and quantitatively analyse the effect of glucosamine sulfate and chondroitin sulfate supplements on condylar remodelling in conjunction with bite-jumping functional appliance therapy in rats.
Materials and methods: The study involved 140 three-week-old, female rats which were divided into a control group (CG), a supplementation group (SG), a functional appliance (bite-jumping) group (FG) and a bite-jumping appliance and supplement recipient group (FSG). The animals were sacrificed at Day 0, Day 7 and at Day 21 after appliance placement, as well as seven days following appliance removal. The condylar head from each animal was blindly scanned using micro-computed tomography (μCT). Qualitative evaluation and volumetric measurements of the condyles, including total condylar volume (TCoV), posterior condylar volume (PCoV), total cartilage volume (TCaV) and posterior cartilage volume (PCaV), were undertaken using VGStudioMax software.
Results: One hundred and thirty-five rats were analysed, some of which responded to the intervention with a protruded bite (Class III response) while others responded with a retruded bite (Class II response). The TCoV and PCoV of the CG decreased during the experimental period. The functional appliance alone and the combination of the functional appliance with the supplement had a significant effect on TCoV and PCoV over the intervention period ($p < 0.01$), peaking at Day 7. There was no statistically significant difference in TCaV between animals that experienced Class II and Class III bite responses at Days 21 and 28 ($p > 0.05$). However, at Day 21, the PCaV increased significantly in those animals which displayed a Class II bite response ($p < 0.05$). The shape of the condyles in FG and FSG varied significantly from that of the condyles in CG and SG.
Conclusion: Supplement therapy was found to enhance the normal biological response to functional appliance therapy in a rat model, particularly after the functional appliance was removed. Further research using an immuno-histochemical analysis of a modified bite-jumping appliance and improved food delivery is recommended.
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Introduction

Condylar cartilage proliferation, condyle growth and glenoid fossa remodelling induced by mandibular advancement[1] constitute the orthopaedic components of Class II correction in functional appliance therapy.[2] Previous research has tried to biochemically stimulate condylar cartilage growth, during which, compounds such as bisphosphonate,[3] growth hormones,[4] anabolic steroids[5] and growth factor-1 and 2[6] have been tested. Although these compounds are potentially efficacious, prescribing long-term hormonal use during functional therapy for healthy adolescents has only limited clinical applicability.

Glucosamine (2-amino-2-deoxy-D-glucose) is an amino monosaccharide precursor of the disaccharide unit of glycosaminoglycan, which is the building block of proteoglycans. The proteoglycans form the matrix of all connective tissues, including bones, muscles and cartilage.[7,8] Glucosamine, taken as a supplement, is the raw material used in the production of proteoglycans and glycosaminoglycans by chondrocytes.[8] Glucosamine exists in a sulphate (GS) or a hydrochloride form; however, the former is the most commonly used and extensively studied. Chondroitin sulfate (CS), a polymer of repeating disaccharide units (galactosamine sulfate and glucuronic acid), is the predominant component of articular cartilage and is a natural component of several other body tissues, including tendons, bones such as the condyle, and cartilage such as vertebral and condylar disks.[9] Bovine trachea, porcine by-products and shark cartilage are the main sources of CS supplements.[10]

Several studies have suggested that GS and CS supplements increase the synthesis of proteoglycans[11] and deliver anti-catabolic,[12] chondro-protective[11,13] and mild anti-inflammatory effects,[14-16] which may contribute to the beneficial results seen in-vitro and in vivo models.[17-21] However, there is limited orthodontic evidence of pharmaceutical enhancement showing that the use of GS and CS improves condylar cartilage adaptation secondary to functional appliance therapy. Therefore, the aim of the present investigation was to quantitatively and qualitatively analyse the effects of GS/CS supplementation on condylar cartilage remodelling during functional appliance therapy.

Materials and methods

The present study involved 140 female, three-week-old, Sprague-Dawley rats (ethics approval 07/31B Ref OA/7-2007/4674). The animals were divided into four groups: a control group without intervention (CG, N = 40), a bite-jumping functional appliance group (FG, N = 30), a GS/CS supplement group (SG, N = 40), and a functional appliance supplement group which received a bite-jumping appliance and GS/CS supplements (FSG, N = 30). The animals of the SG and FSG were individually caged, so that the consumption of supplements could be accurately monitored. The FG and CG were housed four animals to a cage, which were kept clean in a room with controlled temperature/humidity and light.

Throughout the study, the CG and FG were fed orally with 4 ml of commercial veterinary nutritional supplement (Nutrigel, Troy Laboratories, NSW, Australia) every 24 hours. Nutrigel is a highly palatable oral supplement used in veterinary medicine to provide either partial or full nutritional support for mammals. The FSG and SG received 4 ml of Nutrigel homogenised with 250 mg of GS and 200 mg of GS/CS supplements (1.4 gm to 1.6 gm of GS/Kg, 1.15 gm to 1.3 gm of CS/Kg). The animals were permitted unrestricted access to water.

The rats in the FG and FSG were allowed to adapt to their new environment for two weeks (the acclimatisation period) before being fitted with a bite-jumping appliance which meant that the appliance was placed when the rats were five weeks old (Figure 1). This procedure was planned so that appliance insertion would coincide with peak pubertal growth in the ensuing weeks.[22] On the day of appliance placement (when the experimentation period began), 10 animals from the CG and 10 animals from the SG were sacrificed by CO_2 asphyxiation and provided the baseline records. The animals in the FG and FSG were anaesthetised by the inhalational of isoflurane (1% to 2%) with oxygen, following which their incisors were conditioned with a self-etch primer (SEP – 3M Unitek, USA). A dental composite resin (Z100 dental composite resin capsules 3M ESPE, USA) was injected into a paediatric anterior strip crown to form bite-jumping fixed ramps. All animals were weighed to monitor weight fluctuation and were allowed to postoperatively recover before being returned to their cages. At Day 7, 10 animals from each group were sacrificed and, similarly, at Day 21 another 10 animals
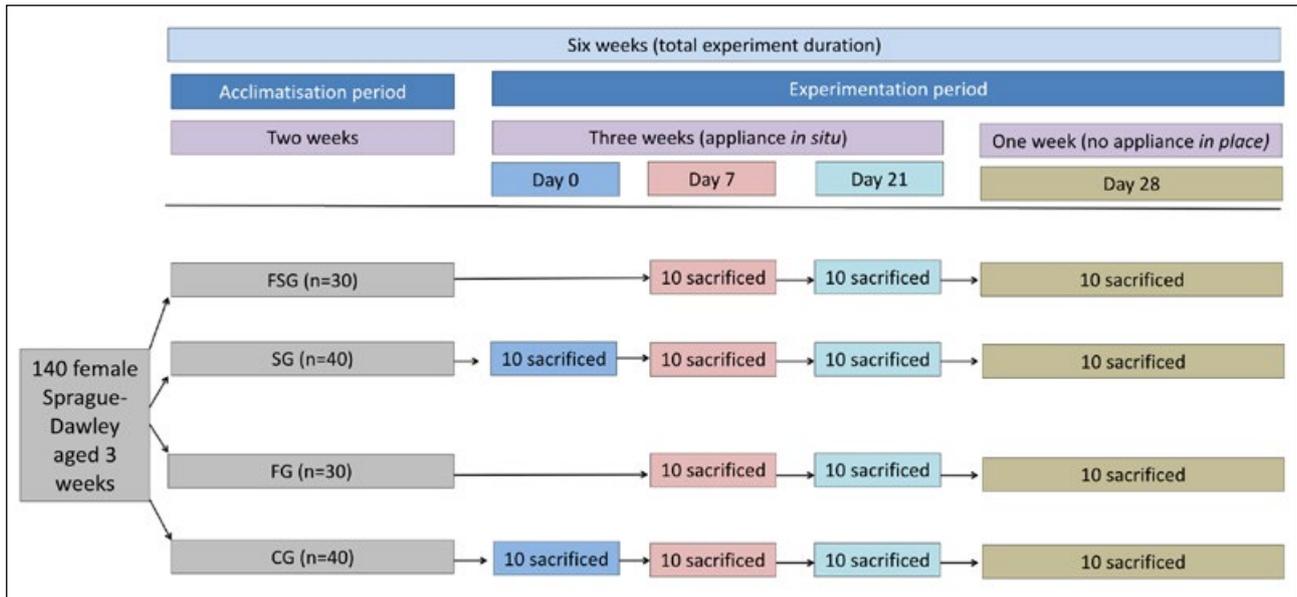
Figure 1. Study design.

from each group were sacrificed. In addition, at Day 21 the bite jumping appliances were removed from the animals remaining in the FSG and FG groups. The 10 surviving rats in each group were ultimately sacrificed at Day 28.

The right hemisection of each rat's mandible was dissected out and fixed in 10% buffered formalin. Each sample was stained for six days in a 200 mM gadolinium chloride solution prior to a blind scanning process using the SkyScan, a high-resolution desktop microtomograph (SkyScan, Aartselaar, Belgium). The acquired three-dimensional (3D) images were reoriented and segmented using VGStudioMax software (Volume Graphics GmbH, Heidelberg, Germany). Qualitative evaluation and volumetric measurements of the condyles, including total condylar volume (TCoV), posterior condylar volume (PCoV), total cartilage volume (TCaV) and posterior cartilage volume (PCaV), were undertaken using VGStudioMax software.[23]

Statistical analysis

Data collection and measurements were performed by a single operator. Measurements of the 23 samples were repeated three times to test for measurement error. Statistical analysis (ANOVA) was applied using SPSS® version 14 for Windows (SPSS, Inc., IL, USA). Weight loss/gain was analysed using a one-way analysis of variance. The results were displayed using box plot diagrams, and the 'whiskers' stretched out to the maximum and minimum values, except for unusual results that are indicated separately.

Results

The analysis was performed on 135 of the 140 specimens, indicating an excellent completion rate (96.4%). There was no significant difference ($p > 0.05$) in the repeated measures (Table I). In addition, there was no significant difference in animal weight between the groups, except in the FG, which showed a statistically significant weight loss by the time of sacrifice ($p < 0.001$).

The effect of the bite-jumping functional appliance and supplementation on the condylar head and cartilage was first analysed for statistical significance. Although some animals responded to the appliance with a protruded bite, some adapted with a retruded bite. Therefore, a secondary analysis was carried out to determine the outcomes based on the classes of the bite and their relationship to the intervention. Three classes of the bite were identified: (a) dental protrusion (a Class III bite response), (b) dental retrusion (a Class II bite response) and (c) neutral response (animals without a bite-jumping appliance).

Qualitative analysis

The shape of the condyle appeared consistent in the CG and SG. The axial slices of the condylar cartilage of the CG showed a condensed thick layer at Day 7;

Table I. Error of measurement.

	TCoV (mm³)	PCoV (mm³)	TCaV (mm³)	PCaV (mm³)
Standard error of measurement	0.0802	0.0508	0.0863	0.0408
Coefficient of variation (CV)	4%	5%	24%	26%
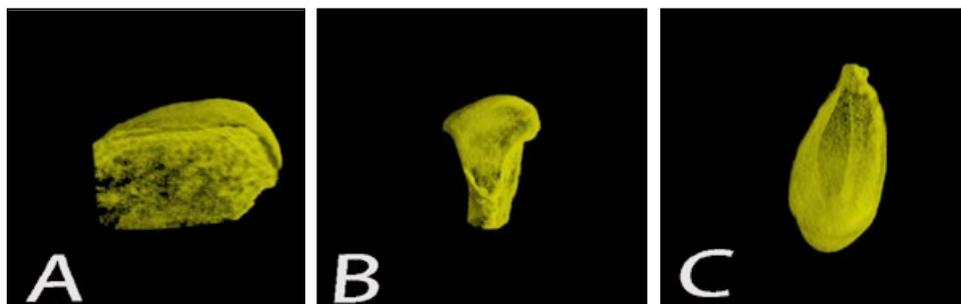
Figure 2. 3D cartilage imaging at Day 0 (a) lateral view, (b) posterior view, and (c) superior view.
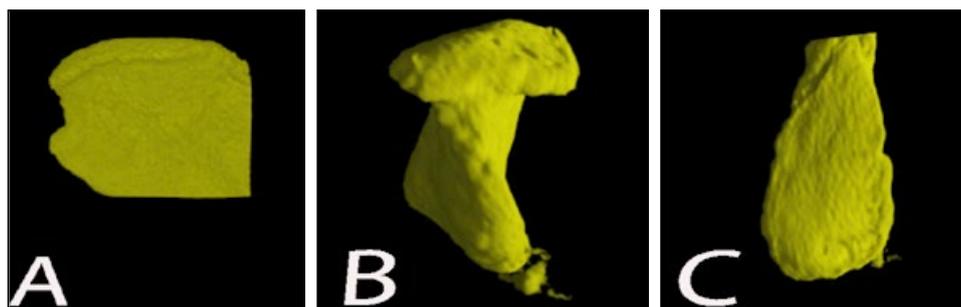
Figure 3. Condyle at Day 21 in animals that received supplementation and had a Class III bite response: (a) lateral view, (b) posterior view, and (c) superior view.
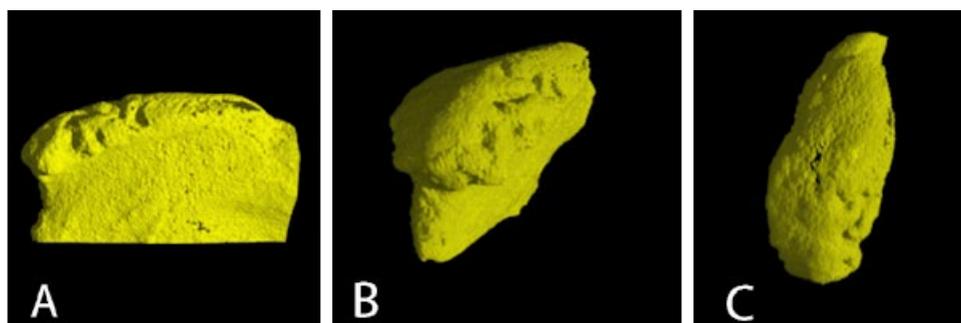
Figure 4. Condyle at Day 28 in animals that received supplementation and had a Class II bite response: (a) lateral view, (b) posterior view, and (c) superior view.

however, over time, by Day 28, the cartilage became thinner and the condylar bone appeared denser. The overall anatomy of the condyle was smooth, with consistent anatomic characteristics as seen in Figure 2.

The shape of the condyles in the FG and FSG varied significantly from the shape of the CG condyles. Changes were evident by Day 7 and increased throughout the experiment period (Figure 3). The animals with a Class III response had altered remodelled morphological characteristics of their condylar cartilage compared with those that displayed a Class II response and those of CG animals. The period after appliance removal noted increased remodelling of the cartilage, particularly in animals with a Class II bite response and those that had been receiving supplement therapy (Figure 4). The increased thickness in the axial sections of the cartilage in the SG and FSG was noticeable on Day 28. There was irregular topography on the superior condylar surface as cartilage filled the remodelling regions (Figure 5).
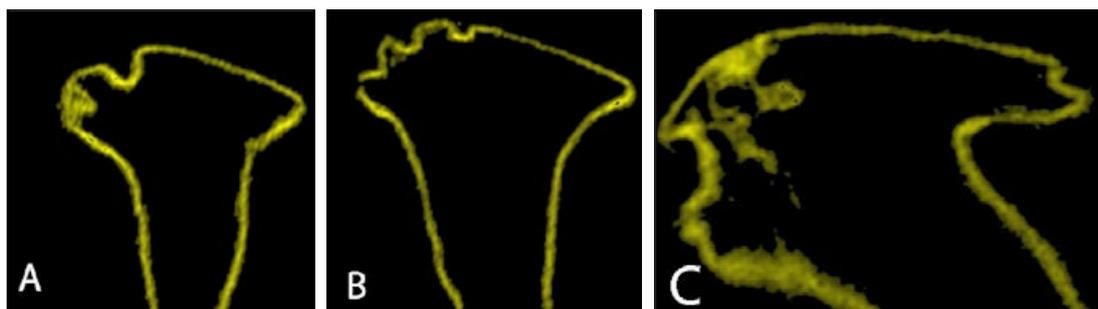
Figure 5. 2D cartilage imaging of condyle at Day 28, after both bite-jumping appliance and supplementation.
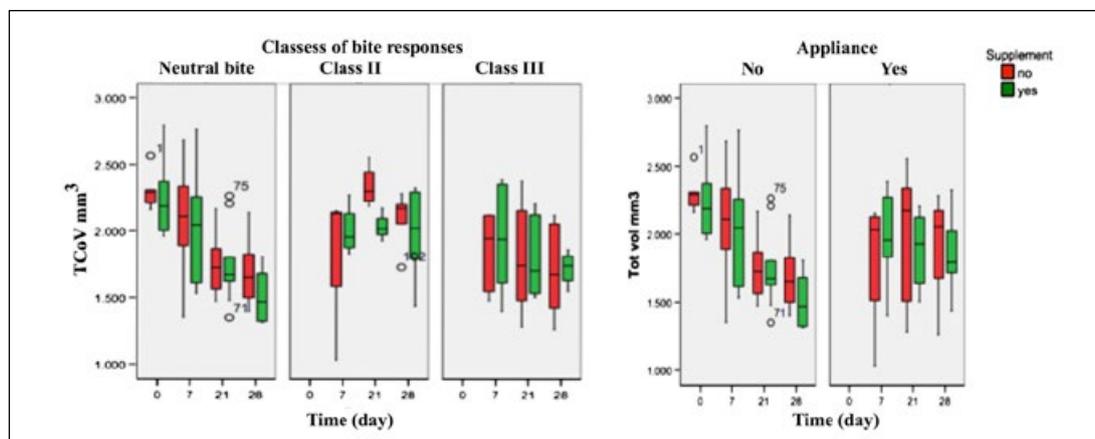
Figure 6. TCoV analysis.

Quantitative analysis

Changes in condyle volume

TCoV changes in relation to the time and class of the bite as well as the interaction of the time and class of the bite were found to be statistically significant ($p < 0.05$) (Figure 6).

The animals with a neutral class of the bite demonstrated a general decrease in TCoV from the commencement of the experimental period through to Day 28. The animals with Class II and Class III bite responses had sustained TCoV during the experimental period. However, differences in relation to the class of the bite and time were found in animals that demonstrated a Class II bite response, where the TCoV increased mildly by Day 21 but decreased following appliance removal. The animals with a Class III bite response showed no changes in their TCoV during the bite-jumping period (from Day 1 through Day 21); however, the TCoV reduced following appliance removal. The presence of the supplements caused a mild increase in the TCoV, but was statistically not significant ($p = 0.065$).

PCoV changes showed a trend similar to the TCoV changes except that the animals with a Class III bite response had decreased PCoV during the bite-jumping period, which was rebound after appliance removal. Supplementation produced a marginal change that was statistically not significant ($p = 0.058$).

Changes in cartilage volume

Supplementation had a statistically significant effect on TCaV, regardless of the use of a bite-jumping appliance (Table II). The effect of a bite-jumping appliance along with supplementation on TCaV was also found to be significant alone and in relation to the various time points of the investigation ($p < 0.05$). Equally important, the presence of a bite-jumping appliance alone had a statistically significant effect on the TCaV as well as its effect over time ($p < 0.05$). A similar analysis was completed to ascertain the effects of the classes of the bite on TCaV. A significant result was found in the interaction between time and classes of the bite, and the classes of the bite and supplementation ($p < 0.05$).

Table II. Total cartilage volume analysis (TCaV).

Investigating class of the bite, supplement and time		Investigating appliance, supplement and time	
Source	Sig.	Source	Sig.
Time	0.000	Time	0.000
Classes of the bite	0.072	Appliance	0.019
Supplement	0.000	Supplement	0.000
Time*Classes of the bite	0.002	Time*Appliance	0.000
Classes of the bite*Supplement	0.004	Appliance* Supplement	0.014
Supplement *Time	0.275	Supplement *Time	0.474
Time*Class of the bite* Supplement	0.099	Time*Appliance* Supplement	0.009
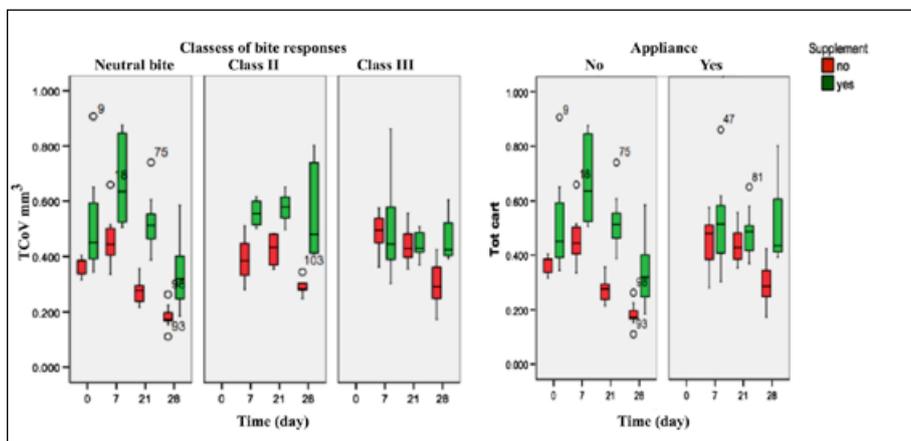
Figure 7. TCaV analysis.

A post hoc comparison of TCaV of animals with a Class II and Class III bite response at Day 21 and 28 failed to find any statistically significant differences ($p > 0.05$). At the end of the acclimatisation period, the baseline record taken from the CG and SG showed an increase in TCaV while the presence of a bite-jumping appliance resulted in an additional increase in the TCaV throughout the experimental period in comparison to CG (Figure 7).

Similar trends were yielded in response to PCaV in relation to the factors mentioned previously. The presence of a bite-jumping appliance ($p < 0.05$) and supplement ($p < 0.05$) therapy had statistically significant effects on PCaV. Importantly, the interaction between the time, bite-jumping appliance and supplementation was significant ($p < 0.05$). Results analysed for the effects of class of bite on PCaV found the class of bite, time by class of bite interaction as well as the time by class of bite by supplement interaction to be statistically significant ($p < 0.05$). A post hoc comparison of PCaV at Day 21 of animals with Class II and Class III bite responses found statistically

significant changes ($p < 0.05$); however, the changes were statistically insignificant by Day 28. The effect of the supplements appears to increase the biological changes of the condylar cartilage secondary to the effects of the bite-jumping appliance, particularly when the appliance was removed, as shown by the changes at Day 28.

Discussion

The decreases in TCoV in the CG and SG in the present study were consistent with previous findings in the literature.[24-26] Topographic changes in cartilage thickness were noted in thin anterior cartilage and thick posterior cartilage in the soft diet groups.[24] These regional differences suggested that the rate of differentiation and maturation of mesenchymal cells into chondrocytes may be influenced by the loading level of the cartilage.[24,25] Therefore, the normal growth pattern of the condyle over the 28-day period was a general reduction in volume, as would be expected. The TCoV of those animals with a Class III bite response was found to have sustainable volumes

throughout the time period. In contrast, those animals with a Class II bite response displayed a general increase in the TCoV that may be attributed to an increase in the functional loading of the condyle. An enhancement of the transition from chondrogenesis to osteogenesis in the mandibular condyle due to bite-jumping appliance therapy has been documented in female Sprague-Dawley rats, with peak osteogenesis occurring at Day 21 after appliance placement.[27]

Several studies have shown that the combination of GS/CS supplementation and mechanical stresses increased the chondrocytes' capability to synthesise proteoglycan.[11,13] In the current investigation, the TCaV and PCaV in the CG increased by Day 7 and then decreased. Bite-jumping appliance therapy alone increased the TCaV and PCaV, with peak changes occurring at Day 7. The TCoV and PCoV increased marginally by Day 21 in accordance with published literature, perhaps illustrating an enhanced transition from chondrogenesis to osteogenesis.[28-30] Animals with a protruded bite (a Class III bite response) have classically shown enhanced cartilage proliferation on the posterior aspects of the condyles, due to the stretching of the retrodiscal elastic band between the condyle and the glenoid fossa.[1,2,31]

An experimental study by Joho and colleagues in monkeys detected significant resorption of the glenoid fossa and the anterior surface of the post-glenoid tubercle following the application of an extra-oral force to the lower mandibular molars.[32] In the present study, similar changes in condylar remodelling were found in Class II and Class III bite responses; however, with mild differences in animals with Class III bite responses, this was probably due to the anatomical differences between the species. The animals used in the present study, and which displayed Class II and Class III bite responses, showed extensive remodelling in the medio-superior aspects of their condyles. This would likely represent the areas of compression during chewing as increased remodelling occurred after appliance removal at Day 28. This result concurs with past reports.[32]

The supplements enhanced the biological response of the condyle to the function of the bite-jumping appliance, particularly in animals with a Class II bite response. In addition, the stimulus noted after the removal of the bite-jumping appliance in both Class II and Class III animals receiving supplementation resulted in increases in cartilage volume, which may indicate that the supplement acted as 'biological growth modifier'. This suggests that the growth of the condyle is not entirely genetically determined but has the capacity for mechanical and also biochemical adaptation and stimulation.[11]

The authors acknowledge that the homogenised Nutrigel paste with GS/CS supplements was deemed successful, due to the ability to monitor and ensure drug administration;[33] however, animals in the FG failed to gain a significant amount of weight, possibly due to a lack of the high caloric and fat content of the rat pellets in their diet. The lack of weight gain may have contributed negatively to the results, with smaller growth changes perhaps occurring in the current investigation. Therefore, it is advised that if a repeat investigation is considered, additional ground rat 'chow' should be added to the food delivered in order to maintain normal gastrointestinal function and sustain weight gain. Although the appliance design used in this study was found to be effective, some animals maintained a protruded bite (a Class III bite response), yielding different regional condylar changes in comparison with those animals with a retruded bite (a Class II bite) response. This result may be due to specific symphyseal joints in the Sprague-Dawley rats.[34] A follow-up study utilising a more forward angulation of the bite ramp could create a more consistent functional protrusion. The overlap of certain morphological characteristics in relation to the animals with Class II and Class III bite responses, respectively, may be due to the animals' ability to functionally alter their mandibular position. Although assessments were made of the animals' functional adaptations, these were made at only specific time points in the experiment and cannot be extrapolated as a fixed adaptation. Animals in a habitual resting position were seen to have a Class II bite response; however, the rats postured forward to eat and chew. The morphological characteristics discussed were generalisations of each class of bite, and overlapping features were found in several samples. Furthermore, it is advised that further investigation is required; for example, a repeat of this experiment so that immuno-histochemical analysis could assess the cellular changes and proteoglycan content of the cartilage secondary to the bite-jumping appliance. An extension of this investigation to include post-pubertal animals is also recommended.

Conclusions

GS/CS supplements increase the volume of condylar cartilage, regardless of the presence of a bite-jumping appliance and, in particular, in those animals with a Class II bite response. The effect of supplementation was enhanced after the bite-jumping appliance was removed. Although the results have proved to be promising, further research is warranted using immuno-histochemical analysis on post-pubertal animals with improved food delivery and a modified bite-jumping appliance.

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