INSTITUTO UNIVERSITÁRIO EGAS MONIZ

MESTRADO INTEGRADO EM MEDICINA DENTÁRIA

THE IMPACT OF MECHANICAL STIMULUS ON THE TEMPOROMANDIBULAR JOINT'S CARTILAGE: A SYSTEMATIC REVIEW

Trabalho submetido por
Beatriz João Queimado Vaz Ponte Duarte
para a obtenção do grau de Mestre em Medicina Dentária

setembro de 2022

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Trabalho orientado por
Prof.ª Doutora Maria Alzira Cavacas

e coorientado por
Prof. Doutor Hélder Nunes Costa

setembro de 2022

Dedicatória

Aos meus pais, Manuela e João,
são o Sol da minha vida.

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Abstract

Introduction: The temporomandibular joint (TMJ)'s fibrocartilage develops in response to the local mechanical environment as a load-absorbing structure, essential for maintaining chondrocyte proliferation and extracellular matrix production. Several animal studies analyse this tissue's response to different types of mechanical stimuli.

Objectives: This systematic review intends to evaluate *in vivo* the morphological and cellular changes in TMJ's fibrocartilage in response to different modalities and intensities of mechanical loading stimulus.

Materials and Methods: PubMed, SCOPUS, and B-On databases were searched until December 2021. Hand-searching through citations was also performed. Only experimental *in vivo* studies that provided knowledge about the effect of the mechanical stimulus in TMJ's fibrocartilage, published in the last ten years, were included. These were grouped into four categories: compressive force loading, anterior mandibular displacement, increased occlusal vertical dimension, and diet hardness. Random effects meta-analysis was also performed for the effect of compressive force loading on the cartilage thickness.

Results: 25 articles were considered for the qualitative synthesis and 6 articles were included in the quantitative synthesis. Different dietary consistencies promoted differences in the thickness and histological content of fibrocartilage. Mandibular advancement was also associated with metabolic changes. The increase in vertical dimension revealed contradictory results. The compressive force loading led to a reduction of 0.49μm/g.day in the fibrocartilage thickness of rats, showing the initial age factor a significant effect on this wear ($p<0.05$).

Conclusions: Mechanical stimulus significantly affects the temporomandibular joint's fibrocartilage, showing not only morphological alterations but also changes in its cellular and metabolic activity, which seem to be related to the period and intensity of the stimulus.

Clinical Relevance: Understanding how the temporomandibular joint's fibrocartilage reacts to different modalities and intensities of mechanical stimulus plays an important role in orthodontic treatments that modify jaw growth and in the prevention of TMJ disease.

Keywords: temporomandibular joint, fibrocartilage, mechanical stimulus, *in vivo.*

Resumo

Introdução: A fibrocartilagem da articulação temporomandibular (ATM) desenvolve-se em resposta ao ambiente mecânico local, atuando como uma estrutura de absorção de carga, essencial para a proliferação dos condrócitos, bem como para a manutenção e produção da matriz extracelular. São vários os estudos realizados em animais que analisam a resposta deste tecido a diversos tipos de estímulos mecânicos.

Objetivos: Avaliar as alterações morfológicas e celulares que ocorrem na fibrocartilagem da ATM perante diferentes modalidades e intensidades de estímulo mecânico, *in vivo.*

Materiais e Métodos: As bases de dados *PubMed*, *SCOPUS* e *B-On* foram pesquisadas até dezembro de 2021. Realizou-se também uma pesquisa manual. Apenas estudos *in vivo* nos quais foi avaliado o efeito do estímulo mecânico na cartilagem da ATM, escritos nos últimos dez anos foram incluídos. Agruparam-se estes em quatro categorias: carga compressiva, desvio anterior da mandíbula, aumento da dimensão vertical oclusal e consistência dietética. Foi também realizada uma meta-análise de efeitos aleatórios para o efeito da carga compressiva na espessura da cartilagem.

Resultados: Foram incluídos 25 artigos na análise qualitativa e 6 na análise quantitativa. Diferentes consistências dietéticas promoveram diferenças na espessura e conteúdo histológico da fibrocartilagem. O avanço mandibular também se associou a alterações metabólicas. Já o aumento de dimensão vertical revelou resultados contraditórios. A carga compressiva contribuiu para uma redução da espessura da fibrocartilagem de ratazanas de 0.49μm/g.dia, mostrando o fator idade inicial um efeito significativo neste desgaste ($p<0.05$).

Conclusões: O estímulo mecânico produz efeitos significativos na fibrocartilagem da ATM, não só morfologicamente, mas também na atividade celular e metabólica, os quais parecem estar relacionados com o tempo de atuação e intensidade do estímulo.

Relevância Clínica: Compreender como a fibrocartilagem da ATM responde a diferentes tipos e intensidades de estímulo mecânico é crucial nos tratamentos ortodônticos que modificam o crescimento mandibular e na prevenção da patologia articular.

Palavras-chaves: articulação temporomandibular, fibrocartilagem, estímulo mecânico, *in vivo.*

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List of Abbreviations

ADAMTS- a disintegrin and metalloproteinase with thrombospondin motif

AMD- anterior mandibular displacement

Ang- angiopoietin

CG- comparison group

CF- confidence interval

Coef.- meta-regression coefficient

ECM- extracellular matrix

EG- experimental group

g- gram

GAG- glycosaminoglycan

HD- hard diet

I- loading intensity

I^2- heterogeneity index

IGF- insulin-like growth factor

Ihh- indian hedgehog protein

IL- interleukin

iOVD- increased occlusal vertical dimension

MAPK- mitogen-activated protein kinase

MMP- matrix metalloproteinase

OA- osteoarthritis

OPG- osteoprotegerin

PRISMA – Preferred Reporting Items for Systematic Reviews and Meta-Analyses

RANK- receptor activator of nuclear factor kappa

RoB- Risk of bias

SD- standard deviation

SE- standard error

SYRCLE- Systematic Review Centre for Laboratory Animal Experimentation

T- cartilage thickness

t- loading period

TMJ- temporomandibular joint

TNF- tumoral necrosis factor

VEGF- vascular endothelial growth factor

µm- micrometre

µm/g.day- micrometre per gram and day

3D- tridimensional

I. Introduction

The stomatognathic system is a complex unit constituted by diverse structures that work in synergy to promote the essential functions of mastication (chewing, sucking, swallowing), communication (speaking, facial expressions performing), breathing and also contributing to dentofacial aesthetics (Roberts & Stocum, 2018; Stocum & Roberts, 2018). These structures include osseous components, teeth, soft tissues, masticatory muscles and temporomandibular joints (Gedrange et al., 2017).

The temporomandibular joint (TMJ) is a diarthrosis, also defined as a ginglymoarthrodial joint, between the mandible and the cranium. In detail, it is a synovial joint formed by the mandible's condyle and the temporal bone's articular eminence, interposed by a fibrocartilaginous disc. As a bilateral articulation, the right and left joints are not independent and must work in coordination with each other (Bordoni & Varacallo, 2019).

Guided by the arrangement of bones, muscles, ligaments and dental occlusion, TMJ carries out a wide range of complex and physiological movements on different orthogonal planes and multiple rotation axes (Vîrlan et al., 2021). Furthermore, it is one of the most heavily loaded joints, particularly when the mandible is in occlusion (Roberts & Goodacre, 2020; Stocum & Roberts, 2018).

1. Brief anatomy and physiology of temporomandibular joint

An inexhaustive explanation of the anatomy and physiology of the temporomandibular joint will be given to introduce some context to this work's main topic: the temporomandibular joint's fibrocartilage.

The osseous structures that form TMJ are the articular fossa of the temporal bone's squamous portion and the mandibular condyle. While the first is fixed to the cranium, the second consists of the moving component of the joint (Bordoni & Varacallo, 2019; Norton, 2016).

The articular fossa is limited posteriorly by the postglenoid process and anteriorly by the articular eminence. The articular eminence is formed by two slopes: an anterior non-articular slope and a posterior articular slope. The last one, named preglenoid plane, is

covered by fibrocartilage and presents a slight inclination, making the movements of the articular disc and the condyle possible and easier (Bordoni & Varacallo, 2019; Vîrlan et al., 2021).

The mandibular condyle fits the articular fossa and slides along the posterior slope of articular eminence during mandibular functional movements (Fan et al., 2021). It consists of an ovoid surface, also covered by fibrocartilage, larger in its mediolateral section than in the anteroposterior area (Bordoni & Varacallo, 2019; Norton, 2016).

Both articular fossa and mandibular condyle are exposed to masticatory forces, responding to changes in the mechanical loading environment (Nickel et al., 2018; Roberts & Goodacre, 2020).

Positioned between the two bones, there is a biconcave fibrocartilaginous disc. This structure increases the congruity between the two osseous surfaces, allowing smooth jaw movements. It divides the TMJ into two compartments: the superior compartment (1.2mL), limited by the articular fossa and the articular disc, which forms a plain-gliding plan providing for the translational movement; and the inferior compartment (0.9mL), limited by the articular disc and the mandibular condyle, which forms a hinge plan and allows rotational movement of the TMJ (Bordoni & Varacallo, 2019; Norton, 2016). In addition, the articular disc also acts in load distribution and aids in joint lubrication (Willard et al., 2017).

The temporomandibular joint is surrounded by an articular capsule, that attaches to the disc near the condylar head. It consists of fibrous connective tissue and its inner lined by a highly vascular synovial membrane that produces the synovial fluid. Each joint compartment is lined by its corresponding synovial membrane (Norton, 2016; Willard et al., 2017).

Synovial fluid acts as a lubricant liquid, facilitating joint movements, and is responsible for nourishing joint tissues. Thus, it serves as a medium for transporting metabolic requirements to and waste products from articular surfaces (Willard et al., 2017).

The capsule is also reinforced by several articular ligaments that provide stability to the joint, namely: the temporomandibular ligament, a capsular structure that reinforces the

capsule laterally; and the sphenomandibular and stylomandibular ligaments, which are considered accessory ligamentous structures (Bordoni & Varacallo, 2019; Norton, 2016).

TMJ is even related to different muscles, which are responsible for the joint's movement and protection. The muscles in direct contact with the joint are masseter, temporal, lateral or external pterygoid, and medial or internal pterygoid (Bordoni & Varacallo, 2019; Norton, 2016). Rather than describing the anatomical attachments, a brief description of their functions as an effect on joint dynamics will be made.

From the mentioned muscles, only the lateral/external pterygoid participates in the open jaw movement. Along with this, other muscles contribute to the mandibular condyle's forward and downward movement along the articular eminence's posterior slope, specifically geniohyoideus, mylohyoideus and digastric. The temporal muscle, the deep and superficial masseter, and the medial/internal pterygoid muscles provide the opposite movement as the jaw closes by the posterosuperior condyle movement (Bordoni & Varacallo, 2019; Norton, 2016).

The neurovascular supply is also an important part of any joint. The arterial supply of TMJ is ensured by branches of the external carotid artery, mainly the superficial temporal artery, the maxillary artery, and the masseteric artery. Furthermore, the venous drainage takes place in the retrodiscal region, by the pterygoid plexus (Bordoni & Varacallo, 2019; Norton, 2016).

Finally, as to the innervation of this joint, the capsule receives it from the masseteric nerve and the auriculotemporal nerve, which correspond to the second and third branches of the trigeminal nerve (cranial nerve V), respectively (Bordoni & Varacallo, 2019; Norton, 2016).

2. Temporomandibular joint's cartilage

In contrast with other synovial joints, such as the elbow or the knee, in which the articular surfaces are covered by hyaline cartilage, the temporomandibular joint's articular surfaces consist of a modified periosteum with a layer of fibrocartilage underneath (Roberts & Goodacre, 2020; Willard et al., 2017).

Fibrocartilage is a secondary growth site capable of growth, adaptation, and regeneration over a lifetime. Contrary, hyaline cartilage acts as a primary growth center and interstitial growth ceases when the growth plate fuses, having poor healing potential (Roberts & Goodacre, 2020).

It is considered a "transitional tissue" between hyaline cartilage and dense fibrous connective tissue. While hyaline cartilage only contains type II collagen, fibrocartilage predominantly contains type I collagen, in addition to type II. Histologically, it is a readily visible tissue in which collagen fibers are embedded in a small amount of extracellular matrix (ECM), whereas, in hyaline cartilage, the collagen fibers are masked by a large quantity of ECM, making it hardly visible (Armiento et al., 2019; Benjamin & Ralphs, 2004).

The rich content of ECM in tightly embraided collagen fibers gives fibrocartilage a great resistance to sheer forces by moderating the peak loads (Armiento et al., 2019; Benjamin & Ralphs, 2004). It acts as a cushioning mass and is found in three places of the TMJ: the articular disc, the articular surfaces of the mandibular condyle, and the articular fossa (**Figure 1**) (Roberts & Goodacre, 2020; Stocum & Roberts, 2018).

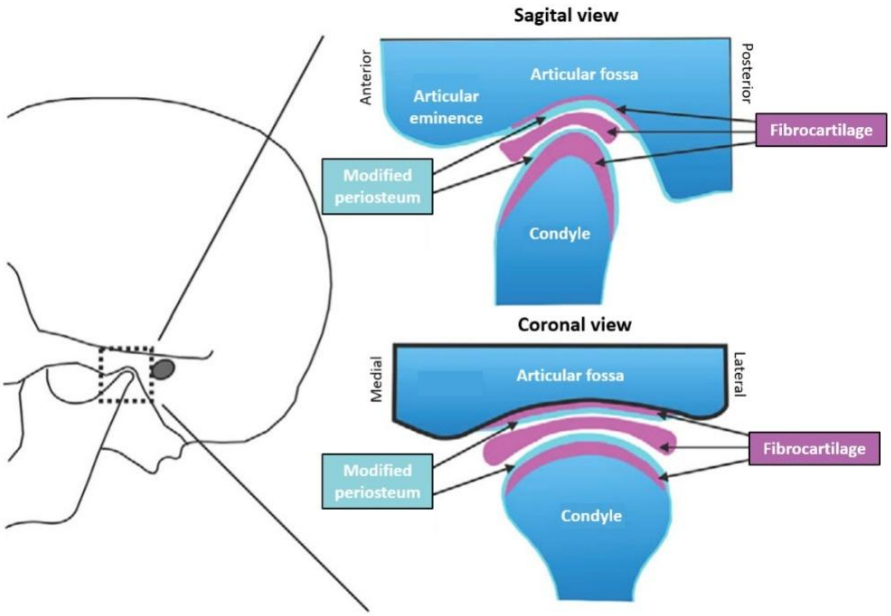

Figure 1- Schematic diagram of the articular surfaces of temporomandibular joint (Adapted from Roberts & Goodacre, 2020).

Some authors (Stocum & Roberts, 2018; Willard et al., 2017) affirm that there's a lack of studies concerning the cellular and biochemical composition of the articular fossa's fibrocartilage. Kato et al. (2015) and Uekita et al. (2015) have demonstrated that the cartilage covering both articular surfaces responds to mechanical loading, showing histological and immunochemical alterations. However, these alterations are much more evident in the condyle than in the articular fossa, and the histological samples of the last one's cartilage are less exuberant. Owtad et al. (2011) also concluded that cellular and molecular activity rates are lower in the articular fossa. Therefore, we will focus this dissertation on the mandibular condylar fibrocartilage study.

3. Mandibular condylar fibrocartilage

Cartilaginous tissues have an important function in the growth of the craniofacial skeleton (Delatte et al., 2004). Due to its unique characteristics and crucial role in mandibular growth, condylar cartilage has been a wide target of study over the decades.

It is often referred to as secondary cartilage, differing from primary cartilage in many aspects: embryonic origin, cell proliferation, histological organization and stimulus response (Mérida Velasco et al., 2009; Shen & Darendeliler, 2005).

3.1. Embryonic origin

Primary cartilages, such as synchondroses in the cranial base and nasal septum, are similar to the epiphyseal cartilage of long bones. They form earlier, being part of primary skeletal cartilage, and act as a scaffold for ossification (Delatte et al., 2004; Mérida Velasco et al., 2009; Shen & Darendeliler, 2005).

Secondary cartilages, namely condylar cartilage, appear later in ontogenetic development. It emerges from the periosteum of the intramembranous bone of the mandible after osteogenesis and independently of the primary skeletal cartilage (Mérida Velasco et al., 2009; Shen & Darendeliler, 2005; Shibata et al., 2013).

3.2. Cell proliferation

Regarding cell proliferation, secondary condylar cartilage growth occurs through differentiation of mesenchymal tissue overlying the condyle's cartilage, and not by mitosis of cartilage progenitor cells in the epiphyseal plate as in primary cartilage. First, mesenchymal cells split themselves into smaller new cells, that enlarge to full size and migrate in the direction of the interior of the condyle; then, as the migration occurs, these cells differentiate and become immature cartilage cells (Mérida Velasco et al., 2009; Shen & Darendeliler, 2005; Wadha & Kapilla, 2008).

This means that, instead of growth occurring within existing tissue (interstitial growth), it occurs from the exterior (appositional growth) (Mérida Velasco et al., 2009; Shen & Darendeliler, 2005; Wadha & Kapilla, 2008).

3.3. Histological organization

While primary cartilage's histological organization mainly comprises a chondrocyte population, various cell populations at different maturation stages can be found in secondary condylar cartilage. It consists of a heterogeneous tissue, in which mesenchymal cells differentiate into chondrocytes or osteoblasts and undergo a maturation process, leading to a characteristic zonal architecture (Delatte et al., 2004; Khaleel et al., 2020; Mizoguchi et al., 2013).

The terminologies used for the histological description of condylar cartilage slightly vary within the investigation community (Mérida Velasco et al., 2009; Mizoguchi et al., 2013). However, four morphological layers can be clearly distinguished, from the surface to the depth, namely: fibrous layer, proliferative layer, mature layer and hypertrophic layer (**Figure 2**) (Khaleel et al., 2020; Willard et al., 2017).

The articular fibrous layer, the most superficial, is continuous with the outer layer of the periosteum. It contains scattered flat fibroblast-like cells arranged mostly parallel to the condyle surface and surrounded by dense collagen type I fibers. For this reason, this cell layer consists of dense fibrous connective tissue and has a load-bearing function, acting as a protective covering for the underlying cartilaginous tissue (Khaleel et al., 2020; Mizoguchi et al., 2013; Willard et al., 2017).

The proliferative layer, immediately below, is marked by high cellularity. It can be divided into two sublayers, according to the cellular morphology: the upper sublayer named polymorphic cell sublayer, and a lower sublayer, the flattened cell sublayer. This layer has no specific cell arrangement (Khaleel et al., 2020; Mizoguchi et al., 2013).

It plays an important role as a "cell reservoir", mainly the cells in the polymorphic sublayer. These cells have significant proliferative activity and multilineage potential, differentiating into osteoblasts for the overlying fibrous layer, as well as chondrocyte precursors for the underlying mature layer. Although collagen synthesis is low in this layer, it is characterized by the expression of collagen type I (Khaleel et al., 2020; Mizoguchi et al., 2013; Willard et al., 2017).

The following layer is the mature layer, as it contains chondrocytes at various stages of maturation. For that reason, the shape of cells varies from flattened to spherical with progressive depth. Nonetheless, they are still capable of proliferating. There's a significant amount of ECM and the synthesis of proteoglycans, glycosaminoglycans and collagens is major in this layer. It is characterized by the expression of both collagen types I and II and also aggrecan, offering resistance against mechanical forces (Khaleel et al., 2020; Mizoguchi et al., 2013; Willard et al., 2017; Wadha & Kapilla, 2008).

At last, there is the hypertrophic layer, the deepest one near the junction with the subchondral bone. In this layer, chondrocytes become hypertrophic, showing an increase in volume, and are invaded by the bone marrow vasculature, along with osteogenic precursor cells and chondroclastic cells. The surrounding matrix begins to calcify and is degraded by differentiated chondroclasts, and a newly secreted bone matrix is then deposited onto the cartilage remnants, through endochondral ossification. It is believed that the hypertrophic chondrocytes undergo apoptosis; however, it is also a hypothesis that some transform into osteogenic cells. As to ECM molecules, collagen type X is typical of this layer (Khaleel et al., 2020; Mizoguchi et al., 2013).

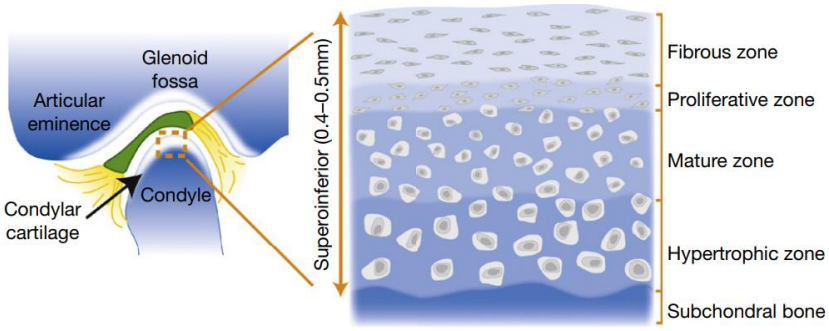

Figure 2 - Schematic diagram of the cellular distribution in mandibular condylar cartilage (Willard et al., 2017).

The four cell layers are characterized by cellular composition and the distribution of ECM molecules, namely collagens, proteoglycans and glycoproteins. These molecules provide structural support to the cellular activity of the cartilage, contributing to its fundamental biomechanical properties (Khaleel et al., 2020).

Collagen is the principal solid component of the ECM and its fibers form a three-dimensional network that provides stability, tensile strength and resistance to shear forces (Khaleel et al., 2020; Kuroda et al., 2009).

As mentioned before, one of the main characteristics of condylar fibrocartilage is the colocalization of collagen types I and II. Collagen type I is present throughout the cartilage but is mostly found in the fibrous and proliferative layers, whereas type II, characteristic of hyaline cartilage, is in mature and hypertrophic collagen (Khaleel et al., 2020; Mizoguchi et al., 2013; Willard et al., 2017).

Other collagen types can be found in the condylar fibrocartilage, yet in less quantity. Collagen type III is also present in the most superficial layer, representing its fibrous nature. Collagen type X, which can also be found in hyaline cartilage, is present in mature and hypertrophic layers (Willard et al., 2017).

The second largest group of ECM components is proteoglycans. They are embedded in the collagen network and consist of macromolecular polysaccharides with a linear chain structure. These macromolecules provide resistance to compressive strength. Aggrecan is the main one and is mainly found in mature and hypertrophic layers, but versican and decorin are also present throughout the four cell layers (Khaleel et al., 2020; Kuroda et al., 2009; Mizoguchi et al., 2013; Willard et al., 2017).

The glycosaminoglycans (GAGs) molecules, namely chondroitin, keratan and dermatan sulphates, are negatively charged molecules that are covalently attached to proteoglycans' protein core. They also contribute to the structural and functional integrity of the fibrocartilage (Khaleel et al., 2020).

It is important to refer that these are not the only components of condylar cartilage's ECM. Apart from water, which forms the major percentage of cartilage weight, it also contains other components in minor quantity, such as integrin, chondronectin and anchorin, that contribute to chondrocytes' attachment (Khaleel et al., 2020).

Based on the histological description, we can conclude that the condylar fibrocartilage is certainly influenced by mechanical loading, as this tissue's properties are unique and fundamental to the articular cartilage and subchondral bone functions (Khaleel et al., 2020).

3.4. Stimulus response

Both primary and secondary cartilages respond to general extrinsic factors such as hormones during growth. Still, secondary cartilage only follows these systemic stimuli after additional modulation by local growth factors, namely mechanical loading (Mérida Velasco et al., 2009; Shen & Darendeliler, 2005).

Mechanical loading generates signals in the chondrocytes, triggering various metabolic events responsible for this tissue's synthesis and degradation (Zhang et al., 2014). Therefore, secondary condylar cartilage requires an optimal level of mechanical stimulation for its maintenance and turnover throughout life (Khaleel et al., 2020; Owtad et al., 2013; Sun, 2010).

4. Effect of mechanical loading on the condylar fibrocartilage

Towards alterations in the mechanical forces that act on TMJ, condylar cartilage produces a biological response, that will influence cartilage and bone shape (Kuang et al., 2013; Utreja et al., 2016).

Clinically, the use of mechanical forces is often correlated with the orthodontic treatment of jaw discrepancies. These might be attributed to the mandibular size and position

anomalies, resulting in mandibular retrognathism or prognathism. By shifting the jaw to an anterior position, in mandibular retrognathism, or restricting its movements with compressive forces, in mandibular prognathism, functional appliances alter the jaw loading conditions of TMJ and consequently modify jaw growth (Karamesinis & Basdra, 2018).

Changes in the occlusion also lead to differences in the biomechanical environment of TMJ. For instance, increasing the occlusal vertical dimension promotes the rotation of the mandibular condyle, altering the relationship between this structure and the articular fossa, and consequently, the forces exerted on condylar cartilage and bone (Abduo & Lyons, 2012; Gopi Chander & Venkat, 2011).

Another aspect that might affect the TMJ loading is diet consistency, as mastication influences the mandibular movements and the occlusal loading, changing the forces applied to TMJ and the fibrocartilage turnover (Scheidegger et al., 2018).

Moderate loading allows the proper balance between anabolism and catabolism of fibrocartilage, critical for maintaining this tissue. However, if mechanical forces exceed the adaptive capacity of TMJ, catabolism is favoured over anabolism, disturbing fibrocartilage homeostasis. This results in damage to the ECM, leading to irreversible degradation of this tissue and dysfunctional remodelling of the condylar bone, characteristic of temporomandibular joint osteoarthritis (TMJ-OA) (Caldwell & Wang, 2015; Mueller & Tuan, 2011; Statham et al., 2022; Sun, 2010).

TMJ-OA is a progressive degenerative joint disease with a high prevalence that affects the hard and soft tissues of TMJ. It is characterized by cartilage thinning, extracellular matrix degradation, decreased number of chondrocytes, and subchondral bone resorption, leading to pain and masticatory dysfunction (Bianchi et al., 2020; Li et al., 2021; Wang et al., 2015).

Although having a multifactorial etiology, including hormonal imbalance, genetics and ageing, excessive mechanical stress has been considered one of the major causes of TMJ-OA (Bianchi et al., 2020; Wang et al., 2015).

It is crucial to understand how temporomandibular joint's fibrocartilage responds to different intensities and modalities of mechanical stimulus, for orthodontic treatments

that aim to modify jaw growth, and in the prevention of TMJ disease (Betti et al., 2018; Karamesinis & Basdra, 2018).

Due to ethical reasons, several non-human *in vivo* studies have been published regarding the impact of mechanical loading on the TMJ structures of animals. They represent a valuable tool in preclinical research, once the animals used allow the installation of mechanical devices that alter the occlusion and mastication, and the results might be extrapolated to humans (Xiang et al., 2021; Zhao et al., 2022).

No animal model is totally similar to the human TMJ, so various animal species are used as models to study this joint. Rodents, namely mice and rats, are the most common animals used, as they are relatively inexpensive and easy to handle. However, larger animals, such as rabbits, pigs, and sheep are often required, once they are anatomically more similar to humans concerning joint size and cartilage thickness. Moreover, they provide large enough tissue for reliable *in vitro* mechanical testing (Almarza et al., 2018; Cougo et al., 2021; Zhao et al., 2022).

Although previous systematic reviews have been published addressing the impact of various mechanical stimuli in fibrocartilage, none has attempted to evaluate the effect of the mechanical stimulus that can be applied in a clinical context to modify jaw growth.

5. Objectives

This systematic review aims to evaluate the histomorphological alterations in the temporomandibular joint's fibrocartilage in response to mechanical loading. Non-human *in vivo* studies relative to clinically applicable mechanical stimuli that altered the magnitude of TMJ loading were selected, including compressive force loading, anterior mandibular displacement (AMD), increased occlusal vertical dimension (iOVD), and diet hardness. Therefore, we aim to answer the following question: "What is the impact of mechanical stimulus on temporomandibular joint's cartilage?". Furthermore, a meta-analysis was also performed to assess the cartilage wear resulting from the compressive force loading, adjusted to the applied strength and respective period.

II. Materials and Methods

1. Protocol and registration

The present systematic review and meta-analysis were developed following the Preferred Reporting Items for Systematic Review and Meta-Analysis (PRISMA 2020) guidelines (Liberati et al., 2009). The PRISMA checklist is provided in **Appendix I** and **Appendix II.**

This review protocol was registered at the International Prospective Register of Systematic Reviews (PROSPERO) with the ID number: CRD42022340742 (**Appendix III**).

2. Review Question

According to PRISMA guidelines, a specific PICO(T) question was formulated, with the following statements:

- **P** (Population)- temporomandibular joint cartilage in non-human animal models subjected to mechanical loading;
- **I** (Intervention)- mechanical stimulus;
- **C** (Comparison)- temporomandibular joint cartilage in non-human animal models not subjected to mechanical loading;
- **O** (Outcome)- histological and cellular alterations of the temporomandibular joint's cartilage;
- **T** (Time)- mechanical stimulus period.

3. Search strategy

A comprehensive electronic search was conducted in PubMed, B-On, and Scopus until December 31st, 2021. Based on the referred PICOT framework, a search strategy was used with the following terms and Boolean operators: "temporomandibular joint" OR "TMJ" AND "cartilage" AND "mechanical".

The search strategy mentioned above was used in all databases, but specific filters were applied according to the database:

- In PubMed: "Title/Abstract", "2011-2021", "English, Portuguese", "Full text";
- In SCOPUS: "Title/Abstract/Key-words", "2011-2021", "English", "Article", "Publication stage: final";
- In B-ON: "Abstract", "2011-2021", "English", "Integral text", "Academic journals", "Temporomandibular joint; mandibular condyle".

Hand-searching the list of references from other relevant articles was also performed to identify potential articles not covered by the previous search strategy, maximizing the number of eligible studies.

4. Eligibility criteria: inclusion and exclusion criteria

In order to answer the formulated question, the systematic review was performed following eligibility criteria stipulated *a priori.* The inclusion and exclusion criteria are presented in **Table 1.**

Table 1- Inclusion and exclusion criteria used in the research for the systematic review.

Inclusion criteria	Exclusion criteria
• *In vivo* experimental studies; • Effects on temporomandibular joint's cartilage; • Clinically applicable mechanical stimuli that alter the magnitude of TMJ loading: - Compressive force loading; - Differences in diet hardness - hard vs soft diet; - Increased occlusal vertical dimension; - Anterior mandibular displacement.	• Non-original studies (reviews, author responses, comments) or secondary research (systematic review and meta-analysis); • *In vitro*, 3D models or cadavers' experiences; • Studies missing control group; • Effects on other elements of TMJ which do not include cartilage; • Animals with previous TMJ pathology; • Animals being subjected to other simultaneous clinical interventions; • Animals subject to unbalanced or pathological mechanical loading; • Studies that consider sex differences; • Studies not written in English or Portuguese.

5. Study selection

After removing the duplicates, all the remaining articles were assessed carefully and independently by two calibrated reviewers (B.D. and M.C.): first, titles and abstracts were analysed, excluding the non-relevant studies, and subsequently, a full-text screening of the pre-selected articles was employed according to the eligibility criteria**.** Simultaneously, a manual search was carried out in the bibliography of the relevant studies. Only full-length articles written in Portuguese or English and published in the last 10 years were accepted and included. The articles which did not meet the formulated criteria were excluded.

6. Data extraction

The studies were organized into four groups, according to the type of mechanical loading: compressive force loading, diet hardness, increased occlusal vertical dimension, and anterior mandibular displacement.

Two independent reviewers (B.D. and M.C.) collected information of interest from the included studies in a Microsoft Office Excel 365 spreadsheet. The collected data included: author and year of publication, sample, experimental and comparison groups data, how the loading was applied (mechanical stimulus type, intensity, and period), and main findings. If required, the corresponding authors of the included studies were contacted via e-mail to clarify missing or unclear data. All disagreements during the selection of studies, data extraction and quality assessment were solved through discussion with a third reviewer (P.M.) to reach a consensus.

7. Risk of bias assessment (quality assessment)

To assess the quality of the articles analysed, we used the Systematic Review Centre for Laboratory Animal Experimentation (SYRCLE)'s risk of bias (RoB) tool (Hooijmans et al., 2014).

This tool is based on the Cochrane RoB tool and was modified to account for bias factors that are distinct in animal intervention studies. It contains ten separate domains (1. Sequence generation; 2. Baseline characteristics; 3. Allocation concealment; 4. Random housing; 5. Blinding; 6. Random outcome assessment; 7. Blinding; 8. Incomplete outcome data; 9. Selective outcome reporting; 10. Other sources of bias) related to 6 types of bias: selection bias, performance bias, detection bias, attrition bias, reporting bias, and other bias, presented in **Appendix IV.**

The risk of bias in the included studies was evaluated independently by the same two reviewers (B.D. and M.C.). For judging each domain, the respective signalling questions were answered with "yes", "no" or "unclear", when the information was not available, referring to low, high or unclear risk of bias, respectively. Domain 5 was classified as "not applicable" in some studies, once it was inapplicable in the review context.

The results were expressed in red, green, and yellow colours, which referred to high, low, and unclear risk of bias, respectively, in a stacked bar chart *(*summary plot*)* and double-entry table (traffic light plot) using Microsoft Office Excel 365 and Robvis tool (McGuinness & Higgins, 2021).

8. Statistical analysis

All statistics and plotting of meta-analyses and meta-regressions were performed using Open Meta Analyst software (Wallace et al., 2012). The extracted measures from the studies and the calculated values from the first were rounded to two decimal digits, as seen in **Appendix V**.

The meta-analyses model parameters were adjusted for random effects using DerSimonian & Laird (1986) method. The uncertainty associated with the mean values was presented as 95% confidence interval (CI). The degree of disagreement associated with the results was evaluated by heterogeneity index I^2, and when greater than 50%, the variance was considered high.

Subgroup meta-analyses were performed due to the presence of two different animal models: rats and mice. Subgroup outcomes were compared through Z test. This statistical test allows for comparing two means obtained from different populations and evaluating any significant differences between them.

The meta-analytic procedures were conducted to evaluate the mean wear of the cartilage, considering the normal physiological thickness, for each animal model subjected to compressive force loading.

Therefore, to assess the mean physiological cartilage thickness at the measurement time of studies, an animal model subgroup meta-analysis was performed with the cartilage thickness values of control groups and respective standard deviations.

Later, a second animal model subgroup meta-analysis was conducted in three steps: initially, a mean difference meta-analysis was performed to calculate the difference between cartilage thickness of experimental and control groups and the associated 95% CI. In studies with several experimental groups, each subjected to a different period of load action, the group subjected to the longest load time with significant results (according to the authors) was used. Next, the standard error was calculated from the 95% CI (see **Equation 1**). In the last step, the cartilage thickness differences, and the associated standard errors were adjusted for treatment differences between studies by making the ratio between each and the product of the applied loading by the respective load action period (μm/g.day). Next, these adjusted values were pooled in a new animal model subgroup meta-analysis.

Equation 1- Equation for the standard error. *SE*: standard error; $\bar{x}$: mean difference value; l: lower limit of 95% CI.

$$SE = \frac{[\bar{x} - l]}{1.96}$$

The percentage of cartilage wear was calculated by comparing the mean value of the cartilage wear obtained from the second meta-analysis to the mean value of physiological cartilage thickness assessed with the first meta-analysis (see **Equation 2**).

Equation 2- Equation for the cartilage wear percentage by loading time. $\overline{\frac{\Delta T}{I.t}}$: mean difference cartilage thickness, adjusted to compressive force loading and period; $\overline{T_C}$: mean cartilage thickness of control groups.

$$Cartilage\ wear\ \% = \frac{\overline{\frac{\Delta T}{I.t}}\ X\ 100}{\overline{T_C}}$$

In the Forest Plots, the square area represents the sample size. The continuous horizontal lines and the blue diamond width represent the 95% CI. The centre of the yellow diamonds indicates the estimate for each subgroup, while the centre of the blue diamond and the red vertical dotted line represent the pooled overall estimate.

In order to evaluate if initial animal age could have a statistically significant effect on the results of the meta-analysis, a meta-regression was also performed between this variable and the values of cartilage wear for the studies using rats, once there was only one study that used mice. The meta-regression resulted in a coefficient (Coef.), which measures the effect of the covariate under study on the cartilage thickness wear. The statistical significance of this covariate was evaluated through Z testing.

In all hypothesis tests, including Z tests, it was considered that there was statistical support for a difference whenever the result of the statistical test presented $p<0.05$.

III. Results

1. Literature identification/ search results

With the described literature search strategy, 521 articles were identified in the universal electronic databases PubMed (n=87), SCOPUS (n=132) and B-On (n=302). These results were exported to a Microsoft Office Excel 365 spreadsheet, in which the duplicates were removed (n=96), leaving 425 records. After screening by title and abstract, 27 articles were selected for a full-text assessment, following the inclusion and exclusion criteria. Of those, 13 articles were excluded, leaving 14 articles included. Hand-searching of the reference lists of the included reports and previous systematic reviews was also conducted, and 14 potential additional records were identified, from which 11 were considered for the qualitative analysis.

Add-together, 25 experimental studies were included in this systematic review, while 6 were considered eligible for the quantitative analysis, as described in **Figure 3**.

2. Qualitative synthesis

The 25 studies that were in accordance with the formulated inclusion criteria presented a wide range of distinct characteristics, namely: the country of origin, the allocation method, the animal population, the intervention type and period, and the outcomes.

The country of origin varied between China (Chen et al., 2019; Du et al., 2020; Huang et al., 2021; Jiang et al., 2017; Jiang et al., 2018; Jing et al., 2013; Li et al., 2013; Liu et al., 2019; Liu et al., 2014; Long et al., 2019; Shi et al., 2018; Wen et al., 2016; Zhang et al., 2021), Japan (Enomoto et al., 2014; Hichijo et al., 2014; Ikeda et al., 2014; Kato et al., 2015; Magara et al., 2012; Uekita et al., 2015), USA (Ravosa & Kane, 2017), Finland (Orajärvi et al., 2011, 2012), Germany (Gredes et al., 2012) and Brazil (de Sá et al., 2017; Figueroba et al., 2014).

The animals were randomly allocated into experimental and control/comparison groups in 16 studies (Chen et al., 2019; de Sá et al., 2017; Du et al., 2020; Enomoto et al., 2014; Figueroba et al., 2014; Gredes et al., 2012; Hichijo et al., 2014; Huang et al., 2021; Ikeda et al., 2014; Jiang et al., 2018; Jing et al., 2013; Liu et al., 2014; Long et al., 2019; Shi et al., 2018; Wen et al., 2016; Zhang et al., 2021), yet no information about the

randomization process was provided. The remaining 9 studies (Jiang et al., 2017; Kato et al., 2015; Li et al., 2013; Liu et al., 2019; Magara et al., 2012; Orajärvi et al., 2011, 2012; Ravosa & Kane, 2017; Uekita et al., 2015) did not mention any implemented randomization procedure.

Regarding animal population, 19 studies used rats population (Chen et al., 2019; de Sá et al., 2017; Figueroba et al., 2014; Hichijo et al., 2014; Huang et al., 2021; Ikeda et al., 2014; Jiang et al., 2017; Jiang et al., 2018; Kato et al., 2015; Li et al., 2013; Liu et al., 2014; Long et al., 2019; Magara et al., 2012; Orajärvi et al., 2011, 2012; Shi et al., 2018; Uekita et al., 2015; Wen et al., 2016; Zhang et al., 2021), 3 used mice (Du et al., 2020; Enomoto et al., 2014; Liu et al., 2019), 2 used rabbits (Jing et al., 2013; Ravosa & Kane, 2017), and the other one left used pigs (Gredes et al., 2012), with sample sizes ranging from 12 to 84 animals, resulting in a total of 909 animals (743 rats, 84 mice, 62 rabbits and 20 pigs). Male animals were used in 17 studies (de Sá et al., 2017; Du et al., 2020; Enomoto et al., 2014; Figueroba et al., 2014; Hichijo et al., 2014; Huang et al., 2021; Ikeda et al., 2014; Jiang et al., 2017; Jiang et al., 2018; Kato et al., 2015; Li et al., 2013; Long et al., 2019; Magara et al., 2012; Ravosa & Kane, 2017; Uekita et al., 2015; Wen et al., 2016; Zhang et al., 2021), while females were used in 7 studies (Chen et al., 2019; Gredes et al., 2012; Liu et al., 2019; Liu et al., 2014;Orajärvi et al., 2011, 2012; Shi et al., 2018), and in one other study (Jing et al., 2013) both sexes were used. The age at the beginning of the experiments varied from two weeks old to twelve weeks old, but most of the studies (n=7) used animals aged three weeks old.

All studies represented the consequences of several different types of mechanical stimulus that cause alterations in the physiological mechanical loading of TMJ cartilage. To enable a sensible comparison of results, the 25 studies were further organized into four distinct groups, according to the mechanical loading: compressive force loading, with 7 studies (Du et al., 2020; Huang et al., 2021; Jiang et al., 2018; Li et al., 2013; Magara et al., 2012; Wen et al., 2016; Zhang et al., 2021); anterior mandibular displacement (AMD), with 4 studies (Chen et al., 2019; de Sá et al., 2017; Gredes et al., 2012; Jing et al., 2013); increased occlusal vertical dimension (iOVD), with 3 studies (Figueroba et al., 2014; Liu et al., 2019; Long et al., 2019); and diet hardness, with 11 studies (Enomoto et al., 2014; Hichijo et al., 2014; Ikeda et al., 2014; Jiang et al., 2017; Kato et al., 2015; Liu et al., 2014; Orajärvi et al., 2011, 2012; Ravosa & Kane, 2017; Shi et al., 2018; Uekita et al.,

2015). The intervention period for the subgroups ranged from one day to twenty-six weeks, being that various studies investigated more than one-time interval.

The outcome parameters of interest concerning the cellular and morphological alterations in the temporomandibular joint's cartilage included: cartilage thickness; the number of chondrocytes; extracellular matrix components (collagen I, II, X, proteoglycans), inflammatory factors, matrix metalloproteinases and growth factors expression.

Various investigation methods assessed the effects caused by the referred mechanical loading in the cartilage: histology and histomorphometry, to measure cartilage thickness; immunohistochemistry, isolation of RNA and qRT-PCR, and TUNEL staining, to perform cell count and analyse the expression of several genes.

The main characteristics of the included experimental studies are summarized in **Table 2**, **Table 3**, **Table 4** and **Table 5**.

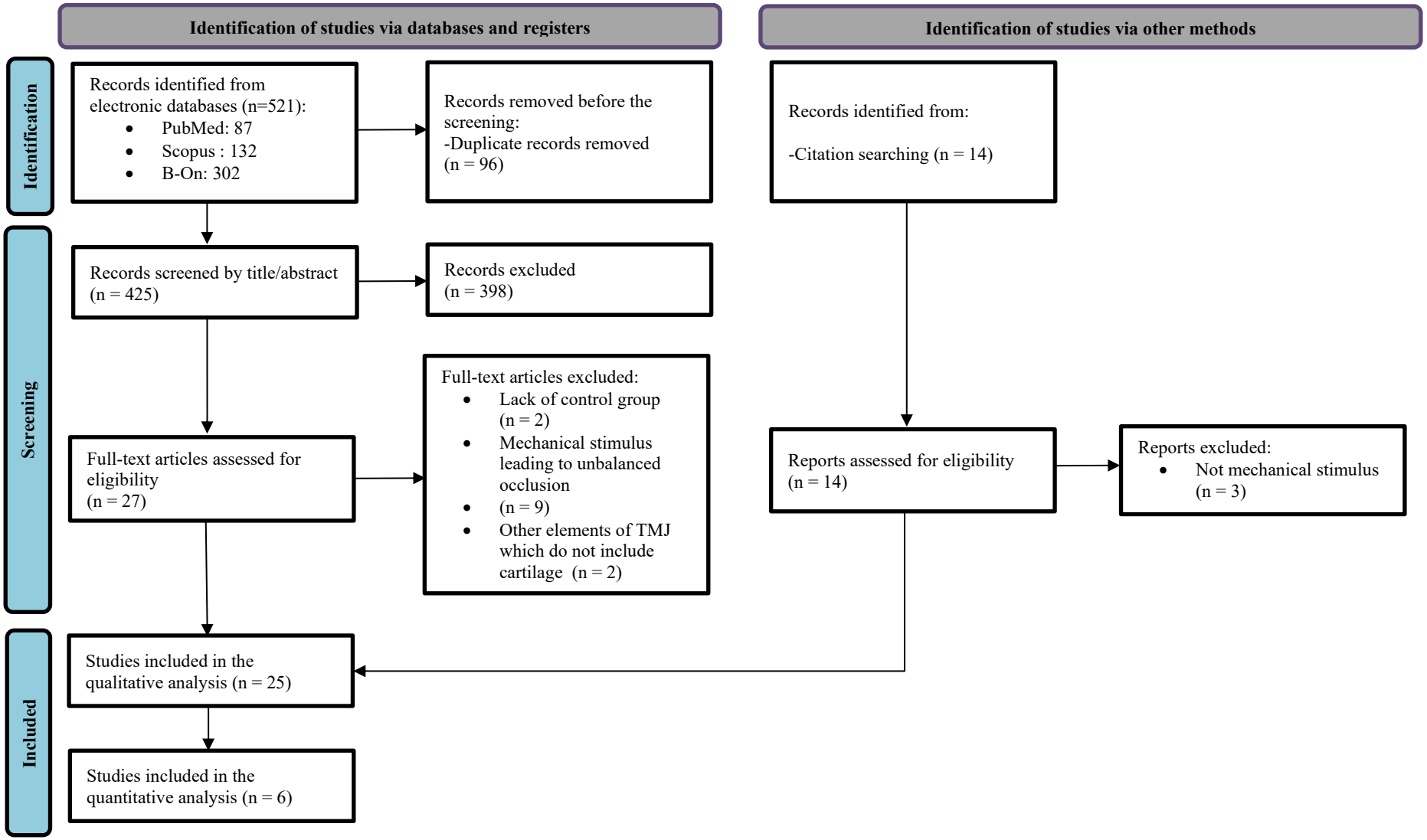

Figure 3- Flowchart of the literature search and study selection, according to PRISMA guidelines.

2.1. Systematic Review Tables

Table 2 - Characteristics of the experimental studies included in the systematic review: compressive force loading.

Study (Author, year)	**Sample (Population/Age/Gender/Weight)**	**How loading was applied (Magnitude of the compressive force loading)**	**Main findings in experimental groups**
Du et al. 2020	12 mice (*C57BL/8-week-old/male/20-25g)* 2 groups	EG= 40g CG=0g	- Thinner total cartilage thickness and less amount of ECM in the central area, after 1 week; - Increased IL-1β, MMP-3 and MMP-13 levels in the central area, after 1 week.
Huang et al. 2021	42 rats (*Sprague-Dawley/8-week-old/male)* 2 groups	EG= 80g CG= 0g	- Thinner cartilage and decreased number of chondrocytes in the central area, after 2 weeks; - Increased TNF- α, IL-1β and IL-6 levels in the central area, after 2 weeks.
Jiang et al. 2018	48 rats (*Sprague-Dawley/6-week-old/male/180-200g)* 4 groups	EG1; EG2= 80g CG1; CG2= 0g	- Thinner total cartilage in the central area, after 4 days, more evidenced after 1 week; - Decreased number of chondrocytes and increased apoptosis after 4 days in the central area, more remarkable after 1 week; - Increased TNF-α and IL-1 levels in the central area, after 4 days and 1 week; - Reduced collagen II and X levels after 4 days in the central area, and lower levels after 1 week.
Li et al. 2013	64 rats (*Sprague-Dawley/8-week-old/male)* 8 groups	EG1; EG2; EG3; EG4= 40g CG1; CG2; CG3; CG4= 0g	- Thinner total cartilage slightly after 3 days, but much more evidenced after 14 days; the thickness moderately increased at 21 days; - Disordered and disarranged, and reduced number of chondrocytes, from 3 days until 14 days, significantly remarkable in the last period; - Dramatic apoptosis at 3 days, but largely reduced at 7 and 14 days; - Decreased collagen II and X expression and reduced amount of ECM, after 14 days.

Magara et al. 2012	32 rats (*Wistar/male/8-week-old)* 3 groups	EG1; EG2= 50g CG= 0g	- Thinner hypertrophic cell layer and an acellular region in mature cell layer in the central area, after 5 days.
Wen et al. 2016	45 rats (*Sprague-Dawley/6-week-old/male/180-220g)* 9 groups	EG1; EG2; EG3= 40g EG4; EG5; EG6= 80g CG1; CG2; CG3= 0g	- Thinner total cartilage thickness in the posterior part of central area after 1 day, more remarkable in heavy compressive groups and 1 week after; - More lacunas in proliferative cell layer, from where chondrocytes penetrated into hypertrophic cell layer and subchondral bone in the posterior part of central area, after 1 day; - Cell disorder and disarrangement and decreased number of chondrocytes in the posterior part of central area, gradually with the increase of load from 1 day to 1 week.
Zhang et al. 2021	84 rats (*Sprague-Dawley/7-week-old/male)* 4 groups	EG1; EG2= 80g CG1; CG2=0g	- Thinner total cartilage, after 4 days, more evidenced after 1 week; - Increased TNF-α expression within 4 days, more remarkable after 1 week; - Decreased proteoglycan and collagen II expression, after 4 days, and even more after 1 week.

Legend: CG, control group; ECM, extracellular matrix; EG, experimental group; IL, interleukin; MMP, matrix metalloproteinase; TNF, tumoral necrose factor.

Table 3 - Characteristics of the experimental studies included in the systematic review: anterior mandibular displacement (AMD).

Study (Author, year)	**Sample (Population/Age/Gender/Weight)**	**How loading was applied (AMD appliance type)**	**Main findings in experimental groups**
Chen et al. 2019	50 rats *(Sprague-Dawley/5-week-old/female)* 8 groups	EG1; EG2; EG3; EG4= bite jumping device cemented to upper incisors CG1; CG2; CG3; CG4= no appliance	- Lubricin expression increased from 1 to 2 weeks and decreased at 3 weeks, in hypertrophic and proliferative cell layers of the posterior area.
De Sá et al. 2017	30 rats *(Wistar/5-week-old/male)* 6 groups	EG1; EG2; EG3= oblique plane cemented to upper and lower incisors CG1; CG2; CG3= no appliance	- Total cartilage thickness decreased after 1 week, increased after 3 weeks, and after 4 weeks decreased again, in the posterior and central areas; - Proliferative cell layer thickness always increased, while mature and hypertrophic cell layers thickness decreased in the posterior and central areas at 1 weeks and then increased at 3 weeks.
Gredes et al. 2012	20 pigs *(Sus scrofa domestica/10-week-old/female)* 2 groups	EG= oblique plane cemented to upper and lower last premolars and first molars CG= no appliance	- Increase in collagen II, MMP-8 and VEGF expression; decrease in collagen X expression, after 2 weeks; - No differences in collagen I and MMP-13 expression, after 2 weeks.
Jing et al. 2013	60 rabbits *(Japanese white /8-week-old/1000-1500g)* 12 groups	EG1; EG2; EG3; EG4; EG5; EG6 = oblique plane cemented to upper central incisors CG1; CG2; CG3; CG4; CG5; CG6= no appliance	- Ang-1 and Ang-2 expression mainly increased, predominantly in the hypertrophic layer of the posterior area, within 12 weeks.

Legend: Ang, angiopoietin; CG, control group; EG, experimental group; MMP, matrix metalloproteinase; VEGF, vascular endothelial growth factor.

Table 4 - Characteristics of the experimental studies included in the systematic review: increased occlusal vertical dimension (iOVD).

Study (Author, year)	**Sample (Population/Age/Gender/Weight)**	**How loading was applied (OVD)**	**Main findings in experimental groups**
Figueroba et al. 2014	20 rats *(Rattus norvegicus albinus /12-week-old/male/ 350-400g)* 2 groups	EG= 0,9mm on upper molars CG= 0mm on upper molars	- Cellular disarrangement, decreased proteoglycan density and increased IL-1α, IL-1β, IL-6 and TNF-α levels in all the three areas of all layers, after 2 weeks.
Liu et al. 2019	36 mice *(C57/6J /6-week-old/female/17-19g)* 6 groups	EG1; EG2; EG3= 1,5mm on upper incisors and 3mm on lower incisors CG1; CG2; CG3= 0mm on upper and lower incisors	- Increase in cartilage thickness of superficial layers, while the hypertrophic layer became thinner in the central area, with the increasing time; - Increase in chondrocytes number and expression of Cyclin D1 and Aggrecan in the superficial layers, and in the extracellular matrix amount in the central area, from 3 to 11 weeks; - The cells in the superficial layers were flat and tightly bonded, but there were no morphological differences in the hypertrophic layer in the central area, with the extension of time; - Increase in Collagen II in the central area at 3 weeks, but decreased at 7 and 11 weeks;
Long et al. 2019	48 rats *(Sprague-Dawley/9-week-old/male)* 8 groups	EG1; EG2; EG3; EG4= 1,5mm on upper first molars and 1mm on upper third molars CG1; CG2; CG3; CG4= 0mm on upper first and third molars	- Cell disorganization, clustering of chondrocytes, the appearance of lacunas and the disappearance of nucleolus, after 3 days; - Increase in the Ihh expression after 1 week, but then decreased with the extension of time; - Chondrocytes shrink, decrease in glycosaminoglycan content, decrease of cartilage thickness and enlargement of cartilage lacuna, from 2 to 4 weeks; - Decrease in MMP-13 and Caspase-3 after 2 weeks, but then increased at 4 weeks; it gradually extended from the hypertrophic layer to the superficial part of endochondral ossification; - Reduction in the number of chondrocytes, after 4 weeks.

Legend: CG, control group; EF, experimental group; Ihh, indian hedgehog protein; IL, interleukin; MMP, matrix metalloproteinase; OVD, occlusal vertical dimension; TNF, tumoral necrosis factor.

Table 5 - Characteristics of the experimental studies included in the systematic review: diet hardness.

Study (Author, year)	**Sample (Population/Age/Gender/Weight)**	**How loading was applied (Diet type)**	**Main findings in SD groups**
Enomoto et al. 2014	36 mice *(CD-1/3-week-old/male)* 4 groups	SD1; SD2=liquid HD1; HD2= pellet	- Thinner hypertrophic cell layer thickness in the central area in SD groups, after 1 week and even more after 4 weeks; - Decreased collagen X in hypertrophic cell layer in the central area in SD groups, after 1 week, and more remarkable after 4 weeks.
Hichijo et al. 2014	14 rats *(Wistar/3-week-old/male)* 2 groups	SD=powder HD=solid	- Thinner total cartilage in the central area, with the cell layers not easily distinguished, in SD group after 11 weeks; - Cell disarrangement and reduced number of chondrocytes in the central area, in SD groups after 11 weeks; - Significantly lower expression of IGF-1r in the central area, in SD groups after 11 weeks.
Ikeda et al. 2014	20 rats *(Wistar/3-week-old/male)* 2 groups	SD=liquid HD=solid	- Thinner total cartilage, especially in hypertrophic cell layer, in all 3 areas, in SD group after 7 weeks; - No significant differences in MMP-13 expression between SD and HD groups, after 7 weeks.
Jiang et al. 2017	80 rats *(Sprague-Dawley/2-week-old/male)* 2 groups	SD=powder HD=pellet	- Thinner total cartilage in all the 3 areas (more remarkable in the anterior area), in SD group after 4 weeks; - Decreased VEGF and MAPK levels in all the three areas, in SD group after 4 weeks; - VEGF staining mainly in proliferative and mature cell layer, and lightly in hypertrophic cell layer in all the three areas in SD group, while more remarkable in the last cell layer, in HD group after 4 weeks.
Kato et al. 2015	24 rats *(Wistar/3-week-old/male)* 6 groups	SD1; SD2; SD3=liquid HD1; HD2;HD3= solid	- Cartilage thickness was similar between SD and HD groups after 1 week; fibrous, proliferative and hypertrophic cell layers thickness decreased in SD groups, after 4 weeks and more remarkably after 8 weeks; - Reduced number of proliferating cells in SD groups, mainly after 1 and 4 weeks.

Liu et al. 2014	20 rats (*C57BL/6J /6-week-old/female/17-19g)* 2 groups	SD=ground HD= pellet	- Cartilage thickness and collagen II, Aggrecan, ADAMTS-5, OPG and RANK levels were similar between SD and HD groups, after 3 weeks.
Orajärvi et al. 2011	16 rats *(Sprague-Dawley/3-week-old/female)* 2 groups	SD=powder HD=pellet	- Thinner proliferative and mature cell layers in the central area, in SD group after 7 weeks; - No differences in fibrous cell layer thickness between SD and HD groups, after 7 weeks; - Decreased MMP-8 expression in the central area, in SD group after 7 weeks.
Orajärvi et al. 2012	16 rats *(Sprague-Dawley/3-week-old/female)* 2 groups	SD=powder HD=pellet	- Cartilage thickness and MMP-13 expression were similar in all the 3 areas between the HD and SD groups, after 7 weeks; - Decreased Collagen II and X area in all the 3 areas, in SD group after 7 weeks.
Ravosa & Kane 2017	12 rabbits *(Oryctolagus cuniculus/4-week-old/male)* 3 groups	SD=powder HD1=pellet HD2=pellet + 2,5cm hay blocks	- Decreased hypertrophic cell layer thickness from SD to HD1 and HD2 groups, after 26 weeks; - Increased proteoglycan levels in SD group, after 26 weeks.
Shi et al. 2018	40 rats *(Sprague-Dawley/5-week-old/female/180-200g)* 2 groups	SD=powder HD=pellet	- Thinner total cartilage in the central area, in SD group after 4 weeks; - Decreased chondrocyte volume in proliferative and hypertrophic cell layers of the central area, in SD group after 4 weeks; - Reduced proteoglycan, collagen II and X expression and Ihh levels in the central area, in SD group after 4 weeks.
Uekita et al. 2015	48 rats *(Wistar/3-week-old/male)* 8 groups	SD1; SD2; SD3; SD4=liquid HD1; HD2; HD3; HD4 =solid	- Thinner total cartilage in SD groups, after 2 weeks, and even more after 8 weeks; - Decreased collagen I and II expression in both mature and hypertrophic, and type X in hypertrophic cell layer in SD groups; no differences in these components in fibrous and proliferative cell layers between SD and HD groups, at 2 weeks; - Fewer collagen fibrils in ECM of SD groups, from 1 to 8 weeks.

Legend: ADAMTS, a disintegrin and metalloproteinase with thrombospondin motif; HD, hard diet; IGF, insulin growth factor; Ihh, Indian hedgehog protein; MAPK,mitogen-activated protein kinase; MMP, matrix metalloproteinase; OPG, osteoprotegerin; RANK, receptor activator of nuclear factor kappa; SD, soft diet; VEGF, vascular endothelial growth factor.

2.2. Summary of main findings

Overall, all cartilage cell layers in the three sagittal section areas (anterior, central and posterior) were affected by mechanical loading, showing histological and cellular changes. Although the different types of stimuli, the methodology of the included studies was very similar in each group.

The compressive force loading experiments were performed with extra-oral appliances consisting of a band tied between a harness around the chest, and anchorage hooks bonded to both lower incisors or a cap supporting both angles of the mandible. The studies in which anterior mandibular displacement was the main topic used oblique planes cemented to incisors, in rats and rabbits samples, or to premolars and molars, in the case of the pig sample. The increased occlusal vertical dimension was achieved in the experiments by resin build-ups bilaterally bonded to maxillary molars, in the rats' samples, or metal tubes bonded to upper and lower incisors on both sides, in the mice experiment. At last, in diet hardness studies, some animals were fed a soft diet (powder, liquid or ground pellet), while the left ones were fed a hard diet (solid pellet).

In general, the animals subjected to compressive force reveal a decrease in cartilage thickness, number of chondrocytes and extracellular matrix components, with an increase in matrix metalloproteinases and inflammatory factors. This also occurs in the animals subjected to a soft diet, contrary to those subjected to a hard diet, in which the cell layers were more easily distinct and organized. Concerning AMD group, histological and immunohistochemical alterations are also visible, so it can be affirmed that this stimulus impacts the TMJ cartilage. Finally, in the iOVD group, the outcomes of the included studies were contradictory.

The main findings also reveal that the referred cellular adaptations were co-related with the experiment period, being noticeable after 1 day and generally more evident with the increase of time, and with the load intensity that the TMJ is subjected to.

2.3. Risk of bias (quality assessment)

After the risk of bias assessment of the 10 domains of each study included in the systematic review, the results were presented in the form of tables - traffic light plots (**Figure 4**, **Figure 6**, **Figure 8** and **Figure 10**)- and graphs- summary plots (**Figure 5**, **Figure 7**, **Figure 9**, **Figure 11**)-, for each of the four mechanical loading groups considered (McGuinness & Higgins, 2021).

Overall, the two domains in which the studies showed more weakness were baseline characteristics (D2), as some studies do not refer to the initial weight of the animals and missing information regarding this topic justifies a high risk of bias; and incomplete outcome data (D8), due to few studies do not include all the animals in the analysis (high risk of bias) or do not report if all the animals were included, leading to an unclear risk of bias. The selective outcome reporting domain (D9) was always classified as low risk of bias, once all study protocols as well as the primary and secondary outcomes were available and reported in the included studies. The blinding domain (D5) was mainly rated with "not applicable", particularly in the compressive force loading studies, since it consists of extra-oral appliances, and in the anterior mandibular displacement studies and the increased occlusal vertical dimension, as the appliances bonded to the animal's teeth were frequently checked by the investigators to prevent any failure.

The allocation concealment (D3), random housing (D4), and random outcome assessment (D6) domains were rated with unclear risk of bias in most of the considered articles, as they miss data concerning these entries. On the other hand, the sequence generation (D1) and blinding outcome assessment (D7) were mainly classified as low risk of bias, since most of the studies refer to a random component in the sequence generation and the blinding of investigators was ensured.

Finally, the last domain concerning other sources of bias (D10) was classified as low risk of bias in half of the studies and unclear in the left half, as these last ones do not refer to if there was an inappropriate influence of funders or conflict of interests.

Analysing the graphs and tables of each loading modality, it is possible to conclude that both in the compressive force loading and in the increased occlusal vertical dimension groups, 2 studies per group (Du et al., 2020; Figueroba et al., 2014; Jiang et al., 2018; Long et al., 2019) did not present any domain classified as high risk of bias, while both

in anterior mandibular displacement and diet hardness groups, there was only 1 study per group (Jing et al., 2013; Shi et al., 2018) without domains rated with a high risk of bias. The left studies (Chen et al., 2019; de Sá et al., 2017; Enomoto et al., 2014; Gredes et al., 2012; Hichijo et al., 2014; Huang et al., 2021; Ikeda et al., 2014; Jiang et al., 2017; Kato et al., 2015; Li et al., 2013; Liu et al., 2019; Liu et al., 2014; Magara et al., 2012; Orajärvi et al., 2011, 2012; Ravosa & Kane, 2017; Uekita et al., 2015; Wen et al., 2016; Zhang et al., 2021) have one or two entries classified in that form.

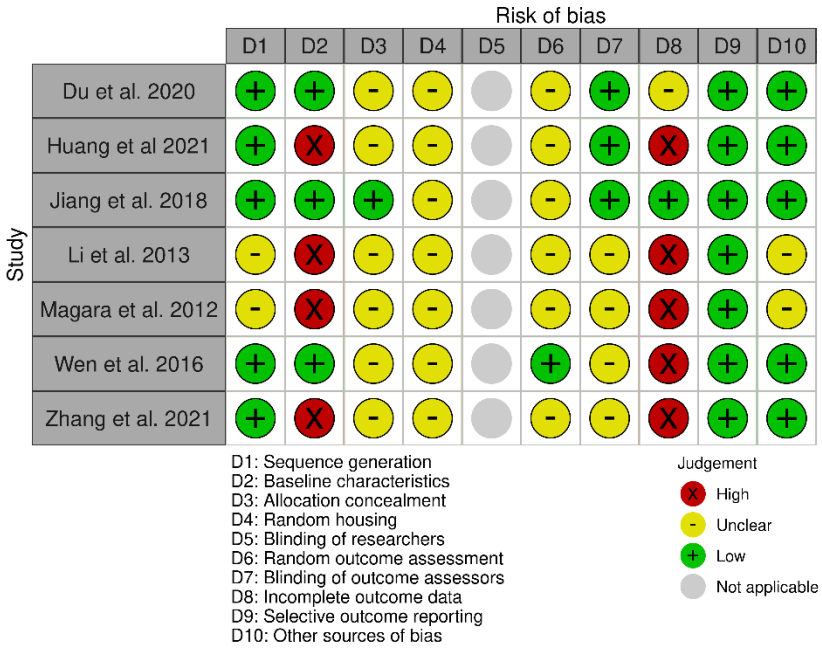

Figure 4 - Traffic light plot (RobVis tool) for compressive force loading's risk of bias assessment.

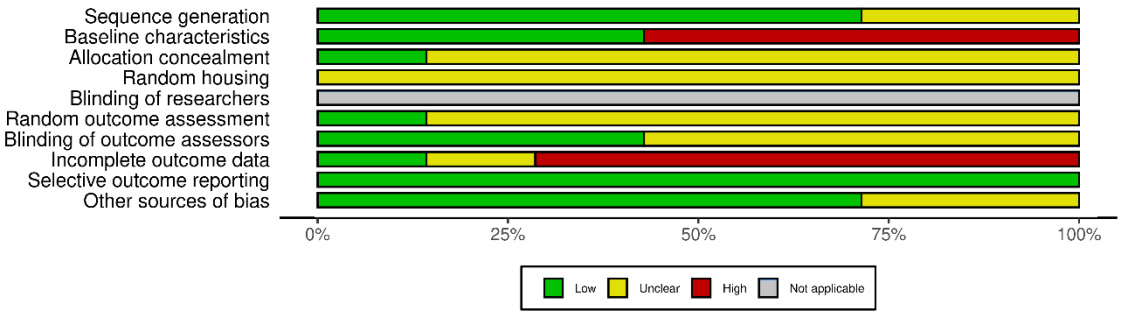

Figure 5 - Summary plot (RobVis tool) for compressive force loading's risk of bias assessment.

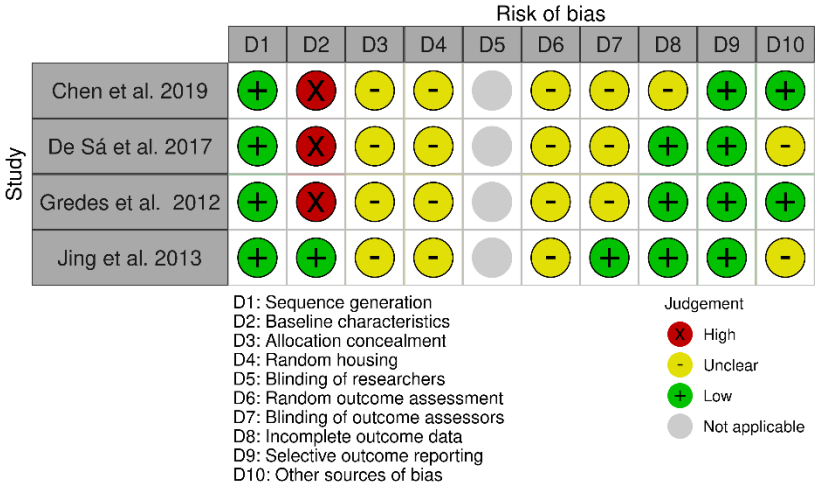

Figure 6 - Traffic light plot (RobVis tool) for anterior mandibular displacement's risk of bias assessment.

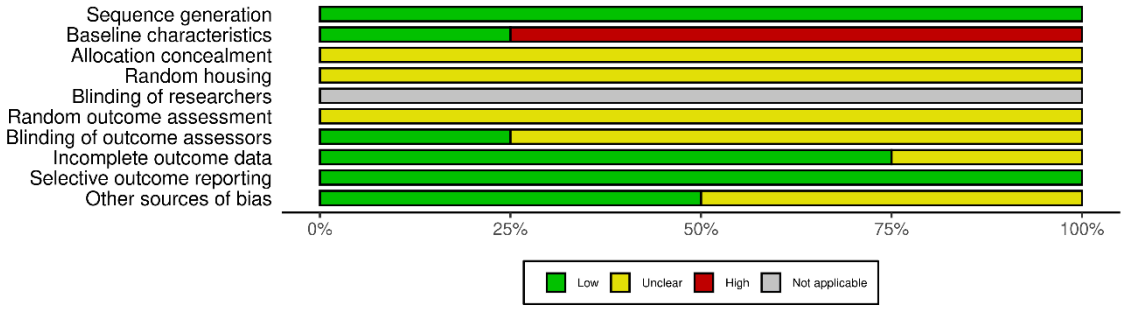

Figure 7 - Summary plot (RobVis tool) for anterior mandibular displacement's risk of bias assessment.

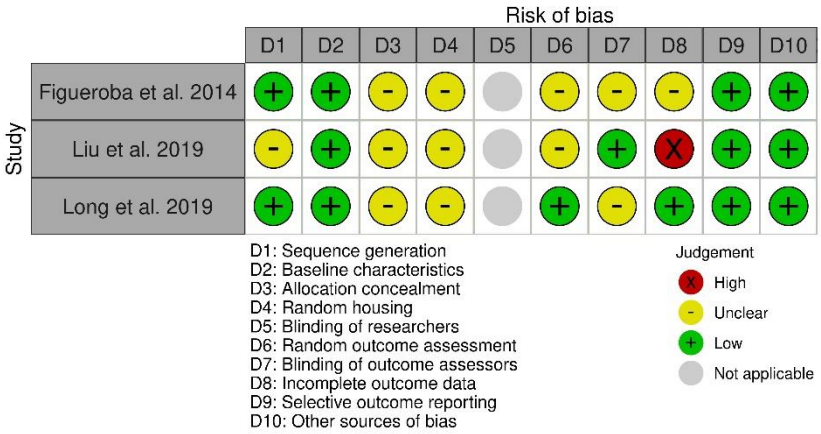

Figure 8 - Traffic light plot (RobVis tool) for increased occlusal vertical dimension's risk of bias assessment.

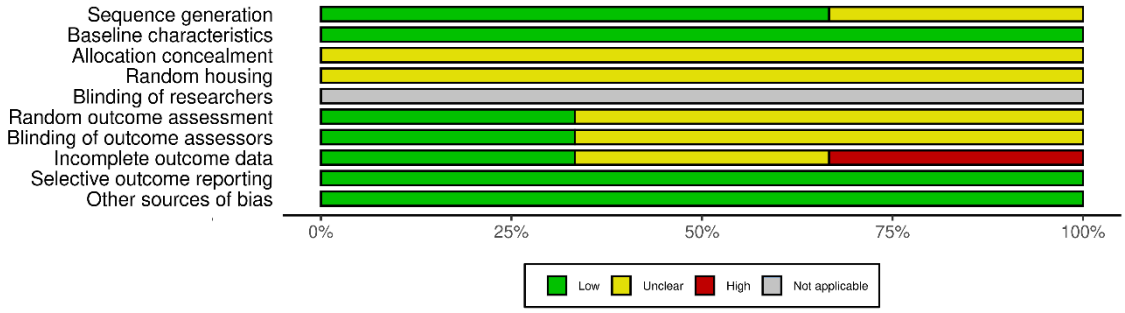

Figure 9 - Summary plot (RobVis tool) for increased occlusal vertical dimension's risk of bias assessment.

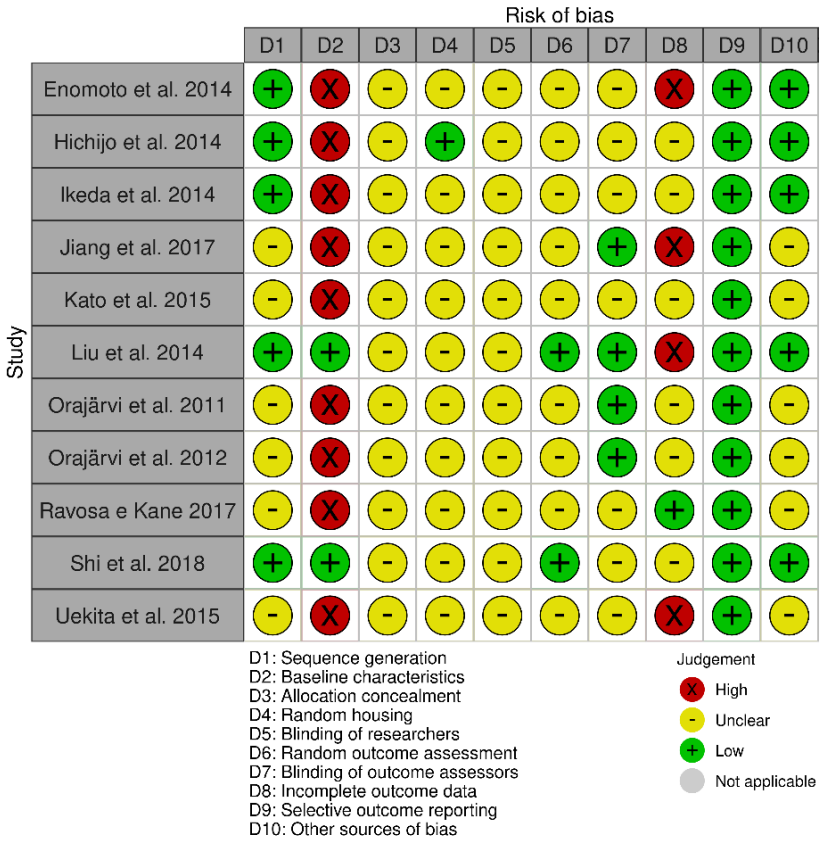

Figure 10 - Traffic light plot (RobVis tool) for diet hardness' risk of bias assessment.

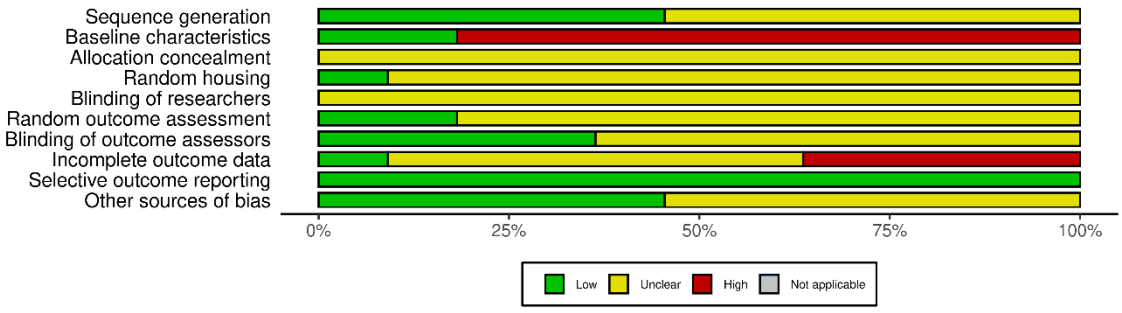

Figure 11 - Summary plot (RobVis tool) for diet hardness' risk of bias assessment.

3. Meta-Analysis (quantitative synthesis)

The Meta-analyses graphics, called Forest Plots, summarise individual information of the included studies results. In this meta-analytic procedure, six articles from the compressive force loading group were included, five respective to rats (Huang et al., 2021; Jiang et al., 2018; Li et al., 2013; Wen et al., 2016; Zhang et al., 2021) and only one respecting mice (Du et al., 2020), performing a total of 140 animals, 70 as controls and other 70 as experimental samples. Since the mice subgroup was composed of one single article, this limited the available evidence and made it impossible to determine heterogeneity.

The first animal model subgroup meta-analysis was performed with the cartilage thickness values of the control group and respective standard deviations, to evaluate the normal physiological cartilage thickness, showed in **Figure 12**.

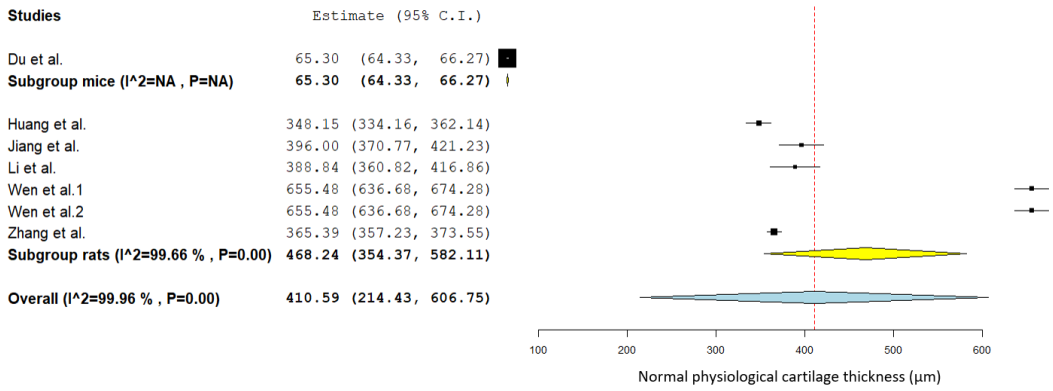

Figure 12 - Forest plot of animal model subgroup meta-analysis for the mean value of normal physiological cartilage thickness (control groups).

The mean value of normal physiological cartilage thickness for rats was 468.24µm (± 113.87), with a heterogeneity of 99.66%, while for mice was significantly lower, 65.30µm (± 0.97) (one mice study included).

To perform the second meta-analysis, first, the difference between cartilage thickness of experimental and control groups was obtained for each study through a mean difference meta-analysis, displayed in **Figure 13.**

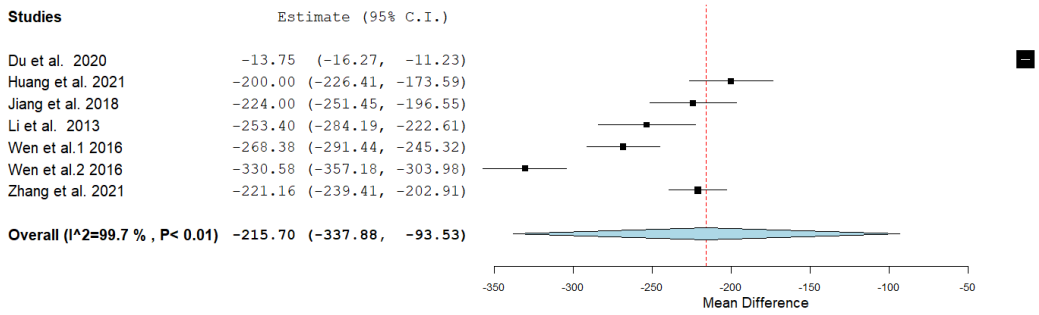

Figure 13 - Forest plot of mean difference meta-analysis for the cartilage thickness between control and experimental groups.

The mean difference values and the respective 95% CI allowed standard error calculated for each study. These values were adjusted to the applied loading, 40 or 80g, and respective period, 7 or 14 days, for each study. The final Forest Plot is displayed in **Figure 14.**

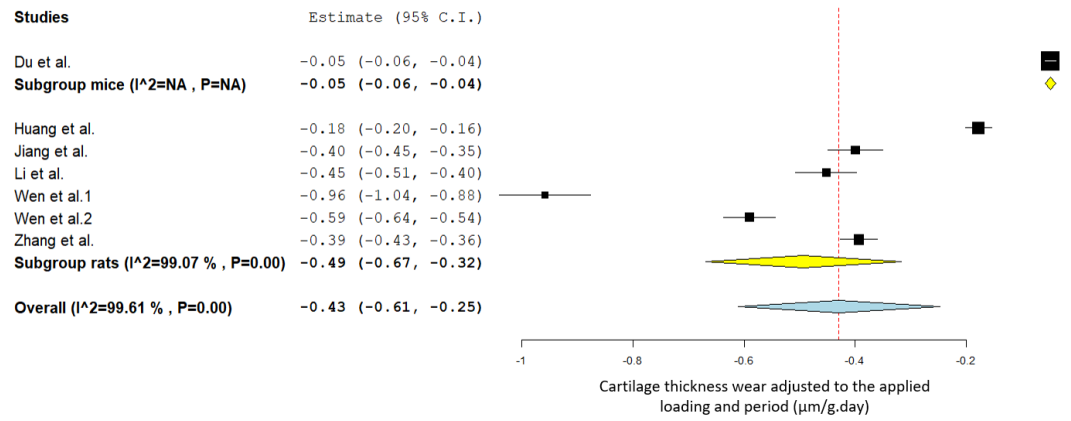

Figure 14 - Forest plot of animal model subgroup meta-analysis for the cartilage thickness wear, adjusted to the applied loading and period.

The mean difference value of cartilage wear was greater in rats (-0.49µm/g.day ± 0.18) than in the single mice study (-0.05µm/g.day ± 0.01), with an associated heterogeneity of 99.61%. As in the first meta-analysis, results revealed a significant difference in the wear cartilage thickness between the two animal models ($p<0.01$, Z test). Within the rats' subgroup, there was an associated heterogeneity of 99.07%, which lead to an investigation of the reasons associated with it, namely the effect of the initial age of the rats, as seen in **Figure 15**.

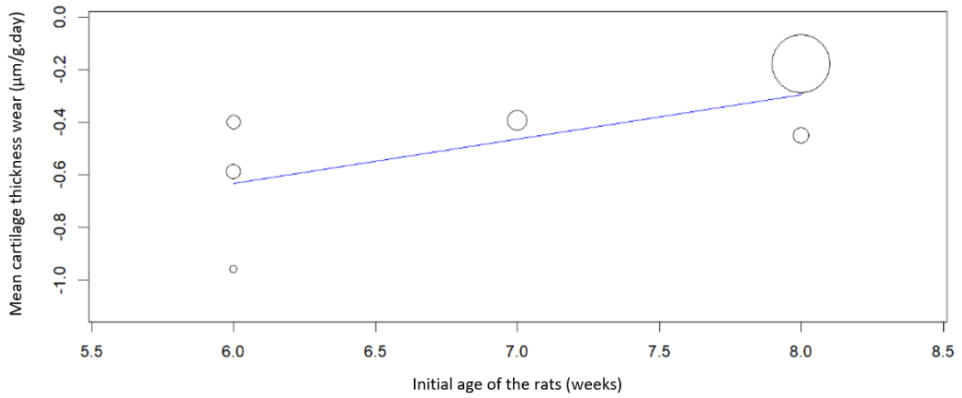

Figure 15 - Meta-regression graphic of the initial age of the rats (weeks) covariate versus mean cartilage thickness wear.

The meta-regression shows a significant tendency for less cartilage wear absolute value with increasing rats' initial age (Coef. =0.169, p=0.042).

With the mean value of rats' physiological cartilage thickness (468.24µm) and cartilage wear (0.49µm/g.day) obtained through the mentioned meta-analyses, the percentage of cartilage wear in rats was calculated, having a value of 0.1%.

IV. Discussion

1. General Considerations

The articular surfaces of the temporomandibular joint are covered by fibrocartilage, which plays an important role as a stress absorber structure and whose metabolism is upregulated by mechanical loading. Considering this, the present review aimed to systematically assess the evidence on the histomorphological alterations in the TMJ's fibrocartilage in response to four modalities of mechanical loading: compressive force loading, anterior mandibular displacement, increased occlusal vertical dimension, and diet hardness. Additionally, a meta-analysis was conducted to estimate the cartilage wear resulting from the compressive force loading, adjusted to the applied strength and respective period.

All these mechanical stimuli allow for modifying jaw growth and are previously tested before putting into clinical practice. The current literature includes *in vivo* and *in vitro* studies on this topic. *In vitro* studies might fail to capture the inherent complexity of organ systems, thus it's not always viable to extrapolate the conclusions to what occurs *in vivo.* Yet, *in vitro* studies should not be discarded, as they allow evaluating the biomechanical properties of fibrocartilage through mechanical loading tests (Chandrasekaran et al., 2017; Chen et al., 2015; Chin & Almarza, 2020). These are crucial in understanding the process of pathogenesis and also in tissue engineering, for the replacement or regeneration of damaged joint fibrocartilage (Gong et al., 2022; Hagandora et al., 2011).

As *in vitro* studies have different experimental designs from *in vivo* ones, it would not be possible comparing both results with each other. Therefore, only *in vivo* studies were considered in this systematic review, assuring more homogeneity in the included studies' methods.

Despite moral and ethical concerns, animals play a critical role in clinical research, serving as a bridge between basic research and clinical practice. The absence of a single and well-established animal model for the study of TMJ, its pathologies and treatments, has been a limiting factor in the investigations. That's why several animal models have been used to study this joint's structures (Cougo et al., 2021; Xiang et al., 2021).

Mice and rats are the most used animal models in TMJ studies due to the low cost and easy handling, but the differences in anatomical structures and joint mechanics between

these species and humans are notorious. Human TMJ is much larger than the rodent one and there is no articular eminence in these animals, being the articular fossa flat and allowing extensive protrusive mandibular movements. Concerning the condylar fibrocartilage, this tissue contains more layers of superimposed chondrocytes and a thicker fibrous layer in humans than in rodents (Chin & Almarza, 2020; Cougo et al., 2021; Suzuki & Iwata, 2016).

Rabbits represent a larger rodent, in which mechanical experiments can also be performed, and in addition, it provides large enough tissues for *in vitro* reliable mechanical testing. This animals' fibrocartilage is similar to that of humans regarding the distribution of type I and type II collagen and proteoglycans, but in contrast is thicker in the mid-region rather than the posterosuperior (Almarza et al., 2018; Cougo et al., 2021).

Pigs are considered the most suitable experimental model for human TMJ, as they closely match humans in terms of anatomy and physiology, particularly in condyle and disc shape. There is further evidence that the mechanical properties of porcine TMJ are also comparable to those of human TMJ (Almarza et al., 2018; Cougo et al., 2021).

Sheep have also been revealed to be an interesting animal model to study TMJ, particularly in the surgical domain, due to many similarities to human beings (Almarza et al., 2018; Zhao et al., 2022). However, no studies concerning the impact of mechanical loading on TMJ's fibrocartilage of sheep were found during the research of this work.

The studies considered in this systematic review approached a wide variety of animals, including mice, rats, rabbits, and pigs. However, the reduced number of experiments in rabbits (n=2) and pigs (n=1) limits the conclusions concerning the effect of mechanical loading within the proper animal species, and even more, compromises the extrapolation of results to humans. The higher costs and more extended maturation periods associated with larger animals might explain the lack of studies using these species (Zhao et al., 2022).

Apart from these, few experiments using mice (n=3) were included. Despite the similarities with rats, mice are smaller, weigh significantly less than rats, and are also more prone to stress from repeating handling (Kent Scientific, 2019). This might be the reason why rats are a preferred model in the compressive force loading and the anterior mandibular displacement groups, in which mechanical loading devices are used, and in

the increased occlusal vertical dimension group. In diet hardness experiments, mice could be favoured over rats as they require less food quantity, but the risk of damage manipulating the fibrocartilage tissue is certainly higher due to its smaller size, which must justify the largest number of rats studies in the diet hardness group too.

Independently from the animal model, all four types of mechanical stimulus promoted histological and cellular changes in condylar fibrocartilage, eventually affecting condylar growth.

2. Compressive force loading

In compressive force loading experiments, it was applied an extra-oral mechanical device that supported the mandible of rats and mice by a chest harness, transmitting compressive forces to the area of the mandibular condyle.

The mechanism of these animal devices is very similar to the chin-cup appliance, an orthodontic treatment modality for the clinical management of mandibular prognathism in growing patients (Karamesinis & Basdra, 2018; Mousoulea et al., 2016; Zurfluh et al., 2015).

Chin-cup appeared in the 19th century intending to restrict the mandibular growth of these patients by applying compressive forces to the mandibular condyle (Zurfluh et al., 2015). Proffit et al. (2019) affirm that human children would not tolerate the amount of force and/or the number of hours per day needed to prevent jaw growth, resulting in TMJ trauma.

Accordingly, in the included studies of this systematic review, rats and mice subjected to compressive force loading have revealed fibrocartilage degradation evidenced by thinner total cartilage, decreased amount of ECM, and reduced number of chondrocytes. Furthermore, the metabolic activity of fibrocartilage was highly marked by the increased levels of inflammatory cytokines such as interleukin-1β (IL-1β), interleukin-6 (IL-6) and tumor necrosis factor-α (TNF-α). These catabolic factors suppress extracellular matrix synthesis and stimulate the expression of matrix metalloproteinases (MMPs), which are responsible for cleaving collagen fibers and degrading aggrecan (Mueller & Tuan, 2011). These alterations are coincident with the pathological changes of TMJ-OA, characterized

by chronic pain and dysfunction of the TMJ along with the reduced quality of life (Bianchi et al., 2020; Li et al., 2021; Wang et al., 2015).

The studies also show that these pathological effects are even more notorious with the increase in time and load intensity. To avoid this, clinically lighter forces in the direction below the mandibular condyle are currently used, which leads to the backward and downward mandibular rotation, and creates a new growth pattern in the condyle, not restricting it (Proffit et al., 2019).

Through a meta-analysis, it was possible to reach an average value of the cartilage thickness reduction in rats, adjusted to the applied loading and respective period. It included five studies using rats and only one using mice, the reason why the last one was not considered. However, it is possible to verify a noteworthy difference between the cartilage thickness of mice and rats, which goes along with the size differences between these animals and justifies the exclusion of the mice's study in the calculi of the percentage of cartilage wear.

Within rats' studies, both mean cartilage thickness and mean difference values revealed a heterogeneity above 99%. This might be due to the initial age of the rats, a covariate that has shown to have a significant effect on the cartilage wear through a meta-regression. Nonetheless, other aspects might be contributing to this heterogeneity, namely the results of both Wen et al. (2010) studies, which stand out compared with the remaining ones. This discrepancy is unclear because these studies' methods were similar to other included studies. We suggest that it might be due to the mentioned higher weight of the animals, advocating a possible correlation of the animal's weight and the cartilage thickness, as it occurs in mice. However, only one left study mentioned the weight of the rats, so it is impossible to confirm this hypothesis.

Additionally, we assessed the percentage of thickness wear resulting from the compressive forces loading, adjusted to the applied force and period, meaning that per each gram of compressive force, a reduction in the order of 0.1% per day is observed in the studies period. This value can eventually be more easily extrapolated to the human beings' case and understood in terms of the magnitude of the effect.

With this, it is assumed that the relation between the cartilage wear and the strength or the period of the force for a constant loading is linear. However, the main forces that are exerted in TMJ result from dynamic loading during a continuous or intermittent period, and this meta-analysis is limited to a period of 14 days and a loading of 80g; thus, we cannot predict how is the evolution furthermore (Betti et al., 2018).

3. Anterior mandibular displacement

Functional mandibular forward repositioning appliances are extensively used in correcting class II malocclusion of growing patients with retrognathic mandibles (Cremonini et al., 2022; Karamesinis & Basdra, 2018).

The appliances can be fixed or removable, but only fixed ones were used in animal studies. These consisted of oblique planes cemented to only upper or both upper and down teeth, inducing a forward and downward mandibular displacement, when occluding. However, the application methods are unclear, as studies do not mention the distance of this displacement, not allowing a correct interpretation of the results.

The variety of animal species covered in this group was wider, comprising rats, rabbits and pigs. Nonetheless, the limited number of studies (n=4) compromises the comparison of the results, and consequently the conclusions concerning the effect of anterior mandibular displacement in condylar fibrocartilage. The reduced number of studies might be due to the restriction of data publication implemented in this systematic review, as Owtad et al. (2013) mention that several studies regarding this topic have been done.

In each study, the results are consistent with anabolic activity in fibrocartilage, including increased thickness, higher levels of ECM components, and also of vascular growth factors expression. Still, the heterogeneity of the histological and immunohistochemical measurements within the few studies included also limits the conclusions.

4. Increased occlusal vertical dimension

Almarza et al. (2018) and Xie et al. (2013) state that altered loading induced by occlusal alterations can result in dramatic changes in TMJ structures. These might be caused by increasing the occlusal vertical dimension, or loss of occlusal contacts due to extraction or gridding, among others.

Even so, few studies concerning iOVD (n=3) were included in this work, which is a considerable limitation in the comparison of results, especially considering that these are not consistent with each other, further complicating their interpretation.

The two studies performed in rats follow each other and reveal that this mechanical stimulus disturbs fibrocartilage homeostasis by promoting catabolic activity. The higher expression of IL, TNF-α, MMP and Caspase results in damage to this tissue, evidenced by thinner total cartilage, reduced number of chondrocytes and appearance of lacunas. The results of the study using mice are the opposite, as the bite-raising induced a proliferative response in chondrocytes of condylar cartilage, by increasing cells number, the cartilage thickness and the amount of ECM components, such as type II collagen and aggrecan.

This discrepancy may have to do with the methods used in each animal model. In rats, the increased occlusal vertical dimension was achieved by bonding composite resin build-ups to upper molars, while in mice, metal tubes were bilaterally bonded to upper and lower incisors, due to the small size of the molars.

Liu et al. (2019) explain that mice incisors erupt continuously, and these rodents grind their teeth naturally to compensate for the iOVD. Thus, an interruption of this process prevents native wear to the incisors and consequently leads to distraction of joint, promoting chondrocytes proliferation. Another aspect that could matter is the initial age of the animals, as mice were six weeks old and rats nine or twelve weeks old, suggesting that with ageing the regenerative response of cartilage tissue could be lower. However, mice were subjected to a greater loading period, and, on the contrary, the proliferative activity was even more notorious with time.

5. Diet Hardness

Mandibular movements are affected by the consistency of the diet, either its volume or hardness (Scheidegger et al., 2018). In this work, only studies with animal groups fed with either a soft or hard diet were considered. Switching diet groups were excluded, as our main intention was to study the solo effect of each diet type in the TMJ fibrocartilage.

A soft diet implies less broad masticatory movements since the food does not require chewing. Sun (2010) has already affirmed that, equally to excessive mechanical loading, reduced loading resulting from joint immobilization also led to catabolic effects in the fibrocartilage temporomandibular joint.

As the load to which the TMJ fibrocartilage is subjected is minimal within a diet with reduced volume, degradation of this tissue occurs, decreasing cell number, the thickness of fibrocartilage and the amount of ECM components. Results also reveal that these catabolic effects are exacerbated with the increasing experiment period.

This highlights the requirement of a moderate physiologic level of mechanical loading, more specifically of an adequate and balanced diet, for the homeostasis and healthy development of condylar fibrocartilage.

6. Study limitations

Although the included studies aimed to investigate the impact of mechanical stimulus on condylar cartilage, it is important to interpret and analyse the results critically. The effect of ageing, sexual hormones, diet type, regenerative capacity and other "cage" effects will inevitably affect the cartilage response. Thus, it is quite impossible to study the effect of the mechanical stimulus solo, even more, when the remaining characteristics of the animal models, such as sex and initial age, or experiment methods differ between the included studies.

However, it is important to bear in mind that clinically, risk factors do not appear in isolation, but combined. In fact, TMJ-AO is a disease with a multifactorial etiology and age, gender, stress, systemic diseases, hormones, and trauma also contribute considerably to its arising (Zhao et al., 2022).

Another limitation of this study has to do with the use of animal models. Despite most of the studies of the four mechanical loading groups using the rat as the animal model, they replicate a sudden change in occlusal loading and consequently trigger drastic changes in fibrocartilage and TMJ functions. This is not representative of the controlled change in occlusion with orthodontic treatments, representing a limitation in the extrapolation of the results to clinical practice (Chin & Almarza, 2020; Cougo et al., 2021; Suzuki & Iwata, 2016).

The lack of consistency and heterogeneity regarding initial age, baseline characteristics of the animals, experiment period and methods, might constitute possible sources of bias and, consequently, limits this study's conclusions (de Vries et al., 2014).

Finally, it was impossible to perform a wider meta-analysis, including other mechanical stimuli or outcomes, as the studies included in the systematic review were not sufficiently homogeneous regarding effect size measures.

V. Conclusion

The present study covered the most relevant non-human *in vivo* studies on the impact of mechanical stimulus on TMJ fibrocartilage, more specifically on condylar cartilage, published between 2011 and 2021. The literature reveals that mechanical stimulus is relevant in the maintenance of temporomandibular joint's fibrocartilage, playing a fundamental role in its homeostasis and influencing the growth of the condyle.

The anterior mandibular displacement was associated to histological and anabolic changes, while increased occlusal vertical dimension showed different results according to the animal model. The diet influenced the fibrocartilage thickness and metabolism, whether it was soft or hard. Also, the compressive force loading promoted cartilage wear, which is related to the initial age of the animals. This is fundamental in clinical practice, more specifically in the treatments that aim to modify jaw growth and in the prevention of TMJ osteoarthritis.

However, there is still few *in vivo* studies concerning this topic, and these might not be free of failure or lack of information, as concluded through the risk of bias. Thus, it is recommended that future studies be performed, with strict experimental protocols, aiming to mimetize the human TMJ as best as possible.

VI. Future Perspectives

To standardize and obtain more robust results and trustworthy conclusions on the effect of mechanical stimulus in TMJ's fibrocartilage, future *in vivo* studies should follow the same *modus operandi* regarding baseline characteristics, namely the animals' sex preferably male, type of diet, and experimental protocols and methods. Concerning the animal model, it would be ideal if more experiments were performed on larger animals, more like humans in TMJ physiology and metabolism, facilitating the extrapolation of the results into the humans' case.

Also, it would be interesting to perform experiments in which the animals were not sacrificed immediately after applying the mechanical stimulus, including the "post-stimulus period" as a follow-up measure and evaluating the fibrocartilage behaviour within the mechanical loading normalized conditions. By comparing the results of these studies with the ones in which the fibrocartilage response is evaluated immediately after the intervention period, it would be possible to investigate the existence of a regenerative response and its biochemical pathways.

VII. References

Abduo, J., & Lyons, K. (2012). Clinical considerations for increasing occlusal vertical dimension: A review. *Australian Dental Journal*, *57*(1), 2–10. https://doi.org/10.1111/j.1834-7819.2011.01640.x

Almarza, A. J., Brown, B. N., Arzi, B., Ângelo, D. F., Chung, W., Badylak, S. F., & Detamore, M. (2018). Preclinical Animal Models for Temporomandibular Joint Tissue Engineering. *Tissue Engineering - Part B: Reviews*, *24*(3), 171–178. https://doi.org/10.1089/ten.teb.2017.0341

Armiento, A. R., Alini, M., & Stoddart, M. J. (2019). Articular fibrocartilage - Why does hyaline cartilage fail to repair? *Advanced Drug Delivery Reviews*, *146*, 289–305. https://doi.org/10.1016/j.addr.2018.12.015

Benjamin, M., & Ralphs, J. R. (2004). Biology of Fibrocartilage Cells. *International Review of Cytology*, *233*(Iv), 1–45. https://doi.org/10.1016/S0074-7696(04)33001-9

Betti, B. F., Everts, V., Ket, J. C. F., Tabeian, H., Bakker, A. D., Langenbach, G. E., & Lobbezoo, F. (2018). Effect of mechanical loading on the metabolic activity of cells in the temporomandibular joint: a systematic review. *Clinical Oral Investigations*, *22*(1), 57–67. https://doi.org/10.1007/s00784-017-2189-9

Bianchi, J., de Oliveira Ruellas, A. C., Gonçalves, J. R., Paniagua, B., Prieto, J. C., Styner, M., Li, T., Zhu, H., Sugai, J., Giannobile, W., Benavides, E., Soki, F., Yatabe, M., Ashman, L., Walker, D., Soroushmehr, R., Najarian, K., & Cevidanes, L. H. S. (2020). Osteoarthritis of the Temporomandibular Joint can be diagnosed earlier using biomarkers and machine learning. *Scientific Reports*, *10*(1), 1–14. https://doi.org/10.1038/s41598-020-64942-0

Bordoni, B., & Varacallo, M. (2019). Anatomy, Head and Neck, Temporomandibular Joint. *StatPearls*, *April*. http://www.ncbi.nlm.nih.gov/pubmed/30860721

Caldwell, K. L., & Wang, J. (2015). Cell-based articular cartilage repair: The link between development and regeneration. *Osteoarthritis and Cartilage*, *23*(3), 351–362. https://doi.org/10.1016/j.joca.2014.11.004

Chandrasekaran, P., Doyran, B., Li, Q., Han, B., Bechtold, T. E., Koyama, E., Lu, X. L., & Han, L. (2017). Biomechanical properties of murine TMJ articular disc and condyle cartilage via AFM-nanoindentation. *Journal of Biomechanics*, *60*, 134–141. https://doi.org/10.1016/j.jbiomech.2017.06.031

Chen, X., Zimmerman, B. K., & Lu, X. L. (2015). Determine the equilibrium mechanical properties of articular cartilage from the short-term indentation response. *Journal of Biomechanics, 48(1)*, 176–180. https://doi.org/10.1016/j.jbiomech.2014.10.036

Chen, Z., Mai, Z., Tu, S., Lu, H., Chen, L., & Ai, H. (2019). Expression of lubricin in rat posterior mandibular condylar cartilage following functional mandibular forward repositioning. *Journal of Orofacial Orthopedics*, *80*(3), 128–135. https://doi.org/10.1007/s00056-019-00173-x

Chin, A. R., & Almarza, A. J. (2020). Trueness of fit of biphasic transversely isotropic parameters model in the porcine temporomandibular joint disc and mandibular condylar cartilage and regional dependence. *Journal of Biomechanical Engineering*, *142*(8), 1–8. https://doi.org/10.1115/1.4046922

Cougo, M. C. R., Quevedo, A. S. de, & Ponzoni, D. (2021). Modelos animais utilizados para o estudo da articulação temporomandibular: revisão de literatura. *Research, Society and Development*, *10*(12), e420101220586. https://doi.org/10.33448/rsd-v10i12.20586

Cremonini, F., Cervinara, F., Siciliani, G., & Lombardo, L. (2022). Class II Treatment in Growing Patients: Preliminary Evaluation of the Skeletal and Dental Effects of a New Clear Functional Appliance. *Applied Sciences*, *12*(11), 5622. https://doi.org/10.3390/app12115622

de Sá, M. P. N., Ramos, A. L., Zanoni, J. N., de Souza, F. D., Pepelascov, D. E., & Terada, R. S. S. (2017). Histological and morphological evaluation of condylar cartilage of young rats in response to stimulation of mandibular protrusion. *Acta Scientiarum - Health Sciences*, *39*(2), 219–226. https://doi.org/10.4025/actascihealthsci.v39i2.27789

de Vries, R. B. M., Wever, K. E., Avey, M. T., Stephens, M. L., Sena, E. S., & Leenaars, M. (2014). The usefulness of systematic reviews of animal experiments for the

design of preclinical and clinical studies. *ILAR Journal*, *55*(3), 427–437. https://doi.org/10.1093/ilar/ilu043

Delatte, M., Von Den Hoff, J. W., Van Rheden, R. E. M., & Kuijpers-Jagtman, A. M. (2004). Primary and secondary cartilages of the neonatal rat: The femoral head and the mandibular condyle. *European Journal of Oral Sciences*, *112*(2), 156–162. https://doi.org/10.1111/j.0909-8836.2004.00108.x

Du, J., Jiang, Q., Mei, L., Yang, R., Wen, J., Lin, S., & Li, H. (2020). Effect of high fat diet and excessive compressive mechanical force on pathologic changes of temporomandibular joint. *Scientific Reports*, *10*(1), 1–9. https://doi.org/10.1038/s41598-020-74326-z

Dyment, N. A., Jiang, X., Li, Y., Nanda, R., Rowe, D. W., Utreja, A., Villa, M. M., & Yaday, S. (2016). Cell and matrix response of temporomandibular cartilage to mechanical loading. *Osteoarthritis and cartilage, 24(2)*, 335–344. https://doi.org/10.1016/j.joca.2015.08.010

Enomoto, A., Watahiki, J., Nampo, T., Irie, T., Ichikawa, Y., Tachikawa, T., & Maki, K. (2014). Mastication markedly affects mandibular condylar cartilage growth, gene expression, and morphology. *American Journal of Orthodontics and Dentofacial Orthopedics*, *146*(3), 355–363. https://doi.org/10.1016/j.ajodo.2014.05.028

Fan, X. C., Singh, D., Ma, L. S., Piehslinger, E., Huang, X. F., & Rausch-Fan, X. (2021). Is there an association between temporomandibular disorders and articular eminence inclination? A systematic review. *Diagnostics*, *11*(1), 1–20. https://doi.org/10.3390/diagnostics11010029

Figueroba, S. R., Desjardins, M. P., Ferreira, L. E. N., Berto, L. A., Valdrighi, H. C., & Groppo, F. C. (2014). The influence of altered occlusion on pro-inflammatory cytokine levels in the TMJ synovial tissues of rats. *Archives of Oral Biology*, *59*(11), 1164–1171. https://doi.org/10.1016/j.archoralbio.2014.07.003

Gedrange, T., Kunert-Keil, C., Heinemann, F., & Dominiak, M. (2017). Tissue Engineering and Oral Rehabilitation in the Stomatognathic System. *BioMed Research International*, *2017*, 2–4. https://doi.org/10.1155/2017/4519568

Gong, S., Emperumal, C. P., Al-Eryani, K., & Enciso, R. (2022). Regeneration of temporomandibular joint using in vitro human stem cells: A review. *Journal of Tissue Engineering and Regenerative Medicine*, *February*, 591–604. https://doi.org/10.1002/term.3302

Gopi Chander, N., & Venkat, R. (2011). An appraisal on increasing the occlusal vertical dimension in full occlusal rehabilitation and its outcome. *Journal of Indian Prosthodontist Society*, *11*(2), 77–81. https://doi.org/10.1007/s13191-011-0066-9

Gredes, T., MacK, H., Spassov, A., Kunert-Keil, C., Steele, M., Proff, P., MacK, F., & Gedrange, T. (2012). Changes in condylar cartilage after anterior mandibular displacement in juvenile pigs. *Archives of Oral Biology*, *57*(6), 594–598. https://doi.org/10.1016/j.archoralbio.2011.09.017

Hagandora, C. K., Chase, T. W., & Almarza, A. J. (2011). A comparison of the mechanical properties of the goat temporomandibular joint disc to the mandibular condylar cartilage in unconfined compression. *Journal of Dental Biomechanics*, *2*(1), 1–10. https://doi.org/10.4061/2011/212385

Hichijo, N., Kawai, N., Mori, H., Sano, R., Ohnuki, Y., Okumura, S., Langenbach, G. E. J., & Tanaka, E. (2014). Effects of the masticatory demand on the rat mandibular development. *Journal of Oral Rehabilitation*, *41*(8), 581–587. https://doi.org/10.1111/joor.12171

Hooijmans, C. R., Rovers, M. M., De Vries, R. B. M., Leenaars, M., Ritskes-Hoitinga, M., & Langendam, M. W. (2014). SYRCLE's risk of bias tool for animal studies. *BMC Medical Research Methodology*, *14*(1), 1–9. https://doi.org/10.1186/1471-2288-14-43

Huang, Z., Yang, R., Zhang, L., Zhu, M., Zhang, C., Wen, J., & Li, H. (2021). BRD4 inhibition alleviates mechanical stress-induced TMJ OA-like pathological changes and attenuates TREM1-mediated inflammatory response. *Clinical Epigenetics*, *13*(1), 1–13. https://doi.org/10.1186/s13148-021-01008-6

Ikeda, Y., Yonemitsu, I., Takei, M., Shibata, S., & Ono, T. (2014). Mechanical loading leads to osteoarthritis-like changes in the hypofunctional temporomandibular joint in rats. *Archives of Oral Biology*, *59*(12), 1368–1376.

https://doi.org/10.1016/j.archoralbio.2014.08.010

Jiang, L., Xie, Y., Wei, L., Zhou, Q., Shen, X., Jiang, X., & Gao, Y. (2017). Identification of the vascular endothelial growth factor signalling pathway by quantitative proteomic analysis of rat condylar cartilage. *FEBS Open Bio*, *7*(1), 44–53. https://doi.org/10.1002/2211-5463.12155

Jiang, Y. Y., Wen, J., Gong, C., Lin, S., Zhang, C. X., Chen, S., Cheng, W., & Li, H. (2018). BIO alleviated compressive mechanical force-mediated mandibular cartilage pathological changes through Wnt/β-catenin signaling activation. *Journal of Orthopaedic Research*, *36*(4), 1228–1237. https://doi.org/10.1002/jor.23748

Jing, Z., Gu, Z., & Feng, J. (2013). Forward mandibular positioning enhances the expression of Ang-1 and Ang-2 in rabbit condylar chondrocytes. *Molecular Medicine Reports*, *8*(4), 1094–1098. https://doi.org/10.3892/mmr.2013.1620

Karamesinis, K., & Basdra, E. K. (2018). The biological basis of treating jaw discrepancies: An interplay of mechanical forces and skeletal configuration. *Biochimica et Biophysica Acta - Molecular Basis of Disease*, *1864*(5), 1675–1683. https://doi.org/10.1016/j.bbadis.2018.02.007

Kato, T., Takahashi, S., & Domon, T. (2015). Effects of a liquid diet on the temporomandibular joint of growing rats. *Medical Principles and Practice*, *24*(3), 257–262. https://doi.org/10.1159/000370072

Kent Scientific.(2019). Retrieved August 20, 2022, from https://www.kentscientific.com/blog/mice-vs-rats-in-research-whats-the-difference/

Khaleel, A. K., Shaari, R. Bin, Nawi, M. A. A., & Alrifai, A. S. (2020). Biological characteristics and causes of degradation of rats mandibular condylar cartilage. *International Journal of Pharmaceutical Research*, *12*(4), 1625–1635. https://doi.org/10.31838/ijpr/2020.12.04.236

Kuang, B., Dai, J., Wang, Q. Y., Song, R., Jiao, K., Zhang, J., Tian, X. G., Duan, Y. Z., & Wang, M. Q. (2013). Combined degenerative and regenerative remodeling responses of the mandibular condyle to experimentally induced disordered

occlusion. *American Journal of Orthodontics and Dentofacial Orthopedics*, *143*(1), 69–76. https://doi.org/10.1016/j.ajodo.2012.08.024

Kuroda, S., Tanimoto, K., Izawa, T., Fujihara, S., Koolstra, J. H., & Tanaka, E. (2009). Biomechanical and biochemical characteristics of the mandibular condylar cartilage. *Osteoarthritis and Cartilage*, *17*(11), 1408–1415. https://doi.org/10.1016/j.joca.2009.04.025

Li, B., Guan, G., Mei, L., Jiao, K., & Li, H. (2021). Pathological mechanism of chondrocytes and the surrounding environment during osteoarthritis of temporomandibular joint. *Journal of Cellular and Molecular Medicine, 25(11)*, 4902–4911. https://doi.org/10.1111/jcmm.16514

Li, H., Zhang, X. Y., Wu, T. J., Cheng, W., Liu, X., Jiang, T. T., Wen, J., Li, J., Ma, Q. L., & Hua, Z. C. (2013). Endoplasmic reticulum stress regulates rat mandibular cartilage thinning under compressive mechanical stress. *Journal of Biological Chemistry*, *288*(25), 18172–18183. https://doi.org/10.1074/jbc.M112.407296

Liberati, A., Altman, D. G., Tetzlaff, J., Mulrow, C., Gøtzsche, P. C., Ioannidis, J. P. A., Clarke, M., Devereaux, P. J., Kleijnen, J., & Moher, D. (2009). The PRISMA statement for reporting systematic reviews and meta-analyses of studies that evaluate health care interventions: Explanation and elaboration. *PLoS Medicine*, *6*(7). https://doi.org/10.1371/journal.pmed.1000100

Liu, Q., Yang, H. xu, Duan, J., Zhang, H. yun, Xie, M. jiao, Ren, H. tian, Zhang, M., Zhang, J., Lu, L., Liu, X. dong, Yu, S. bin, & Wang, M. qing. (2019). Bilateral anterior elevation prosthesis boosts chondrocytes proliferation in mice mandibular condyle. *Oral Diseases*, *25*(6), 1589–1599. https://doi.org/10.1111/odi.13128

Liu, Y. D., Liao, L. F., Zhang, H. Y., Lu, L., Jiao, K., Zhang, M., Zhang, J., He, J. J., Wu, Y. P., Chen, D., & Wang, M. Q. (2014). Reducing dietary loading decreases mouse temporomandibular joint degradation induced by anterior crossbite prosthesis. *Osteoarthritis and cartilage, 22(2)*, 302–312. https://doi.org/10.1016/j.joca.2013.11.014.

Long, H. qing, Tian, P. fei, Guan, Y. xin, Liu, L. xia, Wu, X. ping, & Li, B. (2019). Expression of Ihh signaling pathway in condylar cartilage after bite-raising in adult

rats. *Journal of Molecular Histology*, *50*(5), 459–470. https://doi.org/10.1007/s10735-019-09840-0

Magara, J., Nozawa-Inoue, K., Suzuki, A., Kawano, Y., Ono, K., Nomura, S., & Maeda, T. (2012). Alterations in intermediate filaments expression in disc cells from the rat temporomandibular joint following exposure to continuous compressive force. *Journal of Anatomy*, *220*(6), 612–621. https://doi.org/10.1111/j.1469-7580.2012.01501.x

McGuinness, L. A., & Higgins, J. P. T. (2021). Risk-of-bias VISualization (robvis): An R package and Shiny web app for visualizing risk-of-bias assessments. *Research Synthesis Methods*, *12*(1), 55–61. https://doi.org/10.1002/jrsm.1411

Mérida Velasco, J. R., Rodríguez Vázquez, J. F., De La Cuadra Blanco, C., Campos López, R., Sánchez, M., & Mérida Velasco, J. A. (2009). Development of the mandibular condylar cartilage in human specimens of 10-15 weeks' gestation. *Journal of Anatomy*, *214*(1), 56–64. https://doi.org/10.1111/j.1469-7580.2008.01009.x

Mizoguchi, I., Toriya, N., & Nakao, Y. (2013). Growth of the mandible and biological characteristics of the mandibular condylar cartilage. *Japanese Dental Science Review*, *49*(4), 139–150. https://doi.org/10.1016/j.jdsr.2013.07.004

Mousoulea, S., Tsolakis, I., Ferdianakis, E., & Tsolakis, A. I. (2016). The Effect of Chin-cup Therapy in Class III Malocclusion: A Systematic Review. *The Open Dentistry Journal*, *10*(1), 664–679. https://doi.org/10.2174/1874210601610010664

Mueller, M. B., & Tuan, R. S. (2011). Anabolic/Catabolic balance in pathogenesis of osteoarthritis: identifying molecular targets. *PM & R : The Journal of Injury, Function, and Rehabilitation*, *3*(6 Suppl 1), S3–S11. https://doi.org/10.1016/j.pmrj.2011.05.009

Nickel, J. C., Iwasaki, L. R., Gonzalez, Y. M., Gallo, L. M., & Yao, H. (2018). Mechanobehavior and Ontogenesis of the Temporomandibular Joint. *Journal of Dental Research*, *97*(11), 1185–1192. https://doi.org/10.1177/0022034518786469

Norton, N. (2016). *Netter's Head and Neck Anatomy for Dentistry* (3rd ed.). Elsevier -

Health Sciences Division.

Orajärvi, M., Hirvonen, O., Yu, S. Bin, Liu, X., Tiilikainen, P., Wang, M., Raustia, A., & Pirttiniemi, P. (2011). Effect of estrogen and altered diet hardness on the expression of estrogen receptor alpha and matrix metalloproteinase-8 in rat condylar cartilage. *Journal of Orofacial Pain*, *25*(3), 261–268.

Orajärvi, M., Puijola, E., Yu, S.-B., Liu, X., Tiilikainen, P., Wang, M., Raustia, A., & Pirttiniemi, P. (2012). Effect of estrogen and dietary loading on condylar cartilage. *Journal of Orofacial Pain*, *26*(4), 328–336. http://www.ncbi.nlm.nih.gov/pubmed/23110273

Owtad, P., Park, J. H., Shen, G., Potres, Z., & Darendeliler, M. A. (2013). The biology of TMJ growth modification: A review. *Journal of Dental Research*, *92*(4), 315–321. https://doi.org/10.1177/0022034513476302

Owtad, P., Potres, Z., Shen, G., Petocz, P., & Darendeliler, M. A. (2011). A histochemical study on condylar cartilage and glenoid fossa during mandibular advancement. *Angle Orthodontist, 81(2)*, 270–276. https://doi.org/10.2319/021710-99.1

Proffit, W. R., Fields, H. W., Larson, B. E., & Sarver, D. M (2019).*Contemporary Orthodontics* (6th ed.). Elsevier.

Ravosa, M. J., & Kane, R. J. (2017). Dietary variation and mechanical properties of articular cartilage in the temporomandibular joint: implications for the role of plasticity in mechanobiology and pathobiology. *Zoology*, *124*(August), 42–50. https://doi.org/10.1016/j.zool.2017.08.008

Roberts, W. E., & Goodacre, C. J. (2020). The Temporomandibular Joint: A Critical Review of Life-Support Functions, Development, Articular Surfaces, Biomechanics and Degeneration. *Journal of Prosthodontics*, *29*(9), 772–779. https://doi.org/10.1111/jopr.13203

Roberts, W. E., & Stocum, D. L. (2018). Part II: Temporomandibular Joint (TMJ)—Regeneration, Degeneration, and Adaptation. *Current Osteoporosis Reports*, *16*(4), 369–379. https://doi.org/10.1007/s11914-018-0462-8

Scheidegger, R., Koletsi, D., & Eliades, T. (2018). The impact of dietary consistency on structural craniofacial components: Temporomandibular joint/condyle, condylar cartilage, alveolar bone and periodontal ligament. A systematic review and meta-analysis in experimental in vivo research. *Archives of Oral Biology*, *94*(June), 33–47. https://doi.org/10.1016/j.archoralbio.2018.06.016

Shen, G., & Darendeliler, M. A. (2005). The adaptive remodeling of condylar cartilage — a transition from chondrogenesis to osteogenesis. *Journal of dental research, 84*(8),691-699. https://doi.org/10.1177/154405910508400802

Shi, Z., Lv, J., Xiaoyu, L., Zheng, L. W., & Yang, X. W. (2018). Condylar Degradation from Decreased Occlusal Loading following Masticatory Muscle Atrophy. *BioMed Research International*, *2018*. https://doi.org/10.1155/2018/6947612

Shibata, S., Sato, R., Murakamib, G., Fukuoka, H., & Rodriguez-Vázquez, J. F. (2013). Origin of mandibular condylar cartilage in mice, rats, and humans: Periosteum or separate blastema? *Journal of Oral Biosciences*, *55*(4), 208–216. https://doi.org/10.1016/j.job.2013.08.001

Statham, P., Jones, E., Jennings, L. M., & Fermor, H. L. (2022). Reproducing the Biomechanical Environment of the Chondrocyte for Cartilage Tissue Engineering. *Tissue Engineering - Part B: Reviews*, *28*(2), 405–420. https://doi.org/10.1089/ten.teb.2020.0373

Stocum, D. L., & Roberts, W. E. (2018). Part I: Development and Physiology of the Temporomandibular Joint. *Current Osteoporosis Reports*, *16*(4), 360–368. https://doi.org/10.1007/s11914-018-0447-7

Sun, H. B. (2010). Mechanical loading, cartilage degradation, and arthritis. *Annals of the New York Academy of Sciences*, *1211*, 37–50. https://doi.org/10.1111/j.1749-6632.2010.05808.x

Suzuki, A., & Iwata, J. (2016). Mouse genetic models for temporomandibular joint development and disorders. *Oral diseases, 22(1)*, 33–38. https://doi.org/10.1111/odi.12353

Uekita, H., Takahashi, S., Domon, T., & Yamaguchi, T. (2015). Changes in collagens and

chondrocytes in the temporomandibular joint cartilage in growing rats fed a liquid diet. *Annals of Anatomy*, *202*, 78–87. https://doi.org/10.1016/j.aanat.2015.08.006

Vîrlan, M., Păun, D., Bordea, E., Pellegrini, A., Spînu, A., Ivașcu, R., Nimigean, V., & Nimigean, V. (2021). Factors influencing the articular eminence of the temporomandibular joint (Review). *Experimental and Therapeutic Medicine*, *22*(4), 1–5. https://doi.org/10.3892/etm.2021.10518

Wadhwa, S., & Kapila, S. (2008). TMJ disorders: future innovations in diagnostics and therapeutics. *Journal of dental education, 72(8)*, 930–947. https://doi.org/10.1002/j.0022-0337.2008.72.8.tb04569.x

Wallace, B. C., Dahabreh, I. J., Trikalinos, T. A., Lau, J., Trow, P., & Schmid, C. H. (2012). End-Users : R as a Computational Back-End. *Journal of Statistical Software*, *49*(5), 1–15.

Wang, X. D., Zhang, J. N., Gan, Y. H., & Zhou, Y. H. (2015). Current understanding of pathogenesis and treatment of TMJ osteoarthritis. *Journal of Dental Research*, *94*(5), 666–673. https://doi.org/10.1177/0022034515574770

Wen, J., Jiang, Y., Zhang, C., Chen, S., & Li, H. (2016). The protective effects of salubrinal on the cartilage and subchondral bone of the temporomandibular joint under various compressive mechanical stimulations. *PLoS ONE*, *11*(5), 1–14. https://doi.org/10.1371/journal.pone.0155514

Willard, V. P., Zhang, L., & Athanasiou, K. A. (2017). 6.9 Tissue engineering of the temporomandibular joint. In *Comprehensive Biomaterials II* (Vol. 6). Elsevier Ltd. https://doi.org/10.1016/B978-0-08-100691-7.00268-8

Xiang, T., Tao, Z. Y., Liao, L. F., Wang, S., & Cao, D. Y. (2021). Animal models of temporomandibular disorder. *Journal of Pain Research*, *14*, 1415–1430. https://doi.org/10.2147/JPR.S303536

Xie, Q., Li, X., & Xu, X. (2013). The difficult relationship between occlusal interferences and temporomandibular disorder - insights from animal and human experimental studies. *Journal of Oral Rehabilitation, 40(4)*, 279–295. https://doi.org/10.1111/joor.12034

Zhang, C., Zhu, M., Wang, H., Wen, J., Huang, Z., Chen, S., Zhao, H., & Li, H. (2021). LOXL2 attenuates osteoarthritis through inactivating Integrin/FAK signaling. *Scientific Reports*, *11*(1), 1–12. https://doi.org/10.1038/s41598-021-96348-x

Zhang, J., Zhang, H.Y., Zhang, M., Qiu, Z.Y., Wu, Y.P., Callaway, D.A., Jiang, J.X., Lu, L., Jing, L., Yang, T., Wang, M.Q. (2014). Connexin43 hemichannels mediate small molecule exchange between chondrocytes and matrix in biomechanically-stimulated temporomandibular joint cartilage. *Osteoarthritis and Cartilage, 22(6)*, 822–830. https://doi.org/10.1016/j.joca.2014.03.017

Zhao, Y., An, Y., Zhou, L., Wu, F., Wu, G., Wang, J., & Chen, L. (2022). Animal Models of Temporomandibular Joint Osteoarthritis: Classification and Selection. *Frontiers in Physiology*, *13*(April), 1–19. https://doi.org/10.3389/fphys.2022.859517

Zurfluh, M. A., Kloukos, D., Patcas, R., & Eliades, T. (2015). Effect of chin-cup treatment on the temporomandibular joint: A systematic review. *European Journal of Orthodontics*, *37*(3), 314–324. https://doi.org/10.1093/ejo/cju048

Appendices

Appendix I- 2020 PRISMA checklist

Section and Topic	Item #	Checklist item	Location where item is reported
TITLE			
Title	1	Identify the report as a systematic review.	
ABSTRACT			
Abstract	2	See the PRISMA 2020 for Abstracts checklist.	**Appendix II**
INTRODUCTION			
Rationale	3	Describe the rationale for the review in the context of existing knowledge.	13-23
Objectives	4	Provide an explicit statement of the objective(s) or question(s) the review addresses.	23
METHODS			
Eligibility criteria	5	Specify the inclusion and exclusion criteria for the review and how studies were grouped for the syntheses.	26-27
Information sources	6	Specify all databases, registers, websites, organisations, reference lists and other sources searched or consulted to identify studies. Specify the date when each source was last searched or consulted.	25
Search strategy	7	Present the full search strategies for all databases, registers and websites, including any filters and limits used.	25-26
Selection process	8	Specify the methods used to decide whether a study met the inclusion criteria of the review, including how many reviewers screened each record and each report retrieved, whether they worked independently, and if applicable, details of automation tools used in the process.	26-27
Data collection process	9	Specify the methods used to collect data from reports, including how many reviewers collected data from each report, whether they worked independently, any processes for obtaining or confirming data from study investigators, and if applicable, details of automation tools used in the process.	27
Data items	10a	List and define all outcomes for which data were sought. Specify whether all results that were compatible with each outcome domain in each study were sought (e.g. for all measures, time points, analyses), and if not, the methods used to decide which results to collect.	27
	10b	List and define all other variables for which data were sought (e.g. participant and intervention characteristics, funding sources). Describe any assumptions made about any missing or unclear information.	27
Study risk of bias assessment	11	Specify the methods used to assess risk of bias in the included studies, including details of the tool(s) used, how many reviewers assessed each study and whether they worked independently, and if applicable, details of automation tools used in the process.	28

Section and Topic	Item #	Checklist item	Location where item is reported
Effect measures	12	Specify for each outcome the effect measure(s) (e.g. risk ratio, mean difference) used in the synthesis or presentation of results.	29-30
Synthesis methods	13a	Describe the processes used to decide which studies were eligible for each synthesis (e.g. tabulating the study intervention characteristics and comparing against the planned groups for each synthesis (item #5)).	27
	13b	Describe any methods required to prepare the data for presentation or synthesis, such as handling of missing summary statistics, or data conversions.	29-30
	13c	Describe any methods used to tabulate or visually display results of individual studies and syntheses.	27
	13d	Describe any methods used to synthesize results and provide a rationale for the choice(s). If meta-analysis was performed, describe the model(s), method(s) to identify the presence and extent of statistical heterogeneity, and software package(s) used.	29-30
	13e	Describe any methods used to explore possible causes of heterogeneity among study results (e.g. subgroup analysis, meta-regression).	29-30
	13f	Describe any sensitivity analyses conducted to assess robustness of the synthesized results.	
Reporting bias assessment	14	Describe any methods used to assess risk of bias due to missing results in a synthesis (arising from reporting biases).	
Certainty assessment	15	Describe any methods used to assess certainty (or confidence) in the body of evidence for an outcome.	
RESULTS			
Study selection	16a	Describe the results of the search and selection process, from the number of records identified in the search to the number of studies included in the review, ideally using a flow diagram.	34
	16b	Cite studies that might appear to meet the inclusion criteria, but which were excluded, and explain why they were excluded.	
Study characteristics	17	Cite each included study and present its characteristics.	35-40
Risk of bias in studies	18	Present assessments of risk of bias for each included study.	42-46
Results of individual studies	19	For all outcomes, present, for each study: (a) summary statistics for each group (where appropriate) and (b) an effect estimate and its precision (e.g. confidence/credible interval), ideally using structured tables or plots.	**Appendix V**
Results of syntheses	20a	For each synthesis, briefly summarise the characteristics and risk of bias among contributing studies.	42-43
	20b	Present results of all statistical syntheses conducted. If meta-analysis was done, present for each the summary estimate and its precision (e.g. confidence/credible interval) and measures of statistical heterogeneity. If comparing groups, describe the direction of the effect.	47-48
	20c	Present results of all investigations of possible causes of heterogeneity among study results.	49

Section and Topic	Item #	Checklist item	Location where item is reported
	20d	Present results of all sensitivity analyses conducted to assess the robustness of the synthesized results.	
Reporting biases	21	Present assessments of risk of bias due to missing results (arising from reporting biases) for each synthesis assessed.	
Certainty of evidence	22	Present assessments of certainty (or confidence) in the body of evidence for each outcome assessed.	
DISCUSSION			
Discussion	23a	Provide a general interpretation of the results in the context of other evidence.	51-57
	23b	Discuss any limitations of the evidence included in the review.	57-58
	23c	Discuss any limitations of the review processes used.	57-58
	23d	Discuss implications of the results for practice, policy, and future research.	59-61
OTHER INFORMATION			
Registration and protocol	24a	Provide registration information for the review, including register name and registration number, or state that the review was not registered.	25
	24b	Indicate where the review protocol can be accessed, or state that a protocol was not prepared.	25
	24c	Describe and explain any amendments to information provided at registration or in the protocol.	
Support	25	Describe sources of financial or non-financial support for the review, and the role of the funders or sponsors in the review.	
Competing interests	26	Declare any competing interests of review authors.	
Availability of data, code and other materials	27	Report which of the following are publicly available and where they can be found: template data collection forms; data extracted from included studies; data used for all analyses; analytic code; any other materials used in the review.	

Appendix II- 2020 PRISMA checklist for abstracts

Section and Topic	Item #	Checklist item	Reported (Yes/No)
TITLE			
Title	1	Identify the report as a systematic review.	Yes
BACKGROUND			
Objectives	2	Provide an explicit statement of the main objective(s) or question(s) the review addresses.	Yes
METHODS			
Eligibility criteria	3	Specify the inclusion and exclusion criteria for the review.	Yes
Information sources	4	Specify the information sources (e.g. databases, registers) used to identify studies and the date when each was last searched.	Yes
Risk of bias	5	Specify the methods used to assess risk of bias in the included studies.	No
Synthesis of results	6	Specify the methods used to present and synthesise results.	Yes
RESULTS			
Included studies	7	Give the total number of included studies and participants and summarise relevant characteristics of studies.	Yes
Synthesis of results	8	Present results for main outcomes, preferably indicating the number of included studies and participants for each. If meta-analysis was done, report the summary estimate and confidence/credible interval. If comparing groups, indicate the direction of the effect (i.e. which group is favoured).	Yes
DISCUSSION			
Limitations of evidence	9	Provide a brief summary of the limitations of the evidence included in the review (e.g. study risk of bias, inconsistency and imprecision).	No
Interpretation	10	Provide a general interpretation of the results and important implications.	Yes
OTHER			
Funding	11	Specify the primary source of funding for the review.	No
Registration	12	Provide the register name and registration number.	No

Appendix III- PROSPERO registration

NIHR | National Institute for Health Research

PROSPERO
International prospective register of systematic reviews

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My other records

These are records that have either been published or rejected and are not currently being worked on.

ID	Title	Status	Last edited
CRD42022340742	The impact of Mechanical Stimulus on the Temporomandibular Joint's Cartilage of non-human animal models: a Systematic Review and Meta-analysis	Registered	31/08/2022

Appendix IV- Risk of Bias Tool (SYRCLE's tool for assessing risk of bias)

Item	Type of bias	Domain	Description of domain	Review authors judgment
1	Selection bias	Sequence generation	Describe the methods used, if any, to generate the allocation sequence in sufficient detail to allow an assessment of whether it should produce comparable groups.	Was the allocation sequence adequately generated and applied? (*)
2	Selection bias	Baseline characteristics	Describe all the possible prognostic factors or animal characteristics, if any, that are compared to judge wether intervention and control groups were similar at the start of the experiment.	Were the groups similar at baseline or were they adjusted for confounders in the analysis? (*)
3	Selection bias	Allocation concealment	Describe the method used to conceal the allocation sequence sufficiently to determine whether intervention allocations could have been foreseen before or during enrolment.	Was the allocation adequately concealed? (*)
4	Performance bias	Random housing	Describe all measures used, if any, to house the animals randomly within the animal room.	Were the animals randomly housed during the experiment?
5	Performance bias	Blinding	Describe all measures used, if any, to blind trial caregivers and researchers from knowing which intervention each animal received. Provide any information relating to whether the intended blinding was effective.	Were the caregivers and/or investigators blinded from knowledge of which intervention each animal received during the experiment?
6	Detection bias	Random outcome assessment	Describe whether or not animals were selected at random for outcome assessment, and which methods to select the animals, if any, were used.	Were animals selected at random for outcome assessment?
7	Detection bias	Blinding	Describe all measures used, if any, to blind outcome assessors from knowing which intervention each animal received. Provide any information relating to whether the intended blinding was effective.	Was the outcome assessor blinded?
8	Attrition bias	Incomplete outcome data	Describe the completeness of outcome data for each main outcome, including attrition and exclusions from the analysis. State whether attrition and exclusions were reported, the numbers in each intervention group (compared with total randomized animals), reasons for attrition or exclusions, and any re-inclusions in analyses for the review.	Were incomplete outcome data adequately addressed? (*)
9	Reporting bias	Selective outcome reporting	State how selective outcome reporting was examined and what was found	Are reports of the study free of selective outcome reporting? (*)
10	Other	Other sources of bias	State any important concerns about bias not covered by other domains in the tool.	Was the study apparently free of other problems that could result in high risk of bias? (*)

*Items in agreement with the items in the Cochrane Risk of Bias tool.

Appendix V- Quantitative characteristics of the studies included in the meta-analyses

Study (Author, year)	**Loading intensity** (g)	**Loading period** (day)	**Experimental group (Subjected to compressive force loading)**			**Control group (Not subjected to compressive force loading)**			**ΔT** (μm)	$\frac{\Delta T}{I.t}$ (μm/g.day)	**SE**	$\frac{SE}{I.t}$
			N	**T** (μm)	**SD**	**N**	**T** (μm)	**SD**				
Du et al. 2020	40	7	6	51,55	± 2,91	6	65,30	± 1,21	-13,75	-0,05	1,29	0,01
Huang et al. 2021	80	14	6	148,15	± 27,99	6	348,15	± 17,49	-200,00	-0,18	13,47	0,01
Jiang et al. 2018	80	7	12	172,00	± 19,11	12	396,00	± 44,59	-224,00	-0,4	14,00	0,03
Li et al. 2013	40	14	5	135,44	± 14,55	5	388,84	± 31,97	-253,40	-0,45	15,71	0,03
Wen et al. 2016_1*	40	7	5	387,10	± 15,32	5	655,48	± 21,45	-268,38	-0,96	11,77	0,04
Wen et al. 2016_2*	80	7	5	324,90	± 21,46	5	655,48	± 21,45	-330,58	-0,59	13,57	0,02
Zhang et al. 2021	80	7	12	144,23	± 28,85	12	365,39	± 14,42	-221.16	-0,40	9,31	0,02

Legend: N, sample size; T, total cartilage thickness; SD, standard deviation; ΔT, mean difference cartilage thickness; SE, standard deviation, I, loading intensity; t, loading period

*The same study presents measurements for different samples

Values rounded to two decimal digits, excepting the loading intensity and period values.