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HISTOLOGICAL, RADIOGRAPHIC AND BIOCHEMICAL CHANGES OF THE TEMPOROMANDIBULAR JOINT IN RATS FOLLOWING MANDIBULAR COMPRESSIVE LOADING

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Στην οικογένειά μου,

στην Ελένη,

στην Άνδρια, στον Δημήτρη και στην μπέμπα μας

που συνεχίζουν να με στηρίζουν

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CONTENTS

PREFACE

The scope of orthodontics is to elucidate craniofacial growth, treat predictably the skeletal discrepancies and align the dentition. To achieve these goals, researchers get motivated to understand the development and function of the bony tissue and the temporomandibular joint (TMJ) alike. It is noteworthy that facial appearance may affect self-esteem and quality of life, hence the orthodontist seeks either to prevent or diagnose early, and then to tackle the most prominent malformations.

Treatment in cases of extreme mandibular growth has been a challenge (Hans et al., 2017; Zere et al., 2018) and research has focused on the anatomy, histology and function of the TMJ (Collins et al., 1946; Bag et al., 2014; Shaffer et al., 2014) seeking the trigger of growth (Baume and Derichsweiler, 1961). In experiments and in clinical practice, the mandible has been pushed backwards, mainly during the period of growth, for protrusion to alleviate (Farias-Neto et al., 2012; Mousoulea et al., 2016; Martina et al., 2019). Clinical observations of animal TMJs and the consequent suggestions after mandibular displacement have been heterogeneous and contradictory. Others claim potential for TMJ disorders and joint structural alterations due to the generation of parafunctional stress, deemed as traumatic (Ingervall et al., 1972; Cholasueksa et al., 2004; Bryndahl et al., 2011).

Mechanical forces have a fundamental role in cellular processes during tissue morphogenesis. In the practice of orthodontic and dentofacial orthopedic, various treatment plans are used which aim at controlling the growth of the lower jaw. Despite the fact that the above-mentioned therapeutic methods have been used at least since the beginning of the twentieth century, the level of forces exerted, as well as their effects on the cellular structures of the facial region are not yet documented.

Many studies have examined the growth of the mandible, especially the mandibular condyles (Kantomaa, 1986; Yonemitsu et al., 2007; Sakurai et al., 2007; Takei et al., 2008). The growth of the condyle is influenced by hereditary and environmental factors, by hormones and metabolism (Copray et al., 1983) and is important for the craniofacial complex. Dental occlusion affects the growth of condyles as they undergo pressure from the chewing forces applied to the teeth (Boyd et al., 1990). In particular, dental malocclusions affect the size of the condyle (Liu et al., 2007), cartilage thickness and cell proliferation. Dental malocclusions also affect the position of the condyle, which should normally be at the center of the glenoid fossa, resulting in the irregular transfer of forces into the TMJ and the creation of dysfunctions (Owen AH III, 1984; Weinberg, 1983; Gerber and Steinhardt, 1990).

Previous studies have examined condyle cartilage under various conditions in experimental animals. In the studies of Desai et al., 1996 and Teramoto et al., 2003, posterior movement of the condyle reduced the number of chondrogenic cells and prevented chondrocyte proliferation and reduced the amount of extracellular matrix. Intermittent posterior displacement of the condyle due to malocclusion precipitates irregular reconstruction of the condyle cartilage and nerve injury (Cholasueksa et al., 2004). Irregular loading of the TMJ can be harmful to the condylar cartilage and the cancellous bone.

However, there are insufficient studies looking for longterm condylar and mandibular alterations.

I.   GENERAL PART

1. REVIEW OF MANDIBULAR AND CONDYLAR GROWTH

There are two principal theories regarding mandibular growth. Sicher (1947) suggested that the growth of the mandible is regulated by signals that arise from the condylar center. Mandibular growth is known to be a result of both extensive remodeling that occurs in all parts of the mandible as it undergoes displacement in space (Enlow, 1973).

Brash (1934), stated that bone growth is a surface phenomenon carried out by surface deposition and surface resorption. While studying the pig's mandibular growth, Brash found superficial three-dimensional osseous changes contributed equally to the growth. The three-dimensional growth pattern involves major growth areas (symphyseal, alveolar, ramal, condylar, coronoidal) that collectively constitute the composite of mandibular growth. These areas are ordinary growing sites that follow the general principles of surface deposition and resorption. The mandible thereby grows in length both anteriorly and posteriorly. The oblique direction of the upward and backward growing condyle contributes significantly to the mandibular elongation.

Rushton (1944), evaluated patients who sustained injury or infection in the condylar area and observed resultant mandibular hypoplasia. He stated that, in the early years it is the function of the condylar growth center to promote continual advancement of bone upon which subperiosteal additions and remodeling may be realized. The hypoplasia was explained by Rushton as a result of no advancement of bone within the periosteum, and because the subperiosteal bone is abnormally localized instead of proportionately distributed.

Sicher (1947), suggested that mandibular growth is the product of cartilagenous condylar growth, subsequently undergoing ossification. He distinguished condylar cartilage from epiphyseal and articular cartilages and stated that the mandibular condyle features an alternative type of cartilage, able to grow both appositionally and interstitially. He believed that mandibular

growth and, mainly, condylar cartilage growth determine the development of the entire face. Individuals with a wide type of facial appearance presumably have slow cartilaginous growth that results in reduced facial height and a wider, shorter face. People with a narrow, long face presumably have cartilage that grows relatively fast, thereby determining this specific type of face.

Robinson and Sarnat (1955) studied the growth pattern of the swine mandible in nine, eight-week-old female Hampshire pigs for twelve weeks. Amalgam implants and serial radiographs were used to study the mandibular growth pattern. They observed that the condyle appeared to be the most active mandibular growth site, contributing about 80% to total ramus height and, to a degree, to the mandibular body length. The posterior border of the ramus was identified as the second most active growth site and also contributed to the total length of the mandible. The interior, anterior and alveolar borders and lateral surfaces of the mandible were considered as less important sites of appositional growth.

Scott (1959) stated that growth of the condylar cartilage thrusts the mandible downward and forward from its contact in the glenoid fossa. Bone resorption at the anterior border of the mandibular ramus was considered a compensatory mechanism whose role is to maintain the proper relationship between the zygomatic arch and the anterior edge of the ramus.

Moss (1960) refined a theory of functional analysis and functional cranial components. Each functional component was described as consisting of two parts, the functional matrix and its skeletal unit. The matrix consists of all muscles that are attached to the mandible, all arteries, veins and the regional nerves, the salivary glands, teeth, skin, adipose and connective tissues, the tongue and the oral and pharyngeal cavities.

The mandible is not a unitary biological entity, but rather is composed of several independent functional cranial

components. The skeletal units of the mandible that correspond to the above mentioned functional cranial components are the alveolar processes, the coronoid process, the angular process, the mandibular corpus, the condylar process and the chin. The primary morphogenetic event in mandibular growth control is the volumetric expansion of the oral functioning space and the oral capsular matrix. Presumably, the above growth process also results in the passive mandibular displacement.

Changes in the shape (remodeling) of the skeletal units are described as secondary, compensatory and mechanically compulsory. Moss and Salentijin (1969) rejected the proposition that the condylar cartilage is a primary site of mandibular growth control. The condyles are believed to be loci where secondary, compensatory growth occurs.

Björk (1963) presented preliminary data on the growth of the mandible in a sample of 45 Danish male volunteers that were kept under annual radiographic review utilizing tantalum implant pins. He reported on a pronounced area of remodeling localizing anteriorly to the mandibular angle. The direction of condylar growth in the sagittal plane was found to vary widely, but most frequently the direction was slightly forward in relation to the posterior tangent to the ramus.

Enlow and Harris (1964) studied the human mandible during the growth period. They showed that the essential principles of area relocation, surface activity determined by regional directions of growth, and the "V" growth principle occur throughout all parts of the mandible. The authors also highlighted the patterns of remodeling occuring consistently at the condylar neck, the coronoid process, the ramus, the body, the chin and the alveolar processes.

Enlow (1975) analyzed in specific detail the mandibular growth process. He argued that remodeling and displacement are the two principal types of movement involved in mandibular growth. In displacement, the whole mandible is moved in a

forward and downward direction. In remodeling, the constituent parts in each region undergo histological alterations to move into new locations, while providing for bone enlargement and function. It is the entire ramus that continuously remodels and adjusts, allowing the lower dental arch to function reasonably along with the upper arch and the glenoid fossa. The condyle and the ramus on the whole provide the exceedingly critical function of progressive adaptation, a prerequisite for mandibular fit to the maxilla and the cranial base (Enlow, 1980).

The mandibular condyle is not regarded by Enlow as a growth center. It does not possess any special regulatory role on growth of the mandible as a whole. Nor is there believed to exist any genetic programming for condylar control upon of lower jaw mandibular growth. According to Enlow, the condyle is only a regional field of growth providing adaptation for its own localized growth circumstances. The growth of the mandible is the result of a composition of various regional factors and functional agents that function to produce the complex shape of the lower jaw.

There is a question regarding the existence of a special type of cartilage in the condylar head. That cartilage is virtually a functional adaptive response to applied compressive forces on the condyle. An intramembranous type of growth could not operate because the periosteal mode of osteogenesis is not pressure-adapted.

The condylar cartilage is actually a secondary type of cartilage, and its function is to provide regional adaptive growth. The specialized cartilage contributes to the condylar multidirectional capacity to grow and remodel according to the various mandibular displacement and/or rotation. This can be explained by the histological picture of four distinct layers (fibrous, proliferative, mature, hypertrophic). The cells in the above zones are not arranged into linear columns, but rather their alignment reflects the capacity for multidirectional proliferation and growth. The proliferation of these cartilage cells produces the

upward and backward relocation of the condyle. The condylar cartilage actually moves by prechondroblast cell division on the articular side with equal amount of cartilage removing from the opposite internal side (Enlow, 1975).

Koski (1968) stated that the condyle is not the leading center of mandibular growth. This was based on the fact that the condylar cartilage is an embryologic latecomer. That it is not an actual articular cartilage, nor an epiphyseal plate. This secondary type of cartilage does not possess any inherent intrinsic growth force, nor does it grow in nonfunctional sites, as has been demonstrated by transplantation experiments.

Also, Baume (1961) and Baume and Derischweiler (1961) advocated that condylar cartilage is responsive to functional stimuli, property that differentiates it from epiphyseal plate cartilage. Baume (1969) categorized the condylar cartilage as a skeletal growth center and regarded the structure as the particular mandibular growth center, pointing to very distinct differences from epiphyseal and synchondrosal centers. To support this interpretation, he referred to the embryological ability of condylar cartilage at about the twelfth foetal week to assume all the functions of a growth center, namely endochondral ossification in concerted with cartilage proliferation.

Meikle (1973) transplanted a complete mandibular joint as isograft into the brain of littermate rats and concluded that the cells of the proliferative zone can form either bone or cartilage, depending on environmental factors, that the proliferative zone's cells differentiate into chondroblasts only in a functional environment executing articular movements and that mandibular growth does not depend on condylar growth, while remaining essential for normal mandibular growth.

McNamara (1973) carried out experiments using monkeys in which an intraoral appliance was inserted to produce forward mandibular position. At the end of the experiment, he observed

changes in the growth pattern of the condylar head and compensatory integration of the dentition. Nevertheless, he did not find any significant increase in the rate of bone deposition along the posterior border of the ramus, only alterations at the angle of the ramus and the angle of the condyle in relation to the occlusal plane. He concluded that there were adaptations and functional changes in the mandibular skeletal units, the dentition and the mandibular musculature, improving the functional relationship of the mandible in a new posture.

Carlson et al. (1980) stated that the TMJ region of young growing individuals has the ability to adapt to environmental change. Prolonged function or displacement of the joint may lead to an increase in the rate of mandibular growth, whereas decreased function or immobilization may lead to a reduction in the rate of mandibular growth. Thus, the TMJ component and the condyle in particular, is believed to have the potential to adapt and affect mandibular growth.

2. FACTORS REGULATING MANDIBULAR CONDYLAR GROWTH

Mandibular condyle is covered by cartilage, consisting of cellular components in extracellular matrix composed of fibrous (mainly collageneous) elements and proteoglycan aggregate (Teramoto et al., 2003). The unique structure of the condylar cartilage comprises distinct layers (Von den Hoff and Delatte, 2008), capable of adaptive remolding in response to masticatory function and external loading (Kiliaridis et al., 1999; Kuroda et al., 2009; Nickel et al., 2018). The condylar cartilage is mainly a load-bearing structure for induced biomechanical stress and its thickness has been suspected to undergo functional adaptation (Utreja et al., 2016). The TMJ performs complex hinge and sliding movement (Bag et al., 2014). During mastication, compressive, shearing, and other complex forces are exerted on the mandibular condyle (Kuroda et al., 2009).

Condylar growth is affected by heredity (He et al., 2012; Carlson, 2015; Coombs et al., 2019; Vieira, 2019), hormones (Baume, 1953; Milam et al., 1987; Baccetti et al., 2005; Robinson et al., 2018; Yu et al., 2020), the environment (Du et al., 2020; Kahn et al., 2020), systemic diseases (Sansare et al., 2011; Chetty et al., 2017; Cedströmer et al., 2020) and stress (Wu et al., 2011; Wu et al., 2012) and is significant in the development of the orofacial complex (Mew, 1986). Customary mastication consists a physiological stress to the TMJ, of great importance for its development in adolescence and the remodeling in adulthood (Bouvier and Hylander, 1984). The lateral condylar displacement in the glenoid fossa as observed in the therapeutic approach of skeletal discrepancies may culminate in abnormal loading of adjacent structures, affecting the physiologic dynamics of condylar cartilage and triggering the release of growth factors (Von den Hoff and Delatte, 2008; Wang, 2019) and inflammatory mediators (Figueroba et al., 2014), to unknown extent and of unspecified clinical significance, a long-standing controversy.

Articular dysfunction may have adverse consequences on the potential for remodeling, resulting in histological alterations and changes in condylar volume. As a result, mandibular retrusion may lead to adverse outcomes in cartilage formation, as has been reported in rats, suggesting dysfunction and disarrangement (Desai et al., 1996; Cholasueksa et al., 2004; Figueroba et al., 2014). However, others claim that TMJ disorder should not be an issue (Zurfluh et al., 2015). Clinical investigations of the effect of orthodontic mandibular displacement in humans during treatment of malocclusion have suggested that the results of treatment appear to be achieved mainly by remodeling of the TMJ (Folke and Stallard, 1966; Ingervall et al., 1972; Meikle, 2007).

According to Rabie and Hägg (2002), mesenchymal cells in the proliferative layer express Sox 9 transcription factor to differentiate into chondroblasts, and, subsequently, to chondrocytes. Chondroblasts engage in condylar cartilage formation by synthesizing type II collagen, the main subtype forming the framework of the growing matrix of the condylar cartilage. Mature chondrocytes progress towards hypertrophy and secrete type X collagen destined for endochondral ossification.

Cells in the outer, hypertrophic zone of the cartilage secrete Vascular Endothelial Growth Factor (VEGF), which regulates neovascularization preceeding carilage formation and modifies the maturation of the cartilaginous matrix. Hypertrophic cartilage continues to remodel along with vascular invasion and the enlargement of bone marrow spaces to be occupied by pluripotent tissue. The mesenchymal stem cells introduced to the mineralization front later differentiate into osteoblasts, engaging in osseous tissue formation. (Rabie and Hägg, 2002)

3. RAT AS AN EXPERIMENTAL MODEL

The term "animal model" lucks a universal definition in the literature. The animal model was defined as an animal with sufficient similarities to humans regarding its physiology and behavior. Its aim is to develop knowledge that may be applied to the human target group. In addition, animal models may serve as models for other animal subjects. However, they are most commonly used to investigate human conditions indirectly through animal studies. (Sjoberg, 2017)

The animal model has a crucial role to preclinical study and constitutes the connection between basic research and clinical practice. The simulation of disease symptoms and pathology is vital part for investigating the mechanisms of disease and developing new treatments. For example, multitudinous animal models have appeared in TMD research, lately. (Xiang et al., 2021)

The role of the TMJ in mandibular movement remains mostly unknown. There is a strong correlation between the morphology of the teeth, the diet, and habitat. The specialized dentitions of the Mammalia associated with dietetic adaptations, are already known. In spite of the fact that the above association extends to the other parts of the masticatory system, the differences in the mandible's morphology, the TMJ and the corresponding muscles have not been studied adequately. (Hiiemäe, 1967)

Despite the unickness of the TMJ in mammals, it varies significantly among different mammalian groups, morphologically, as well as functionally. Currently, the most preffered lab animals are rats, rabbits, pigs, and ruminant ungulates. (Herring, 2003)

In the fields of pharmacology, genetics, immunology, physiology, neuroscience, transplantation, aging and cancer research, the rat appears being a popular animal model. The breeds that have been used derive mainly from the Norwegian rat (Rattus

norvegicus), allegedly originating from the area bordering the Caspian Sea, up to the coastline of Lake Baikal, in Siberia. It migrated to Europe and the United States following the 18th century commercial routes. By the mid – 19's, it had already got established in anatomical, physiological and nutritional projects. Moreover, H. H. Donaldson, W. E. Castle, along with colleagues pioneered developing an initial inbred lineage at the turn of the 20th century, destined for studies in basic genetics and cancer research. At the time, H. H. Donaldson argued on the appropriateness of the rat as a lab animal alleging that the animal of interest had proved compact, pleasing, easy to keep and breed, it might have a litter both numerous and immature, while remaining agile and easy to train. Further development and genetic characterization of inbreds as well as introduction of congenic and recombinant strains have materialized in the United States, Japan and Czechoslovakia. (Castle, 1947; Owen, 1962; Heslop, 1968; Gill, 1985; Gill et al., 1987; Gill et al., 1989)

The resolute transformation of the rat to assume the role of standardized laboratory model was carried out at the Wistar Institute in Philadelphia, USA. The animal to be named Wistar Rat started procreating and disseminating from 1906 through the 1940s, promoted as being of premium quality, consistently reliable, assuredly the original rodent gold standard in research. (Clause, 1993)

The development of the Wistar Rat as a standardized animal may be attributed to the breeding effort of Helen Dean King, combined with the advanced, innovative husbandry of Milton Greenman and Louise Duhring under the lasting guidance and support of Henry Donaldson. Allegedly, the widespread use of the Wistar Rats is a result culminating from Milton Greenman, the highly socially and scientifically knowledgeble biologist and Wistar Institute manager, who foresaw a way for a small institution to provide exceptional service to the Science. He recognized in the rat the potential to become a living substitute to pure chemicals, widely implemented in research. He got inspired while studying the notion of product uniformity, the

required quality standards, and the concept of efficient production, to apply them into scientific practice that resulted in a rodent currently dominating laboratory throughout the world. (Clause, 1993)

The long axis of the murine TMJ is positioned in anteroposterior direction and its osseous wall is lined by fibrous tissue. Transversely, the articular surface is concave, while sagittally is rather straight. The anterior region of the floor of the fossa tilts downwards. The condyle, covered with hyaline cartilage, occupies about half the length and two-thirds the width of the fossa. The articular disk consists of mainly longitudinally stretching collagen fibers. It is thin centrally, but thickens in the front and rear of the condylar head. The capsule connects to the zygomatic bone with lateral ligaments. (Weijs, 1975)

In the adult rat, the condyle slides anteroposteriorly over a distance of about 6 mm, residing mainly to the posterior two-thirds of the fossa during food grinding and the anterior two-thirds when gnawing. Besides, the lateral space between the fossa and the condyle allows for considerable rotational movement around the condylar vertical axis. Evidently, the condyle assumes different positions in molar and incisal occlusion, and the resting position is intermediate to the above. (Weijs, 1975) When the animal starts to bite, complex condylar movements (sliding and rotational) may be registered. (Hiiemae and Ardran, 1968)

The maxillary incisors have a clinical crown length of about 4 mm, each being about 1.5 mm in width. The labial surface appears convex and is covered with enamel. Palatally, they feature dentin wear facets adjoining flat enamel cutting edges. Both teeth have an anteroposterior inclination of about 160°. The mandibular incisors are similarly curved, consisting of a labial enamel band and a posterior body of dentin. Their clinical length is about 7 mm, their width 1.2 mm and also has lingual, elliptical wear facets. The angle between the lower incisor long axis and the occlusal plane approximates 35°. The maxillary

molar occusal surfaces are laterally angled at about 20° to the horizontal plane. Lower molars appear medially inclined, to the same degree. Overall, the maxillary molars show buccal, central and palatal cusps, whereas the mandibular ones feature cusps accommodated in buccal and lingual rows. (Weijs, 1975)

The rat is likely the most selected experimental animal to study cranial growth despite the existing differences with human's anatomy. Therefore, clinical trials should be conducted, as animal studies cannot totally reproduce the normal human function.

4. MANDIBULAR POSTERIOR DISPLACEMENT

Janzen and Bluher (1965) applied for 140 days a constant retracting bilateral force of 150 grams against the mandible through a retracting mechanism in four 9 to 23 month-old growing rhesus monkeys. The force affected mandibular growth and also the rest of the facial complex. A slight increase in ramus height, a decrease in the mandibular angle, a reduced maxillary alveolar development and hypertrophy of the external pterygoid muscles were among the observed anatomic changes. However, the condyle was not allowed to reach its fullest growth potential.

Petrovic et al., (1973) and Petrovic et al., (1986) used chin caps in growing rats and concluded that the rate of mandibular growth was diminished, that the length between the posterior border of the condyle and the mental foramen became smaller and reported on decreased proliferation of condylar cartilage and prechondroblasts. Also, the number of the successivelly arranged sarcomeres of the lateral pterygoid muscle was increased.

Rats wearing chin caps in which the whole lateral pterygoid muscle had been resected demonstrated significantly diminished mandibular growth rate compared to rodents in which the lateral pterygoid was only partially removed. The response to the retractive force was pronounced when the condylar cartilage was in a rapid growth stage.

Tsolakis (1981) concluded that light traction forces are preferable for inhibiting mandibular growth. They significantly diminish the extent of mandibular growth without causing any significant detrimental change in the joint and also avoid muscular over-reaction. In contrast, heavy traction force inhibits growth of the lower jaw and also creates serious functional and structural imbalances in the adjacent tissues, including destructive changes in the TMJ. Thus, there appears to exist a threshold in the magnitude of retrusive force that might cause a

pressure effect on the condyle, without triggering strong contractile activity of the lateral pterygoid muscles, resulting in condylar growth inhibition.

Ghafari and Degroote (1986) summarized that an inclined plane bonded to the upper incisors of rats may be used to produce continuous forward and downward mandibular displacement and result in accelerated ossification of the hypertrophic zone of the cartilage (meaning adaptation and functional adjustment). The observed changes, potentially transient, may be attributed to premature maturation or may be inconsistent depending to alterations in the transmitted force levels according to the mandibular functional position. It is noteworthy that the observations differed from those in intermittent displacement that increased the thickness of the prechondroblastic and chondroblastic zones.

5. RADIOGRAPHIC DATA

A recent systematic review aimed to appraise the quality of the available evidence in animal studies regarding the effects (macroscopic, measurable, dimensional changes) following posterior displacement of the mandible (Lyros et al., 2021).

In growing Wistar rats, the mandible grew shorter anteroposteriorly, the coronoid process became higher, the condylar neck measured thicker and an enlarged retromolar corpus was evident after application of an orthopedic collar device exerting a backward force on the mandible (Asano, 1986). Moreover, the orthopedic effects were limited to the period when the force was applied and the mandible returned to the inherited growth pattern in both the experimental and control groups after the activation of the appliance had ceased, when the mandibles resumed growing at similar rates, in anteroposterior direction, as regulated by genetics (Asano, 1986). Indeed, the mandibular area where growth was more pronounced due to the intervention showed less subsequent growth and remodeling comparing with controls (Asano, 1986). Allegedly, the above-mentioned alterations in mandibular development had not affected the overall growth pattern as evaluated by skull dimensions and body weight (Asano, 1986). Notwithstanding, mandibular retraction did not significantly affect the condylar height and the thickness of the angular process (Asano, 1986). In another similar experiment involving rats, it was confirmed radiographically that the posterior mandibular displacement prevented the mandibular condyle from displacing anteriorly in the temporal fossa (Teramoto et al., 2003).

In rats and rabbits subjected to mandibular/condylar backward retraction with the aid of inclined planes cemented on maxillary incisors or properly modified guiding appliances, lateral radiography disclosed mandibular molars occluding more distally in relation to the maxillary (Desai et al., 1996;

Cholasueksa et al., 2004). Interestingly however, at a later age the radiographic, distalized molar relationship became less pronounced, supposedly an attempt on the part of the subject to establish a new balance within the altered oral environment (Desai et al., 1996).

Mandibular posterior displacement in growing rats by an appropriate occlusal guiding appliance attached to the maxillary incisors resulted in shorter mandibular length on both sides (Farias-Neto et al., 2012). In the experimental group, statistically significantly smaller mandibular lengths were measured radiographically, without any noteworthy difference between the left and right sides (Farias-Neto et al., 2012). Additionally, statistically insignificant differences were observed between experimental groups regarding the ramus height and the intercondylar distance (Farias-Neto et al., 2012).

The cementation of modified inclined planes on the upper and lower molars of rats effected a statistically significantly shorter condylar process and significantly larger angulation of its axis to the mandibular plane in the experimental groups, as evidenced radiographically (Hua et al., 2012). Both the overall mandibular length and the condylar height remained significantly smaller in the experimental groups compared to the controls until the end of the period of study (Hua et al., 2012). By the end of the experiment, also the condylar width measured significantly less in test subjects (Hua et al., 2012). By the midst and the end of the observation period, the condylar posterior surface appeared flattened compared to that of the control groups; additionally, its most posterior point had shifted upward (Hua et al., 2012). Eventually, the decrease in mandibular length in experimental animals was attributed to the remodeling of the condyle (Hua et al., 2012). Ultimately, Wang et al., 2019 found in the rat that a twin inclined device resulting in posterior mandibular displacement may lead to adaptive bone resorption at the posterior region of the condyle. In their control group, the posterior margins of the condylar bone remained round, whereas in the experimental group the lower part of the

posterior margin of the condyle appeared significantly flattened by the end of the observation period as highlighted by 3D reconstruction (Wang et al., 2019).

The predominant animal in the studies has been the rat, although Desai et al. 1996 experimented on rabbits and earlier studies have selected the monkey. The age of the animals is of importance because mandibular growth is related to general growth, and varies in relation to chronological age (Mito et al., 2003; Hunter et al., 2007; Hans et al., 2017). The age of the animals was clearly reported, but varied from 4 weeks (Asano, 1986), 5 weeks (Farias-Neto et al., 2012), 6 weeks in the papers by Hua et al. (Hua et al., 2012) and Wang et al. (Wang et al., 2019) up to 8 weeks (Teramoto et al., 2003; Cholasueksa et al., 2004), and even 9 months in rabbits (Desai et al., 1996). Moreover, bone turnover depends on sexual hormones; additionally, TMJ pathology has been linked to the hormonal profile (Robinson et al., 2018). In the same review, five studies reported on male Wistar rats (Asano, 1986; Teramoto et al., 2003; Cholasueksa et al., 2004; Hua et al., 2012; Wang et al., 2019), while Farias-Neto et al. (Farias-Neto et al., 2012) experimented on female Wistar rats and Desai et al. (Desai et al. 1996) on rabbits of unidentified sex. The method to produce the mandibular displacement included inclined planes cemented on maxillary incisors (Desai et al. 1996; Cholasueksa et al., 2004; Farias-Neto et al., 2012) or the molars (Hua et al., 2012; Wang et al., 2019), and collar extraoral appliances exercising orthopedic traction by attachments on the lower incisors (Asano, 1986; Teramoto et al., 2003). All the included studies had control and experimental groups and the comparison was performed between them to identify differences of statistical significance at the level of 5%, at least. Statistical methodology was stated without much detail, particularly addressing the aspect of statistical normality. Inadequate or inappropriate statistics may contribute to systematic errors and thus potentially undermine the quality of conclusions of the systematic review.

Lateral radiography was rather used to confirm the change in the relationship between the maxillary and mandibular molars in the studies by Cholasueksa et al. (2004) and by Desai et al. (1996). Indeed, in both, the mandibular first molars in the experimental group moved in a distal position relative to the maxillary ones after posterior displacement of the mandible. In addition, the customary posterior mandibular displacement was considered dysfunctional and traumatic as evidenced by the production of proteins indicating damaged nerve fibers in the retrocondylar region (Cholasueksa et al., 2004). Similarly, Desai et al. (Desai et al. 1996) found alterations, albeit statistically insignificant, in the spatial orientation of the temporomandibular disk that allegedly might predispose to anterior disk displacement concomitant with TMJ disorder (Desai et al. 1996). Anterior displacement of the articular disk was proposed by Teramoto et al. (Teramoto et al., 2003), who also found that the condyle in their experimental group sustaining backward compressive force was positioned more posteriorly within the articular fossa during mouth opening, compared to the control group.

Cephalometric measurements by Asano (Asano, 1986) showed that the mandibles that are pushed backward end-up smaller in length, having less volume and weight. He calculated an increase in the size of the anterior mandibular region, coronoid process, the neck of the condyle and also found thickening of the retromolar region in the experimental group in relation to controls. However, the condylar height and the thickness of the angular process remained statistically unaffected. The observed differences occur due to localized differential bone apposition and resorption leading to remodeling and adaptation to accommodate the applied force in the altered environment.

An important finding was that the differences in growth remained after the cessation of the external force and growth direction returned to the inherited growth behaviour, meaning that a lasting effect may be anticipated in similar cases (Asano, 1986). It is noteworthy that the use of various experimental

devices was not found to have any significant influence to general growth or the size of the skull as a whole (Asano, 1986; Farias-Neto et al., 2012; Hua et al., 2012). In agreement with the aforementioned research, Farias-Neto et al. (Farias-Neto et al., 2012) also found decreased mandibular length in cases of functional posterior mandibular displacement, but negligible difference in the height of the ramus. Farronato et al. speculated that differences in condylar heads could be attributed to condylar growth center dysregulation, whereas the reduced condylar neck volume could have been an outcome of growth deficit and also the height of the ramus had not been significantly affected (Farronato et al., 2020).

Similarly, Hua et al. (Hua et al., 2012) reported that when inducing backward movement of the mandible, reductions in the length of the condylar process and the mandible may be expected. Their cephalometric analysis revealed a greater increase of the angle of the condylar process to the mandibular plane and a decrease of the condylar width in the experimental animals. Moreover, they mentioned that the most posterior condylar point had shifted upward and the posterior condylar surface had a tendency to flatten, indicating bone resorption (Hua et al., 2012). Flattening of the entire posterior margin of the condyle became progressively evident and statistically significant compared to controls in the study by Wang et al. (Wang et al., 2019), who experimented in the rat with posterior inclined planes that apply a functional retrusive force. This pattern of change is compatible with progressive adaptation of the condylar bone to mild, continuous and progressive pressure (Wang et al., 2019).

The observed changes of the various mandibular regions may be attributed to the remodeling that happens due to the paranormal, dysfunctional external force and the potential consequent loss of the optimal, customary mastication force by restricted mandibular movement. The explanation of the mechanism that leads to such an outcome should be sought out within molecular pathways and in cellular interactions

(Teramoto et al., 2003; Farias-Neto et al., 2012). In humans, the differences may be expected more pronounced than in the rodents; additionally, bone resorption may be anticipated in the posterior condylar surface and the anterior region of the post glenoid eminence, because of existing anatomical differences (Cholasueksa et al., 2004; Farias-Neto et al., 2012; Hua et al., 2012). Nevertheless, in growing individuals the ultrastructural changes in the posterior area of the condyle due to mechanical stress could be anticipated to reverse spontaneously at earlier stages (Wang et al., 2019).

6. RANKL - OPG - MCSF

Receptor Activator of Nuclear Factor – (KB) Ligand (RANKL) - Osteoprotegerin (OPG) system

Orthodontic force effects areas of compression and tension within the periodontal ligament and the adjacent alveolar bone, manifesting respective bone resorption and formation to guide the dental unit into new position (Rygh, 1976). Osteoprotegerin (OPG) and the receptor activator of nuclear factor – (KB) ligand (RANKL) compete to regulate osteoclastic cell maturation and bone metabolism by shifting the balance between RANK-RANKL and OPG-RANKL binding (Anderson et al., 1997; Wong et al., 1997; Lacey et al., 1998).

RANKL is a member of the membrane-associated Tumor Necrosis Factor superfamily. It promotes hemopoietic precursors to differentiate into mature osteoclasts and stimulates their resorptive function (Udagawa et al., 1999). OPG a cytocine receptor acts as a decoy receptor for RANKL, as it competes with RANK for RANKL binding to enhance osteoclast apoptosis (Burgess et al., 1999; Lacey et al., 2000; Neumann et al., 2005; Nishijima et al., 2006).

Thus, OPG contributes to the regulation of bone metabolism and the balance between RANK-RANKL and OPG-RANKL binding has a fundamental role (Figure 1).

Figure 1. RANK/RANKL/OPG System
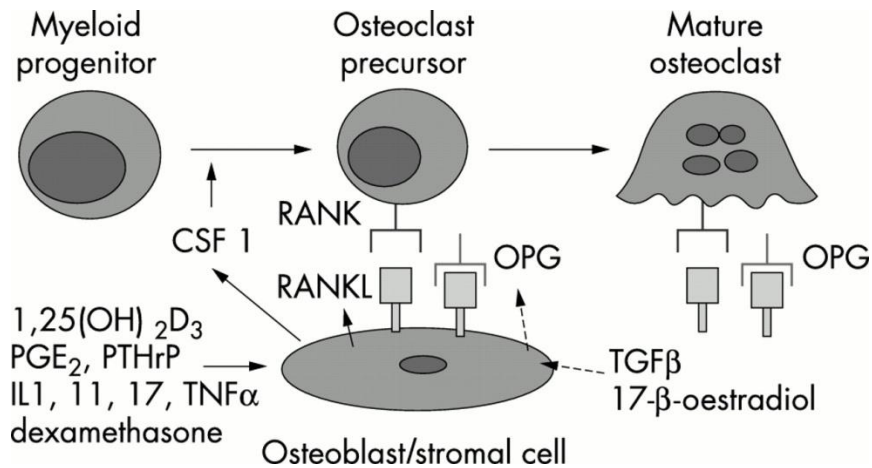
*Macrophage colony-stimulating factor (MCSF)*

The macrophage colony-stimulating factor (MCSF), is a secreted cytokine which stimulates hematopoietic stem cells to differentiate into macrophages or similar cellular types (Stanley et al., 1997). Besides, the eukaryotic cells synthesize the molecule to resist viral assault and tumor enlargement through a chemotactic/phagocytic activity, and pronounced malignant cell cytotoxicity (Nemunaitis, 1993). It belongs to the quartet of experimentally described colony-stimulating factors. MCSF binds to the colony stimulating factor 1 receptor. MCSF binds to the colony stimulating factor 1 receptor (Figure 2) (Metcalf, 2013).

MCSF is produced by a variety of cells, including endothelial cells, fibroblasts, osteoblasts, smooth muscle, and macrophages, and can be detected in plasma (Hamilton, 2008; Pollard, 2009; Hume and MacDonald, 2012). Elevated circulating MCSF levels have been reported in numerous diseases, including cancer, inflammation, and autoimmune disorders (Firestein et al, 1988; Scholl et al, 1996; Bischof et al, 2000; McDermott et al, 2002) and during pregnancy, contributing to placental development) (Bartocci et al, 1986; Pollard et al, 1987; Fixe and Praloran,

1997). Endocrine stimulation by parathyroid hormone (Han et al., 2018) ultimately results in increased plasma calcium levels following bone breakdown.

Concerning its structure, MCSF is a small-sized protein involved in cell signaling. When activated, may be traced in the extracellular space as a disulfide-linked homodimer, believed to have been a product of proteolytic slicing of membrane-bound precursors (Felix et al., 1994).

Figure 2. MCSF System (Sinha et al., 2021)
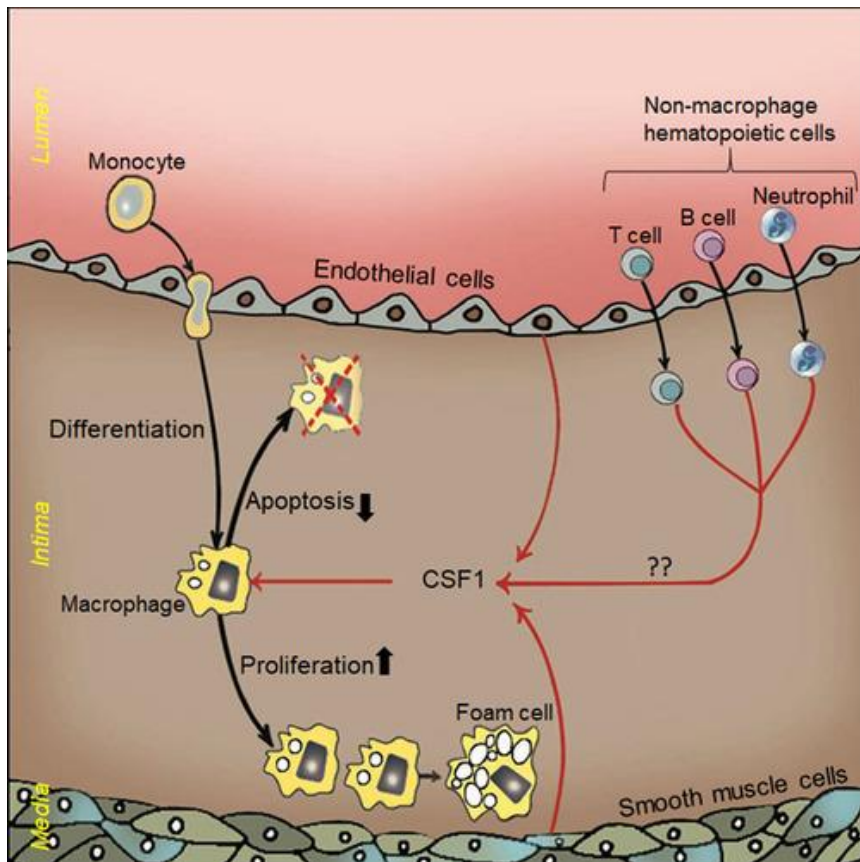
7. HISTOLOGICAL DATA

The mandibular condyle performs translatory and rotary movements, cushioned by a fibrocartilaginous articular disc with disparate thickness (Helland, 1980). In the TMJ, the articular surfaces are load-bearing and are lined with dense, avascular, fibrous connective tissue (Kuroda et al., 2009).

The fibrocartilage and the underlying trabecular bone form a functional entity withstanding the mechanical tension generated by mandibular movement (Kuroda et al., 2011). Bone continuously remodels to provide structural support to the overlying articular structures during jaw movement (Zhang et al., 2013; Aube and Ramirez-Yanez, 2019). The localized architecture and density supposedly accommodate the stress on the cartilage and thus reflect, to some extent, the metabolic state of the whole joint (Jiao et al., 2010).

The cartilage on the condylar head features four distinct layers, namely the fibrous, proliferative, mature and the hypertrophic, to be identified from the articular surface to the underlying bony foundation. The fibrous zone contains fibroblast-like, flat cells, the proliferative zone has heterogeneously distributed mesenchymal precursor cells, while differentiated chondrocytes may be traced in the mature and hypertrophic zones (Kuroda et al., 2009; Jiao et al., 2010).

Bone resorption is an essential component of the development and the remodeling of the skeletal substrate (Siddiqui and Partridge, 2016). The predominant cell in this process appears to be the osteoclast (Ikeda and Takeshita, 2016). The mononucleated precursors invade the mesenchyme surrounding rudimentary bone to proliferate, differentiate into tartrate-resistant acid phosphatase (TRAP) - positive cells, and migrate in conjunction with endothelial cells (Yasuda et al., 1998; Yang et al, 2008; Kim et al., 2015). Subsequently, they invade the calcified cartilage to transform into mature multinucleated osteoclasts (Väänänen et al., 2000; Ikeda and Takeshita, 2016).

These specialized resorptive cells evolve from hematopoietic stem cells under the control of various systemic and local factors (Hofbauer et al., 2000; Inoue et al., 2021). Osteoclast differentiation factor (Yasuda et al., 1998; Rolph and Das, 2020), RANKL, (Boyce and Xing, 2008) osteoprotegerin (Kang et al., 2014), Macrophage Colony - Stimulating factor (MCSF) (Fujikawa et al., 2001), Vascular Endothelial Growth Factor (VEGF) (Niida et al., 1999; Kim et al., 2015), Interleukin - 1 (Lee et al., 2010), and tumor necrosis factor (TNF-α) (Luo et al., 2018) are all implicated in osteoclastic function and the metabolism of the osseous tissue. Besides, proteinases of the matrix metalloproteinase (MMP) family appear to exert direct chemotactic activity on osteoclasts for their recruitment (Engsig et al., 2000).

Asano, (1986) concluded that the condylar cartilage adapted to the mandibular retractive force, while its chondroblastic constituent exhibited limited proliferation ending up hypomaturated.

Teramoto et al. (2003) found expansion of the bone marrow space in the initial stage of the experiment, indicating erosion of the cartilage, starting from the lower hypertrophic zone. Also, a reduction of the thickness of each zone and the number of chondrocytes was observed later on. Nevertheless, in the 7-day experimental group, the thickness of each zone appeared to recover. Besides, the hypertrophic chondrocytes became smaller as the duration of force application increased, only to recover in the 7-day group. In the posterior region, the metachromatically stained area remained unchanged initially. Subsequently, the thickness of each zone and the number of chondrocytes reduced significantly. However, there were no differences in the respective zonal thickness in the 7-day experimental group.

Cholasueksa et al., (2004) calculated that the functional mandibular retrusion in rats resulted in statistically significant reduction of condylar cartilage width at the posterior region.

Kuroda et al., (2009) used micro - computed tomography analysis to suggest that in the experimental animal group, a decrease in the amount of trabecular bone was effected in the condylar anterior region. Moreover, they found statistically significant reductions in the Bone Volume/Total Volume ratio and the Degree of Anisotropy in the same abovementioned area, following distal mandibular displacement.

Hua et al. (2012) did not initially find any statistically significant difference in the early stages of their study regarding the cartilage thickness in all mandibular condylar regions of interest between the studied groups. Later however, on day 30, the mandibular condylar cartilage in the most posterior region was thinner in the experimental groups, comparing to controls, and continued to attenuate until experimental day 60. Contrarily, from day 30 until the end of the experiment on day 60, the condylar cartilage was found thicker in a more anterior region. However, the cartilage measured thinner even more anteriorly, in the experimental groups. Thus, it was hypothesized that the posterior mandibular displacement could culminate in region-specific cartilage alterations and continuing adaptive condylar remodeling.

Figueroba et al., (2014) observed histomorphometrical changes and cellular disarrangement at the articular cartilage in the experimental group. The four cartilage layers measured thicker in the experimental groups undergoing functional posterior mandibular displacement, compared to controls, after 14 days of intervention.

Wang et al. (2019) concluded that the condylar tissue changes meaned occurrence of osteoclastic activity in the posterior region of the condyle and that these adaptive changes showed bone resorption in the posterior condyle.

II.   MAIN PART

1. AIM

The present original research aimed to identify changes (radiographic, biochemical, histological) of the condyle and the mandible in rats that have undergone restriction in mandibular growth by means of an orthodontic / orthopedic device applying mechanical loading, in comparison with control rats without the device.

2. MATERIAL AND METHODS

The study experimental protocol was approved by the Veterinary Directorate and received protocol number 598742/04-10-2019, registered as EL 25 BIO 05, according to Greek national legislation (P.D 56/2013), conforming to European Directive 2010/63/EE and that of the European Council (276/33/20.10.2010) related to the protection of vertebrate animals used in experiments and for other scientific purposes.

Experimental design

In the present experimental study, seventy-two (72) four-week-old male Wistar rats were used. After their initial four-week breeding in the Hellenic Pasteur Institute, all the animals were transferred and housed at the Laboratory for Experimental Surgery and Surgical Research "N. S. Christeas" at the University School of Medicine in Athens. Standardization following National and European legislation determined cage selection (Tecniplast S.P.A., Italy) and stable centrally ventilated (15 air changes/h), environmental conditions at 55% relative humidity, temperature at 20°C ± 2°C , and artificial 12-h span of alternating light-dark cycles. Access to food and water was ad libitum (Figure 3).

Figure 3. Access to food and water was ad libitum.
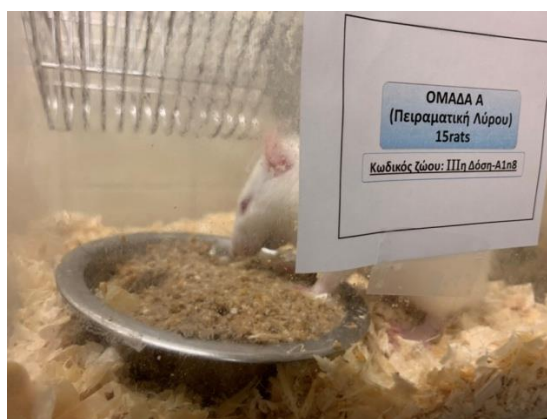
The animals were randomly allocated to equal groups, namely groups A (experimental) and B (control), each been divided into three equally-sized subgroups featuring twelve rats (A1, A2, A3, B1, B2, B3). The online Random Team Generator tool was used for the grouping (Figure 4).

Figure 4. Random Team Generator Tool

Random Team Generator:

Group 1

1 33	2 8	3 22	4 6	5 62	6 68	7 44	8 63	9 59	10 19
11 38	12 43								

Group 2

1 71	2 65	3 15	4 61	5 60	6 9	7 16	8 51	9 52	10 13
11 12	12 11								

Group 3

1 42	2 69	3 20	4 39	5 7	6 24	7 36	8 26	9 53	10 55
11 18	12 34								

Group 4

1 45	2 27	3 47	4 23	5 72	6 54	7 56	8 28	9 58	10 3
11 57	12 66								

Group 5

1 31	2 5	3 21	4 4	5 40	6 35	7 48	8 37	9 25	10 32
11 41	12 14								

Group 6

1 10	2 1	3 50	4 30	5 67	6 70	7 2	8 64	9 29	10 17
11 46	12 49								

Modified orthodontic intraoral devices that have been previously described (Desai et al., 1996) were placed in the experimental animals and led to posterior mandibular displacement. The full-cast metal orthodontic devices were constructed in the laboratory, following a digital intraoral scanning (TRIOS 3, 3Shape intraoral scanner) of an animal selected at random (Figures 5-7).

Figure 5. Digital intraoral scanning

Figure 6. Digital cast

a.

b.
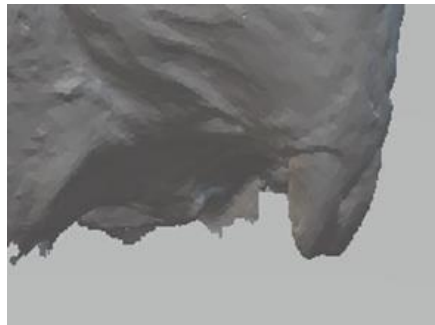
c.

Figure 7. Full - cast metal orthodontic device

a.
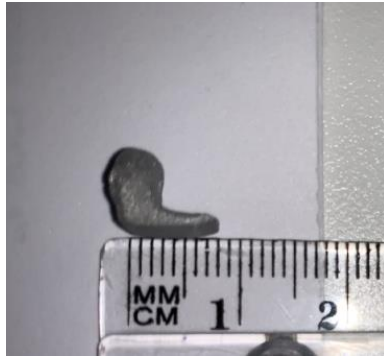
b.
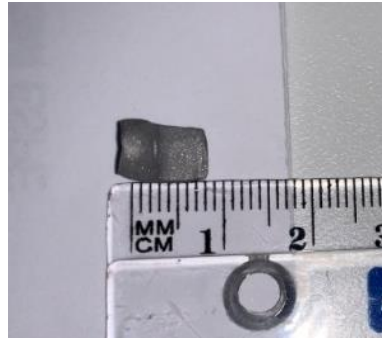
c.
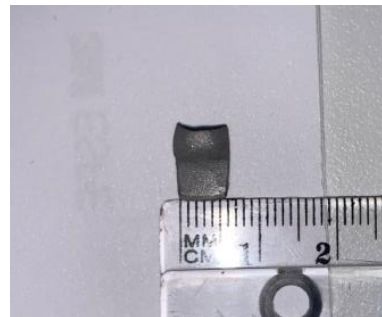
d.
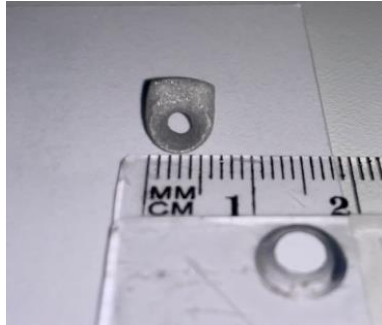
e.

The modified guiding appliances were cemented to the maxillary incisors with zinc phosphate cement (Harvard Cement Normal Setting; Harvard Dental International GmbH, 15366 Hoppegarten, Germany) (Figure 8). During the whole experimental period, all animals (experimental and control) were fed with mashed food, produced by blending pellets with water in standardized proportions to achieve a porridge-like consistency (Figure 9).

Figure 8. The modified orthodontic intraoral device cemented to the maxillary incisors

Figure 9. Standardized preparation of mashed food

a

b.

In total, the experimental period lasted for 90 days. Animals were sacrificed at 30 days (subgroups A1, B1), 60 days (subgroups A2, B2) and 90 days (subgroups A3, B3). At the 60th day of the experiment, orthodontic devices were removed from the subjects still remaining in the experimental subgroup A3. Throughout the entire experimental period, all animals were kept closely monitored for normal growth and development (Figure 10).

Figure 10. Body weight calculation

Three-dimensional radiographic analysis

To determine the three-dimensional morphology of the mandible, initial (day 1 of the experiment) and final (day of sacrifice) CBCTs were performed in every rat (Figure 11). The rats were injected intramuscularly for anaesthesia with ketamine-xylazine combination at a dosage of 0.2 ml/kg. Once the rats were adequately sedated, they were positioned in the head-resting cushion. All rats were scanned with the same CBCT unit (New Tom VGi, Cefla SC, Imola, Italy) using the same field of view (8X8 cm, high-resolution, denture scan) with exposure settings 110kV. Each scan was performed by an Oral and Dentomaxillofacial Radiologist, who assessed the presence or absence of obvious motion artefacts. In cases of obvious motion artefacts the scans were performed again and the volumetric data of all scans were exported as Dicom 3 datasets. Three-dimensional reconstruction and analysis were conducted by using Viewbox software (Viewbox$^{©}$ version 4.1.0.10, dHAL Software, Kifissia, Greece). Table 1 and Figure 12 present the detected mandibular anatomic landmarks while Figures 13a and 13b show the performed linear measurements.

Figure 11. **Cone Beam CT scanning**
a.

b.
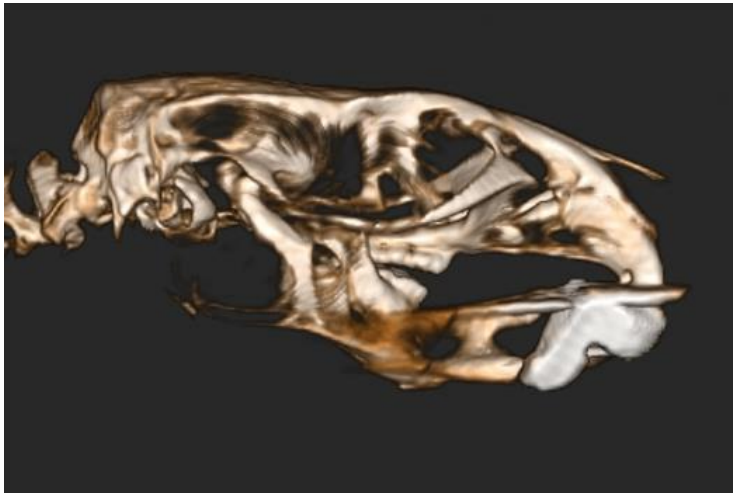
Table 1. Description of anatomic landmarks detected in Cone Beam CT reconstructed images

Anatomic landmarks	Description
Go'	the lowest point of the gonial process
Go	the most posterior point of the gonial process
Menton	the lowest point of the mental process
Coronoid	the tip of the coronoid process
Condylion	the most posterior and highest point of the condylar process
I'	the most anterior point of the alveolar process at the side of the concavity of the lower incisor
Id	the most anterior point of the alveolar process at the side of the convexity of the lower incisor
Incisal	Incisal edge of the lower incisor

Figure 12. Anatomic landmarks detected in Cone Beam CT reconstructed images
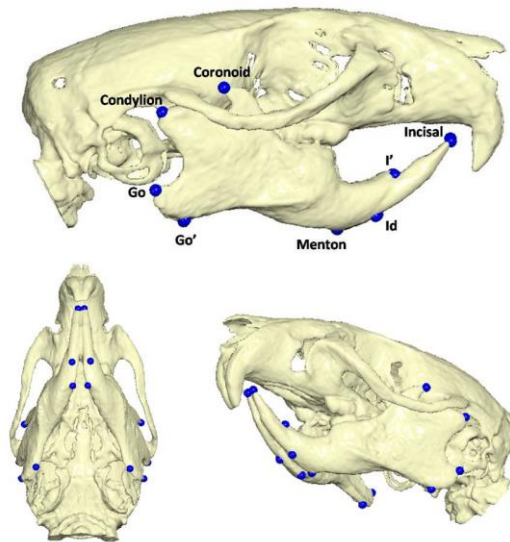
Figure 13a. Linear measurements: Go' - Menton (mandibular body length a); Go - Menton (mandibular body length b); Coronoid - Menton; Condylion/Go' - Menton (Condylion height); Condylion - Go' (Ramus height); Condylion - Menton; Condylion - Id; Condylion - I' (mandibular length); Incisal - Id; Incisal - I'.
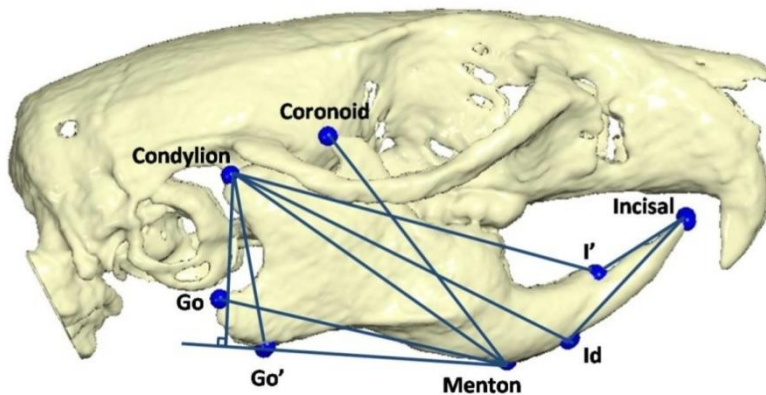
Figure 13b. Linear measurement: Condylion right - Condylion left (Intercondylar distance)

Condylion right

Condylion left
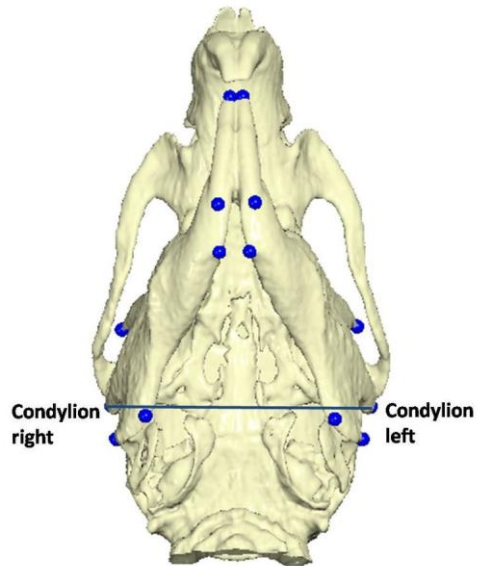
Biochemical analysis

For the measurement of circulating levels of RANKL (Receptor Activator of Nuclear Factor Kappa B Ligand), MCSF (Macrophage Colony Stimulating Factor) and OPG (Osteoprotegerin) proteins, rat serum was collected. In particular, initial (day 1 of the experiment) and final (day of sacrifice), blood collection was performed. The animals were transiently anaesthetized in an ether chamber and blood samples were collected from the eye with a thin sterile laboratory pipette, which was inserted in the eye area, behind the eye ball (Figure 14). Blood was placed in tubes containing heparin and centrifuged at 13,000 rpm for 5 minutes at room temperature. Serum was subsequently collected and stored at -20°C to be further analyzed.

Figure 14. Blood collection
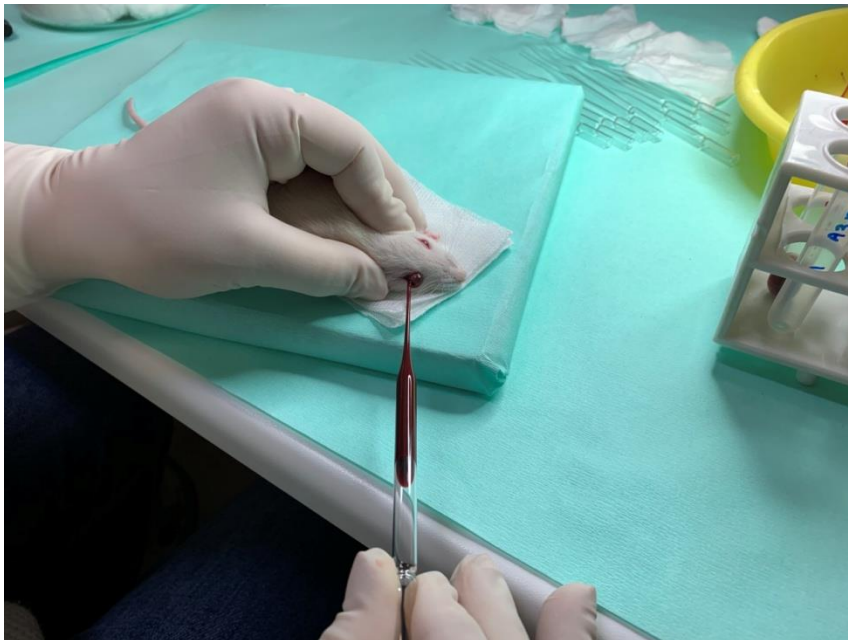
Protein levels were measured using Enzyme-linked immunosorbent assay (ELISA) kits (Elabscience®, USA) according

to manufacturer's protocol. The ELISA kits used were the following: Rat RANκL (E-EL-R0841) Lot: VXI7TPKKPH, Rat MCSF (E-EL-R0601) Lot: 34WBPZTWYT, Rat OPG (E-EL-R3005) Lot: 62SAD9SRJJ (Figure 15).

Figure 15. ELISA kits
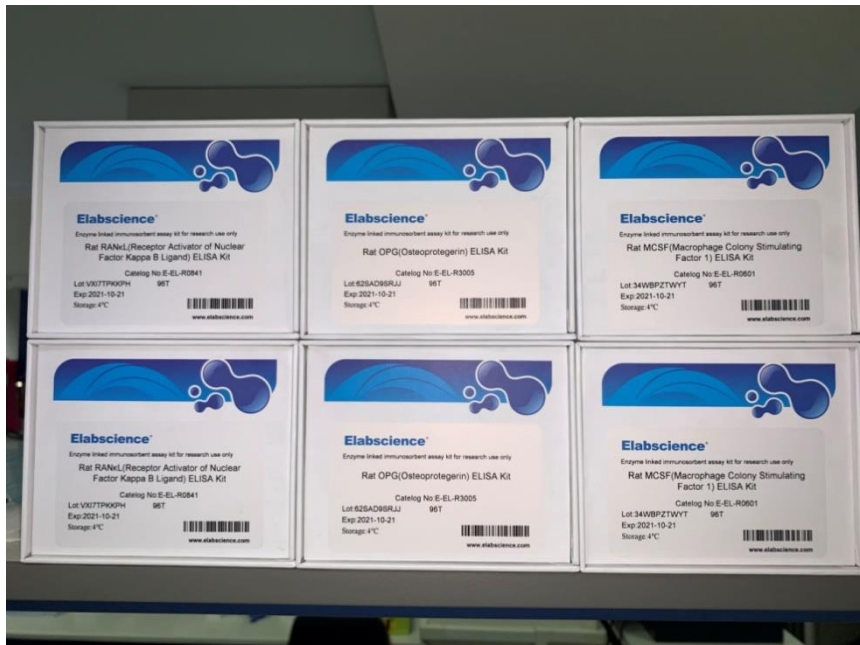
These ELISA kits use the Sandwich-ELISA principle. Briefly, 100μl of serum and the protein standards were added to pre-coated micro-ELISA plates with the corresponding antibody specific to each protein. Then, a biotinylated detection antibody specific for each protein and Avidin-Horseradish Peroxidase (HRP) conjugate were added successively to each micro plate well and incubated. When substrate solution was added to the wells, the wells containing each protein turned blue and the intensity of blue color was proportional to the amount of protein in the sample. Once the reaction was interrupted with a stop solution, the color turned into yellow and the optical density (OD) of yellow color was measured spectrophotometrically at a wavelength of 450nm. The respective protein concentration in each sample was calculated by comparing the OD values of the samples to

the standard curve, which was created using OD values of the known concentration of the standards for each protein (Figure 16).

Figure 16. ELISA procedure

a.

b.
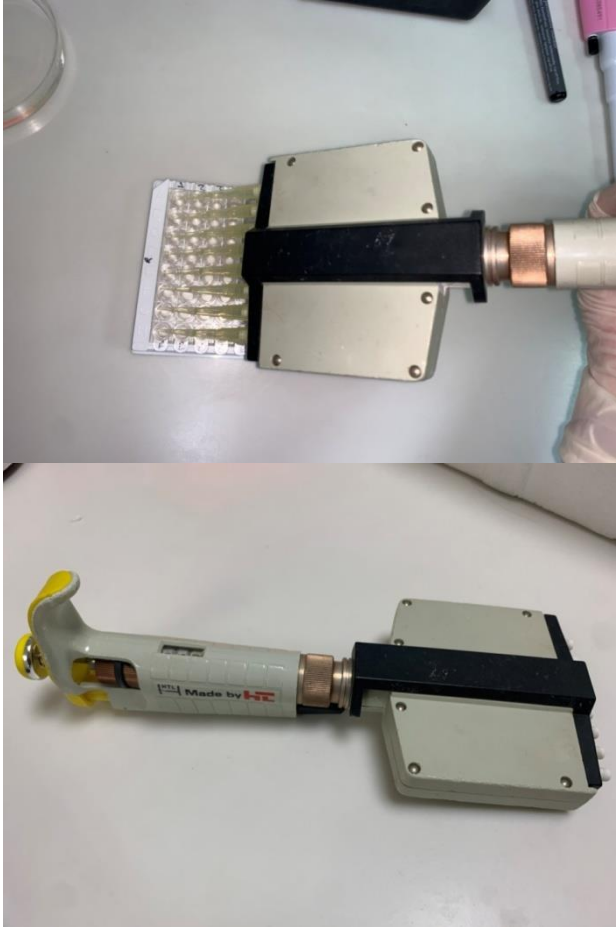
c.

d.

e.
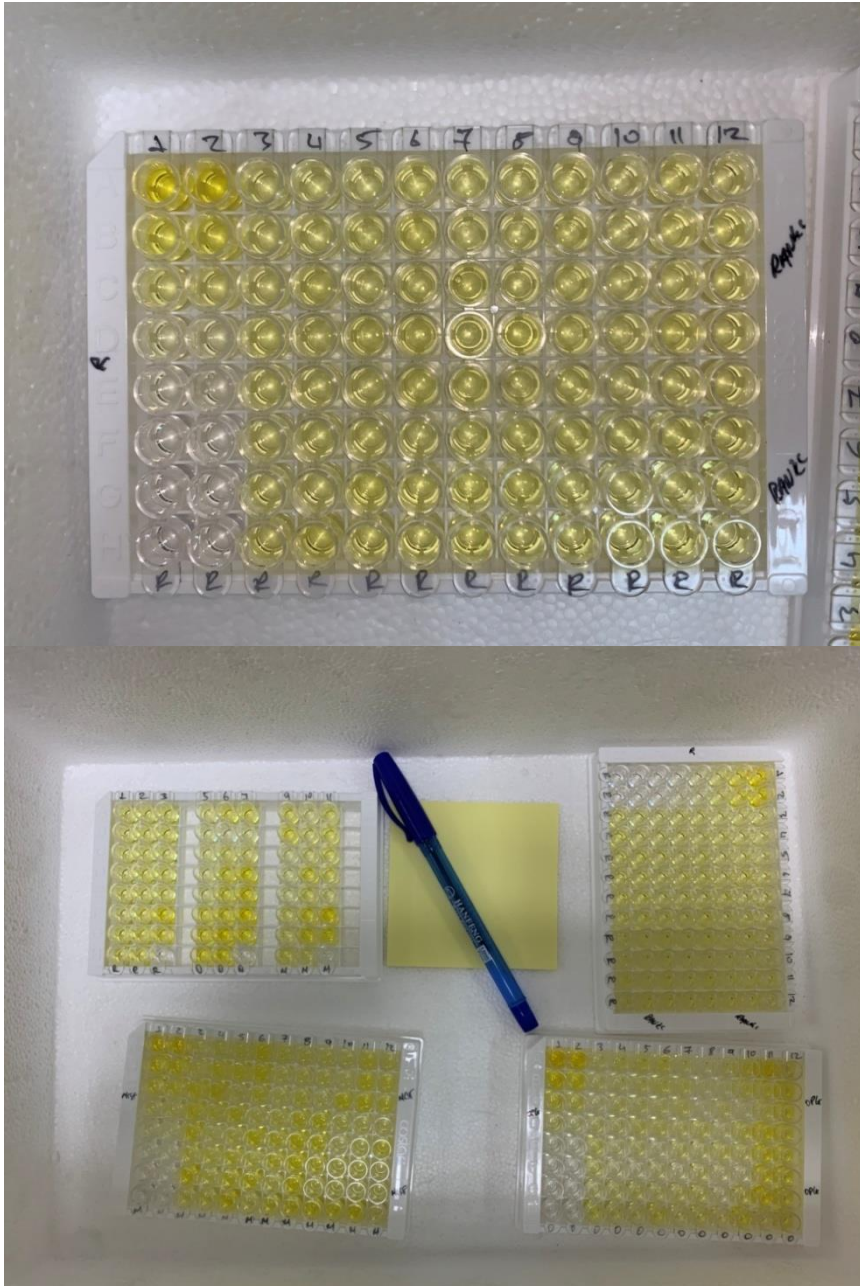
The measurements were carried out in an ELISA photometer (Thermo Scientific Multiskan GO Microplate Spectrophotometer) (Figure 17). The ELISA tests were performed according to the manufacturer's instructions.

Figure 17.   Thermo Scientific Multiskan GO Microplate Spectrophotometer

Histological preparation

Before recovery, the animals were sacrificed by decapitation. Next, their heads were dissected and the soft tissues were removed carefully. Each head was kept intact and separately to be fixed in 10% formalin solution.

Initially, the heads were cut in the middle. Subsequently, the left mandibles were separated from the heads and the condyles were isolated (Figures 18, 19). The specimens were immersed in ethylene diamine tetraacetic acid (MICRODEC EDTA - BASED, DIAPATH S.p.A) solution for 10 days to be decalcified. Eventually, the condyles were embedded in paraffin using conventional methods. Serial 6 mm–thick sections were cut using a fully motorized rotary microtome (ARM3600, Histo - Line Laboratories Co) parallel to the sagittal plane of the mandibular condyle. The sections were stained with hematoxylin and eosin (HE) to observe potential histomorphological changes (Figures 20-22).

The sections were scanned with a 20x fixed magnification lens. The generated TIFF image files were then converted to JPEG for the purposes of digital analysis by using the software Sedeen Viewer Version 5.4.4. (Copyright © Pathcore Inc.2008-2019). Transmitted light microscopy was used for histological evaluation (OLYMPUS CX 23LEDRFS2, Olympus Corporation, Tokyo, Japan).

Quantitative evaluation of condylar cartilage width

The thickness of the articular cartilage was considered at three areas, at both ends (anterior and posterior), and the midline across the sagittal plane passing through the condylar head. All cartilaginous layers (fibrous, proliferating, mature, hypertrophic) in the anterior, middle and posterior areas were measured with a linear calculation tool (Image-Pro Plus v6.0.0.260 Media Cybernetics, Inc., Rockville, MD, USA©) (Figure 23).

Histomorphometry and Quantitative Analysis

Aiming to calculate the bone surface/total surface ratio, two square condylar head regions of interest (anterior and posterior), measuring 1.0 x 1.0 mm, were selected for histological analysis (Figure 23).

Figure 18. Isolation of the mandibles

a.

b.

c.
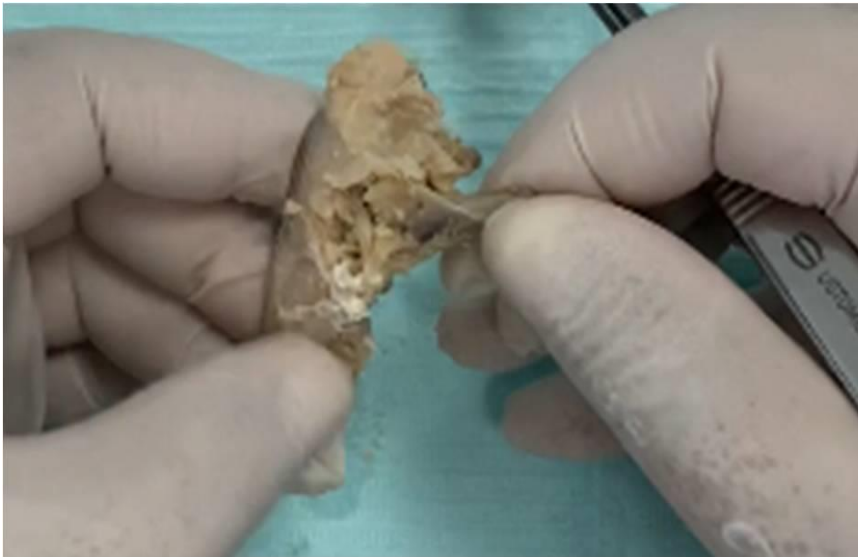
d
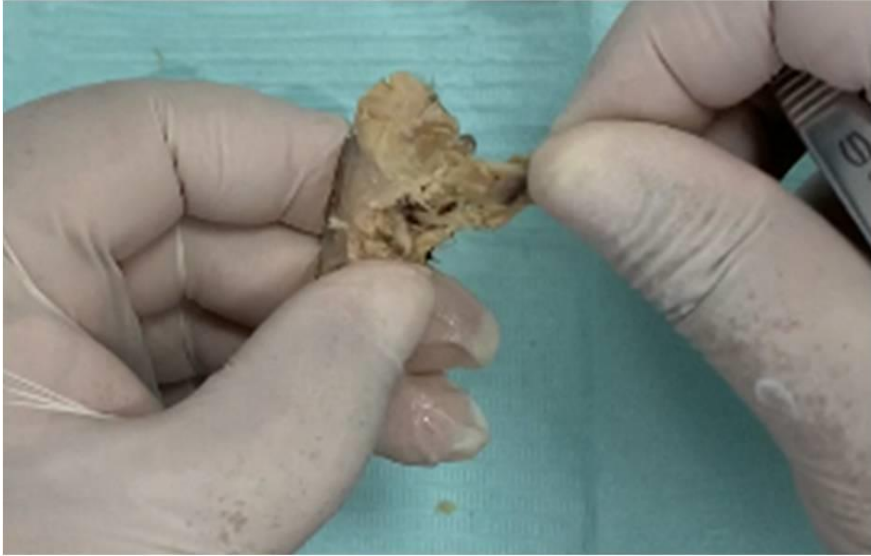
e.

Figure 19. Isolation of the condyles

a.
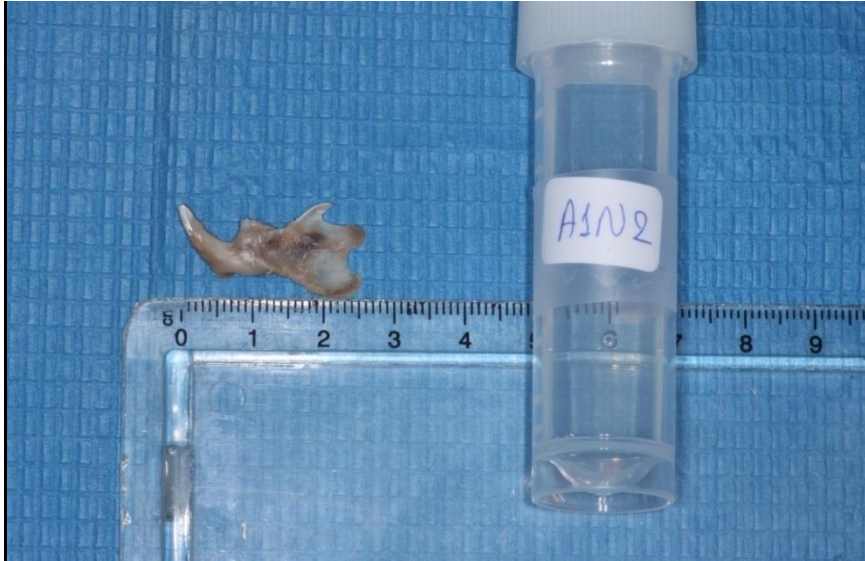
b.
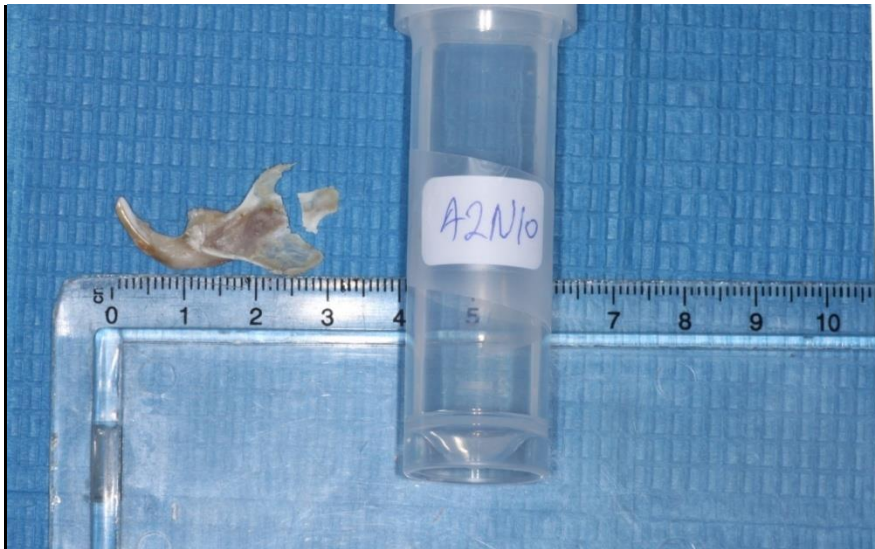
c.

Figure 20. Decalcification of the specimens

a.

b.

Figure 21. Embedding procedure

a.

b.
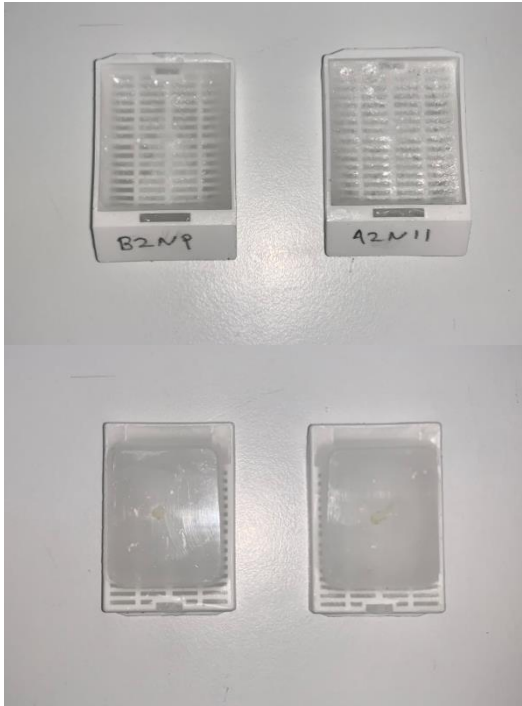
Figure 22. Sectioning procedure
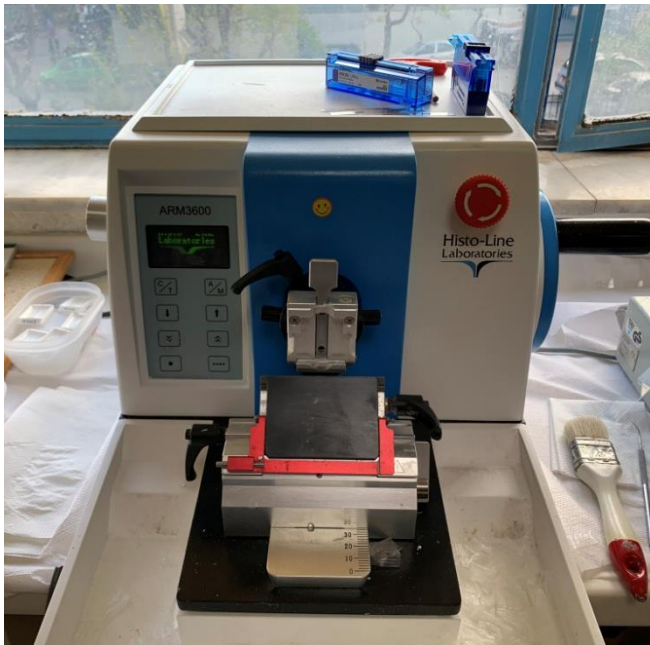
Figure 23. Histological images of the sagittal sections of the condyle, 20x magnification, stained with Hematoxylin and Eosin (HE).

a. **Condylar cartilage thickness (anterior, middle, posterior)**
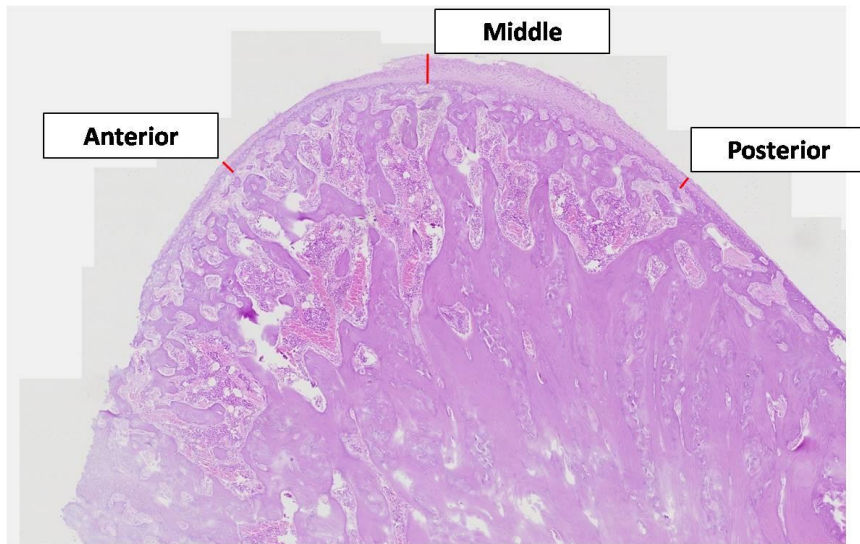
b. **Square condylar head regions of interest (anterior and posterior), measuring 1.0 x 1.0 mm**
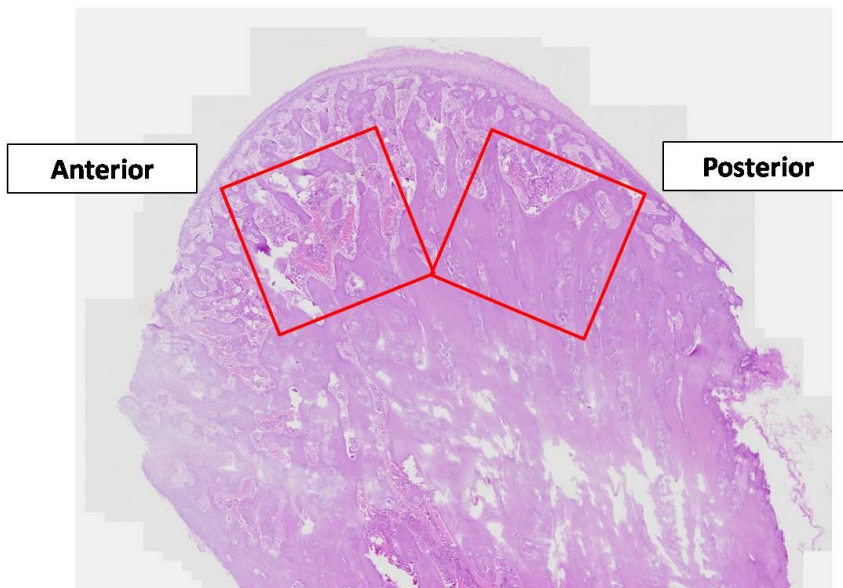
c. Bone surface/Total surface calculation
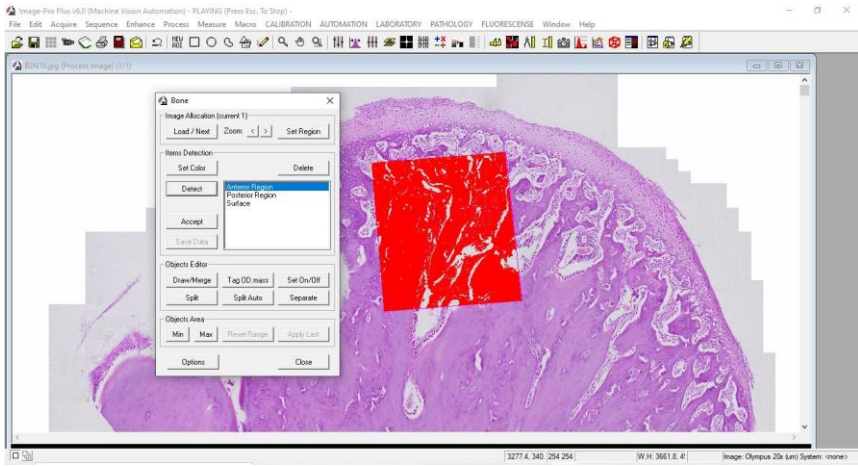
Statistics

The groups should be kept sufficiently small, for ethical reasons, while reliably detecting potentially statistical results. The number of animals was calculated using power analysis. In addition, the size of the respective samples in the study was finalized after allowing for the low probability that some experimental animals might not cope with the stress of the experimental process.

Subgroups consisting of 12 rats were calculated using standard statistical criteria (a = 0.05, b = 0.10), yielding a power of 90% to detect 0.5 mm difference (26.5 vs. 27.0 SD 0.37) for the primary outcome of the study, namely mandibular length (Condylion - I'). Therefore, 72 rats were used, equally divided into experimental and control group.

Three - dimensional analysis

To calculate the intra-observer and inter-observer errors, double measurements, 4 weeks apart, were made independently by two observers that were blinded to the groups undergoing evaluation. Lin's concordance correlation coefficient and Bland and Altman analysis were used for the estimation of inter- and intra-observer agreement (Bland and Altman, 1986; Lin, 1989).

First, seeking to detect any meaningful differences, the dimensional means of the right and left mandibular sides were calculated and were subsequently used for the statistics. Next, differences in measurements related to "group" and "timing (subgroups)" were assessed using linear regression models. Each measurement was regressed on group, timing and their interaction. When initial measurements were assessed, models were adjusted for initial weight when appropriate. Models for the final measurements were adjusted for the initial ones. Estimated changes from the initial measurements (final minus initial) were also investigated using regression models with group, timing and their interaction as dependent variables,

adjusting for initial weight when appropriate. When normality assumption for the residuals was violated, quantile regression was used. Estimates were adjusted for multiple comparisons, using the Bonferroni method.

Biochemical analysis

One way ANOVA or Kruskal-Wallis test was used for comparisons by timing. Differences in markers by group and timing were investigated using regression models with each marker's change from initial measurement (final - initial) as dependent variable and group, timing and their interaction as independent ones. Estimates were adjusted for multiple comparisons, using Bonferroni method.

Histological analysis

Differences in measurements related to group and timing were assessed using linear regression models. Each measurement was regressed on group, timing and their interaction, as well as final weight. Estimates were adjusted for multiple comparisons, using Bonferroni method.

Analysis was performed at the α = 5% level of statistical significance (p – value < 0.05 indicates a statistically significant result). Data were coded and analyzed using the statistical software Stata ver.14 (Stata Statistical Software: Release 14. College Station, TX: StataCorp LP.)

4. RESULTS

Three-dimensional radiographic analysis

Only minor deviation was observed in Lin's concordance correlation coefficient and values of 0.98 or greater were predominant. These values indicate excellent agreement in measurements. The level of agreement (LoA) of Bland and Altman analysis is displayed in Supplementary Tables 1 to 12 (Tables S1 - 12). Descriptive statistics with estimated means and standard deviations (SD) are presented in Tables 2 and 3.

Table 2. Descriptive statistics (mean and standard deviation) for each measurement by subgroups and overall, for experimental group A.

Experimental Group A	Subgroups - Timing				
	A1 - 0d	A2 - 0d	A3 - 0d	Overall	
	Mean (SD)	Mean (SD)	Mean (SD)	Mean (SD)	p-value*
Weight Initial (grams)	117.2 (17.2)	117.6 (17.5)	115.8 (13.4)	116.9 (15.7)	0.956
Go' - Menton Initial (mm)	13.60 (0.52)	14.80 (0.51)	13.70 (0.53)	14.03 (0.74)	**<0.001**
Go - Menton Initial (mm)	16.75 (0.52)	17.14 (0.55)	16.63 (0.46)	16.84 (0.54)	**0.018**
Coronoid - Menton Initial (mm)	16.17 (0.44)	16.48 (0.48)	16.00 (0.56)	16.22 (0.52)	**0.016**
Condylion/Go' - Menton Initial (mm)	8.29 (0.40)	8.20 (0.30)	8.36 (0.33)	8.28 (0.34)	0.137
Condylion - Go' Initial (mm)	8.81 (0.38)	8.52 (0.31)	8.82 (0.26)	8.71 (0.34)	**0.002**
Condylion - Menton Initial (mm)	18.55 (0.42)	18.91 (0.45)	18.48 (0.42)	18.64 (0.46)	**0.005**
Condylion - Id Initial (mm)	20.65 (0.36)	20.95 (0.52)	20.71 (0.49)	20.77 (0.47)	0.140

	A1 - 30d Mean (SD)	A2 - 60d Mean (SD)	A3 - 90d Mean (SD)	Overall Mean (SD)	p-value*
Condylion - I' Initial (mm)	20.85 (0.51)	21.11 (0.48)	20.75 (0.38)	20.90 (0.48)	0.076
Incisal - Id Initial (mm)	7.87 (0.45)	7.94 (0.29)	7.82 (0.28)	7.88 (0.34)	0.741
Incisal - I' Initial (mm)	5.06 (0.24)	5.13 (0.27)	5.27 (0.26)	5.15 (0.26)	0.124
Intercondylar Initial (mm)	17.78 (0.40)	17.43 (0.33)	17.50 (0.48)	17.57 (0.42)	0.068
	A1 - 30d	**A2 - 60d**	**A3 - 90d**	**Overall**	
	Mean (SD)	**Mean (SD)**	**Mean (SD)**	**Mean (SD)**	**p-value***
Weight Final (grams)	256.1 (24.5)	320.7 (25.0)	337.0 (58.6)	304.6 (52.2)	**<0.001**
Go' - Menton Final (mm)	15.86 (0.79)	16.58 (0.69)	16.83 (0.69)	16.43 (0.82)	**0.003**
Go - Menton Final (mm)	19.48 (0.46)	20.81 (0.51)	21.38 (0.77)	20.56 (0.99)	**<0.001**
Coronoid - Menton Final (mm)	18.74 (0.40)	20.13 (0.49)	20.50 (0.98)	19.79 (1.01)	**<0.001**
Condylion/Go' - Menton Final (mm)	9.72 (0.38)	10.58 (0.26)	10.78 (0.51)	10.36 (0.60)	**<0.001**
Condylion - Go' Final (mm)	10.12 (0.40)	11.02 (0.31)	11.32 (0.53)	10.82 (0.66)	**<0.001**
Condylion - Menton Final (mm)	20.93 (0.48)	22.24 (0.48)	22.87 (0.81)	22.01 (1.01)	**<0.001**
Condylion - Id Final (mm)	23.13 (0.54)	24.48 (0.60)	25.10 (0.95)	24.23 (1.09)	**<0.001**
Condylion - I' Final (mm)	23.06 (0.48)	24.35 (0.49)	25.13 (0.75)	24.18 (1.04)	**<0.001**
Incisal - Id Final (mm)	7.40 (0.93)	9.22 (1.62)	12.59 (1.00)	9.73 (2.48)	**<0.001**
Incisal - I' Final (mm)	4.25 (0.97)	5.63 (1.52)	8.90 (1.00)	6.26 (2.29)	**<0.001**
Intercondylar Final (mm)	18.02 (0.35)	18.19 (0.33)	18.08 (0.54)	18.10 (0.41)	0.142

* derived from linear regression models; pairwise comparisons
are provided in tables 4 & 5

Table 3. Descriptive statistics (mean and standard deviation) for each measurement by subgroups and overall, for control group B.

Control Group B	Subgroups - Timing				
	B1 - 0d	B2 - 0d	B3 - 0d	Overall	
	Mean (SD)	Mean (SD)	Mean (SD)	Mean (SD)	p-value*
Weight Initial (grams)	109.4 (12.2)	105.4 (22.1)	121.7 (14.7)	112.2 (17.8)	0.062
Go' - Menton Initial (mm)	13.18 (0.43)	13.40 (0.61)	13.74 (0.38)	13.44 (0.52)	0.075
Go - Menton Initial (mm)	16.34 (0.44)	16.28 (0.66)	16.70 (0.55)	16.44 (0.57)	0.804
Coronoid - Menton Initial (mm)	15.90 (0.39)	15.74 (0.74)	15.98 (0.59)	15.87 (0.58)	0.348
Condylion/Go' - Menton Initial (mm)	8.13 (0.34)	8.26 (0.37)	8.55 (0.37)	8.31 (0.39)	**0.040**
Condylion - Go' Initial (mm)	8.67 (0.34)	8.72 (0.38)	8.97 (0.41)	8.79 (0.39)	0.413
Condylion - Menton Initial (mm)	18.11 (0.50)	18.18 (0.71)	18.50 (0.52)	18.26 (0.59)	0.400
Condylion - Id Initial (mm)	20.36 (0.59)	20.37 (0.81)	20.61 (0.64)	20.45 (0.68)	0.345
Condylion - I' Initial (mm)	20.42 (0.52)	20.44 (0.78)	20.72 (0.58)	20.53 (0.63)	0.546
Incisal - Id Initial (mm)	7.64 (0.26)	7.73 (0.32)	7.95 (0.54)	7.77 (0.40)	0.381
Incisal - I' Initial (mm)	5.17 (0.31)	5.15 (0.22)	5.33 (0.43)	5.21 (0.33)	0.865
Intercondylar Initial (mm)	17.60 (0.47)	17.43 (0.50)	17.65 (0.55)	17.56 (0.50)	0.604
	B1 - 30d	B2 - 60d	B3 - 90d	Overall	
	Mean (SD)	Mean (SD)	Mean (SD)	Mean (SD)	
Weight Final (grams)	282.3 (18.1)	365.3 (35.0)	430.2 (30.1)	359.2 (67.4)	**<0.001**
Go' - Menton Final	17.43 (0.43)	18.74 (0.51)	19.92 (0.80)	18.69 (1.19)	**<0.001**

(mm)					
Go - Menton Final (mm)	20.30 (0.60)	21.63 (0.67)	22.93 (0.56)	21.62 (1.24)	**<0.001**
Coronoid - Menton Final (mm)	19.35 (0.51)	20.63 (0.43)	21.61 (0.47)	20.53 (1.05)	**<0.001**
Condylion/Go' - Menton Final (mm)	9.66 (0.44)	10.56 (0.58)	11.23 (0.23)	10.48 (0.78)	**<0.001**
Condylion - Go' Final (mm)	9.86 (0.46)	10.72 (0.58)	11.32 (0.25)	10.63 (0.75)	**<0.001**
Condylion - Menton Final (mm)	21.60 (0.67)	23.06 (0.33)	23.91 (0.37)	22.86 (1.08)	**<0.001**
Condylion - Id Final (mm)	23.90 (0.72)	25.79 (0.54)	26.45 (0.41)	25.38 (1.23)	**<0.001**
Condylion - I' Final (mm)	23.93 (0.58)	25.69 (0.39)	26.44 (0.48)	25.36 (1.17)	**<0.001**
Incisal - Id Final (mm)	10.05 (0.38)	11.06 (0.37)	11.81 (0.48)	10.98 (0.83)	**<0.001**
Incisal - I' Final (mm)	6.92 (0.23)	7.72 (0.28)	8.13 (0.32)	7.59 (0.58)	**0.001**
Intercondylar Final (mm)	18.02 (0.41)	18.07 (0.48)	18.18 (0.49)	18.09 (0.45)	0.642

* derived from linear regression models; pairwise comparisons are provided in tables 4 & 5

At baseline, only a few differences were evidenced among the subgroups, as expected, due to the sample's prior randomization. Contrarily, the final measurements revealed noteworthy changes (Tables 4-6).

Comparisons between the experimental and the respective control subgroups (A1 vs B1, A2 vs B2, A3 vs B3) revealed a statistically significant restriction of mandibular body length a (Go' - Menton), mandibular body length b (Go - Menton) and mandibular length (Condylion - I'), across all subgroups ($p < 0.001$). The Coronoid - Menton, Condylion - Menton, Condylion - Id, measurements were also found significantly different. Incisal - Id and Incisal - I' measurements measured significantly different between A1 vs B1 and A2 vs B2 subgroups, respectively, albeit differences did not persist after 90 days of

experiment (A3 vs B3 subgroups). The condylion height (Condylion/Go' - Menton), the ramus height (Condylion – Go') dimensions and the Intercondylar distance did not show significant difference.

Table 4. Pairwise group per timing measurements comparisons' p-values derived from linear regression models, adjusted for multiple comparison (Bonferroni).

Measurement	p-values*		
	A1 vs B1	A2 vs B2	A3 vs B3
Weight Initial	0.764	0.222	>0.999
Go' - Menton Initial	0.262	**<0.001**	>0.999
Go - Menton Initial	0.436	**0.003**	>0.999
Coronoid - Menton Initial	>0.999	**0.016**	0.810
Condylion/Go' - Menton Initial	>0.999	**0.027**	0.953
Condylion - Go' Initial	>0.999	**0.001**	>0.999
Condylion - Menton Initial	0.177	**0.005**	0.900
Condylion - Id Initial	>0.999	0.363	0.206
Condylion - I' Initial	0.345	0.059	0.605
Incisal - Id Initial	0.775	>0.999	>0.999
Incisal - I' Initial	0.353	0.873	>0.999
Intercondylar Initial	>0.999	0.590	>0.999
Weight Final	0.202	**0.007**	**<0.001**
Go' - Menton Final	**<0.001**	**<0.001**	**<0.001**
Go - Menton Final	**<0.001**	**<0.001**	**<0.001**
Coronoid - Menton Final	**0.002**	**0.001**	**<0.001**
Condylion/Go' - Menton Final	>0.999	>0.999	0.104
Condylion - Go' Final	0.793	0.056	>0.999
Condylion - Menton Final	**<0.001**	**<0.001**	**<0.001**
Condylion - Id Final	**0.001**	**<0.001**	**<0.001**
Condylion - I' Final	**<0.001**	**<0.001**	**<0.001**
Incisal - Id Final	**<0.001**	**<0.001**	>0.999
Incisal - I' Final	**<0.001**	**<0.001**	0.245
Intercondylar Final	>0.999	>0.999	>0.999

* p-values in bold indicate statistical significance at 5% level

Table 5. Pairwise timing per group measurements comparisons' p-values derived from linear regression models, adjusted for multiple comparison (Bonferroni).

Measurement	p-values*					
	A1 vs A2	**A1 vs A3**	**A2 vs A3**	**B1 vs B2**	**B1 vs B3**	**B2 vs B3**
Weight Initial	>0.999	>0.999	>0.999	>0.999	0.295	0.075
Go' - Menton Initial	**<0.001**	>0.999	**<0.001**	0.211	0.194	>0.999
Go - Menton Initial	0.116	>0.999	**0.031**	>0.999	>0.999	>0.999
Coronoid - Menton Initial	0.192	>0.999	**0.020**	>0.999	0.627	>0.999
Condylion/Go' - Menton Initial	>0.999	>0.999	0.189	0.153	0.089	>0.999
Condylion – Go' Initial	**0.016**	>0.999	**0.007**	>0.999	0.959	0.959
Condylion - Menton Initial	**0.027**	>0.999	**0.014**	0.711	>0.999	>0.999
Condylion - Id Initial	0.205	>0.999	0.831	>0.999	>0.999	0.595
Condylion - I' Initial	0.338	>0.999	0.128	>0.999	>0.999	>0.999
Incisal - Id Initial	>0.999	>0.999	>0.999	>0.999	0.726	>0.999
Incisal - I' Initial	>0.999	0.192	0.618	>0.999	>0.999	>0.999
Intercondylar Initial	0.099	0.415	>0.999	>0.999	>0.999	>0.999
Weight Final	**<0.001**	**<0.001**	0.999	**<0.001**	**<0.001**	**<0.001**
Go' - Menton Final	>0.999	**0.003**	0.212	0.266	**0.001**	0.152
Go - Menton Final	**<0.001**	**<0.001**	**0.009**	**<0.001**	**<0.001**	**<0.001**
Coronoid - Menton Final	**<0.001**	**<0.001**	**0.023**	**<0.001**	**<0.001**	**0.001**
Condylion/Go' - Menton Final	**<0.001**	**<0.001**	>0.999	**<0.001**	**<0.001**	**0.009**
Condylion – Go' Final	**<0.001**	**<0.001**	>0.999	**<0.001**	**<0.001**	**0.028**

Condylion - Menton Final	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**0.005**
Condylion - Id Final	**<0.001**	**<0.001**	**0.012**	**<0.001**	**<0.001**	0.117
Condylion - I' Final	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**0.011**
Incisal - Id Final	**<0.001**	**<0.001**	**<0.001**	**0.007**	**<0.001**	0.594
Incisal - I' Final	**0.003**	**<0.001**	**<0.001**	**0.032**	**0.002**	>0.999
Intercondylar Final	0.198	0.990	>0.999	>0.999	>0.999	>0.999

* p-values in bold indicate statistical significance at 5% level

Differences within the subgroups in the experimental group (A1, A2, A3) and within the control subgroups (B1, B2, B3) are presented in Table 5. Table 6 presents the mean difference (Final minus Initial) for each subgroup regarding each measurement. It appears that the rate of mandibular growth was smaller in the experimental group in comparison to the control group. The comprehensive regression model regarding major measurements is depicted graphically in Figures 24-58.

Table 6. Estimated mean differences (final – initial), 95% Confidence Intervals and p-values (compared to 0 i.e. no change) per group and timing.

Final-Initial	Mean Difference (mm)	95% Conf. Interval		p-value
Go' - Menton				
A1	2.29	1.74	2.84	**<0.001**
B1	4.19	3.63	4.74	**<0.001**
A2	1.82	1.27	2.38	**<0.001**
B2	5.23	4.66	5.79	**<0.001**
A3	3.15	2.60	3.70	**<0.001**
B3	6.26	5.70	6.82	**<0.001**
Go - Menton				
A1	2.73	2.22	3.24	**<0.001**

B1	3.96	3.45	4.47	**<0.001**
A2	3.67	3.16	4.18	**<0.001**
B2	5.34	4.83	5.85	**<0.001**
A3	4.75	4.23	5.26	**<0.001**
B3	6.23	5.72	6.74	**<0.001**
Coronoid - Menton				
A1	2.61	2.18	3.03	**<0.001**
B1	3.38	2.96	3.81	**<0.001**
A2	3.69	3.27	4.11	**<0.001**
B2	4.78	4.35	5.21	**<0.001**
A3	4.52	4.10	4.94	**<0.001**
B3	5.73	5.30	6.15	**<0.001**
Condylion/Go' - Menton				
A1	1.45	1.14	1.76	**<0.001**
B1	1.51	1.20	1.82	**<0.001**
A2	2.39	2.08	2.70	**<0.001**
B2	2.26	1.94	2.57	**<0.001**
A3	2.43	2.12	2.74	**<0.001**
B3	2.71	2.39	3.02	**<0.001**
Condylion - Go'				
A1	1.32	0.98	1.66	**<0.001**
B1	1.17	0.83	1.51	**<0.001**
A2	2.51	2.17	2.85	**<0.001**
B2	1.96	1.61	2.31	**<0.001**
A3	2.51	2.17	2.85	**<0.001**
B3	2.37	2.03	2.72	**<0.001**
Condylion - Menton				
A1	2.42	2.02	2.82	**<0.001**
B1	3.42	3.02	3.82	**<0.001**
A2	3.37	2.97	3.77	**<0.001**
B2	4.77	4.36	5.18	**<0.001**
A3	4.40	4.00	4.80	**<0.001**
B3	5.50	5.10	5.91	**<0.001**
Condylion - Id				
A1	2.51	2.04	2.99	**<0.001**
B1	3.47	2.99	3.95	**<0.001**

A2	3.57	3.10	4.05	**<0.001**
B2	5.29	4.80	5.78	**<0.001**
A3	4.40	3.93	4.88	**<0.001**
B3	5.94	5.45	6.42	**<0.001**
Condylion - I'				
A1	2.25	1.82	2.67	**<0.001**
B1	3.45	3.01	3.88	**<0.001**
A2	3.28	2.85	3.71	**<0.001**
B2	5.12	4.68	5.56	**<0.001**
A3	4.40	3.97	4.82	**<0.001**
B3	5.82	5.39	6.26	**<0.001**
Incisal - Id				
A1	-0.60	-1.37	0.17	0.235
B1	2.40	1.63	3.17	**<0.001**
A2	0.95	0.18	1.72	**0.007**
B2	3.25	2.48	4.02	**<0.001**
A3	4.40	3.63	5.17	**<0.001**
B3	3.85	3.08	4.62	**<0.001**
Incisal - I'				
A1	-0.90	-1.57	-0.23	**0.002**
B1	1.85	1.18	2.52	**<0.001**
A2	0.70	0.03	1.37	**0.033**
B2	2.60	1.93	3.27	**<0.001**
A3	3.60	2.93	4.27	**<0.001**
B3	2.70	2.03	3.37	**<0.001**
Intercondylar				
A1	0.26	-0.09	0.62	0.261
B1	0.37	0.02	0.72	**0.033**
A2	0.79	0.44	1.14	**<0.001**
B2	0.55	0.19	0.91	**0.001**
A3	0.59	0.24	0.94	**<0.001**
B3	0.59	0.23	0.94	**<0.001**

Figure 24. Estimated mean and 95% Confidence Interval per group and timing in initial weight

Adjusted Predictions of Timing#RatGroup with 95% CIs
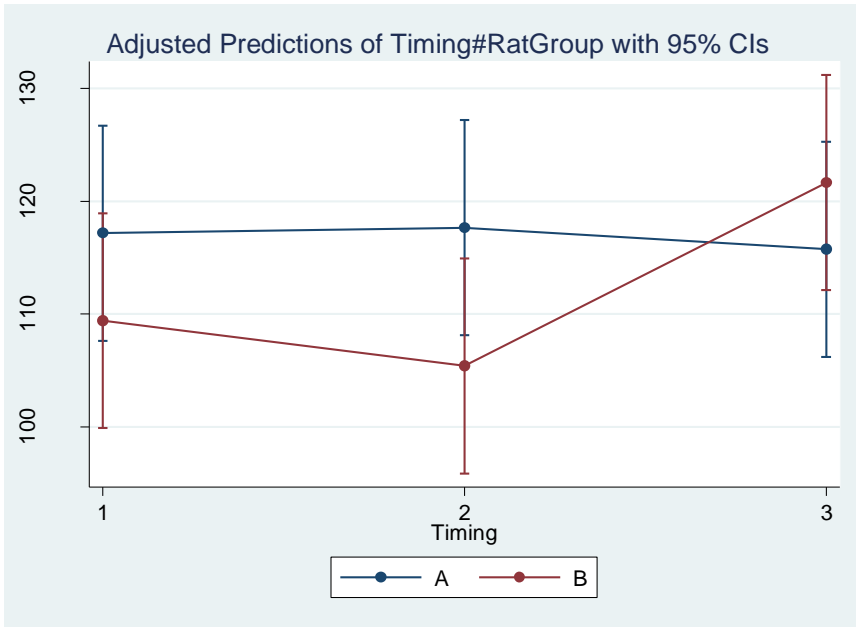
Figure 25. Estimated mean and 95% Confidence Interval per group and timing in final weight

Adjusted Predictions of Timing#RatGroup with 95% CIs

Figure 26. Estimated mean and 95% Confidence Interval per group and timing in GO' - Menton Initial

Predictive Margins of Timing#RatGroup with 95% CIs

Figure 27. Estimated mean and 95% Confidence Interval per group and timing in GO' - Menton Final

Predictive Margins of Timing#RatGroup with 95% CIs

Figure 28. Estimated mean change (Final – Initial) and 95% Confidence Interval per group and timing in GO' - Menton

Predictive Margins of Timing#RatGroup with 95% CIs

Figure 29. Estimated mean and 95% Confidence Interval per group and timing in Go - Menton Initial

Predictive Margins of Timing#RatGroup with 95% CIs

Figure 30. Estimated mean and 95% Confidence Interval per group and timing in Go - Menton Final

Figure 31. Estimated mean change (Final – Initial) and 95% Confidence Interval per group and timing in Go - Menton
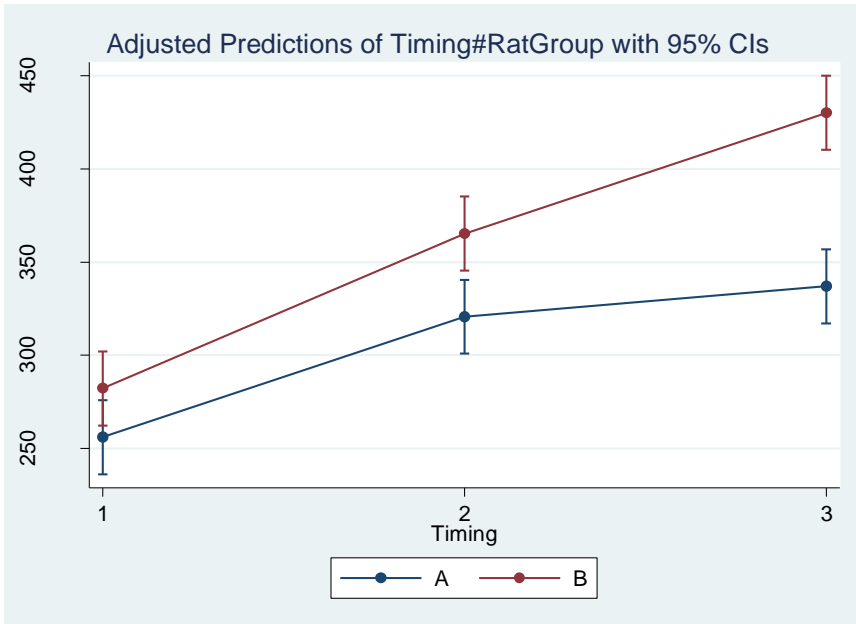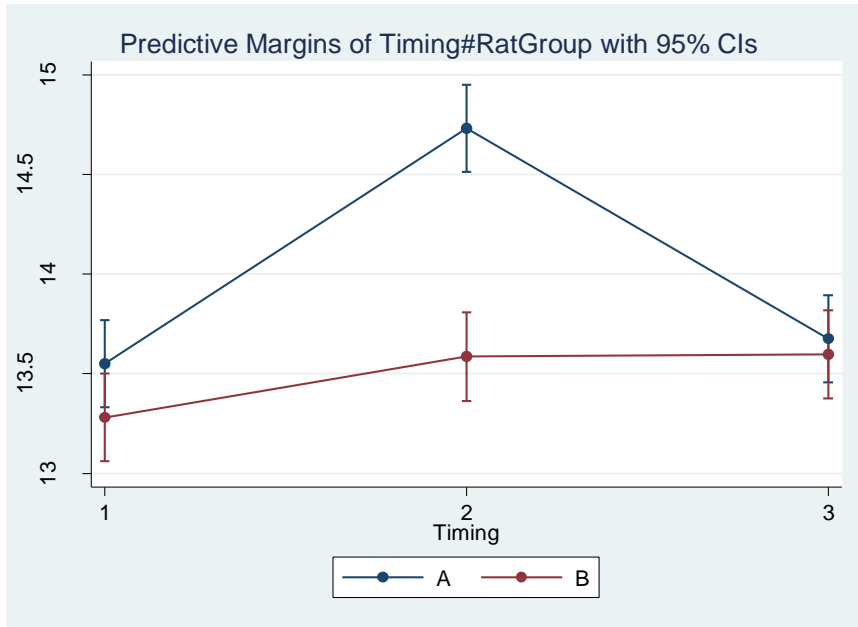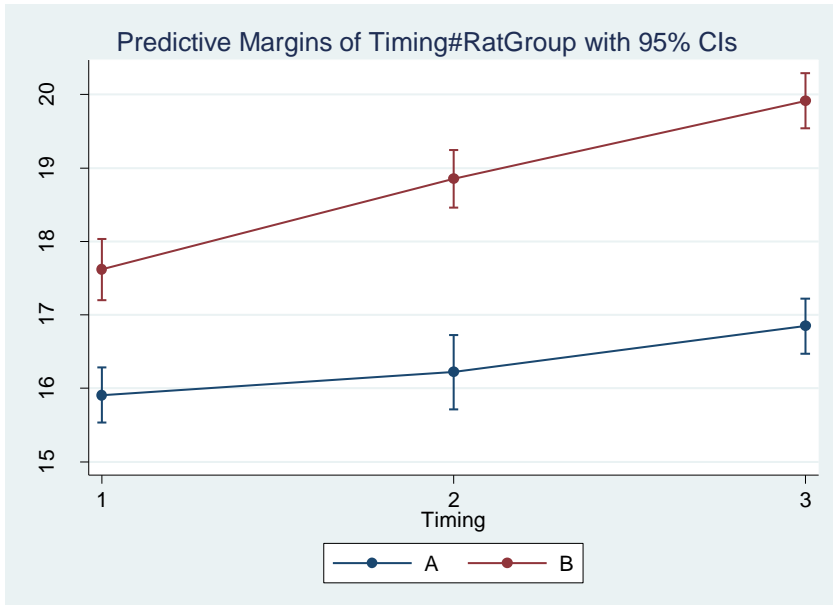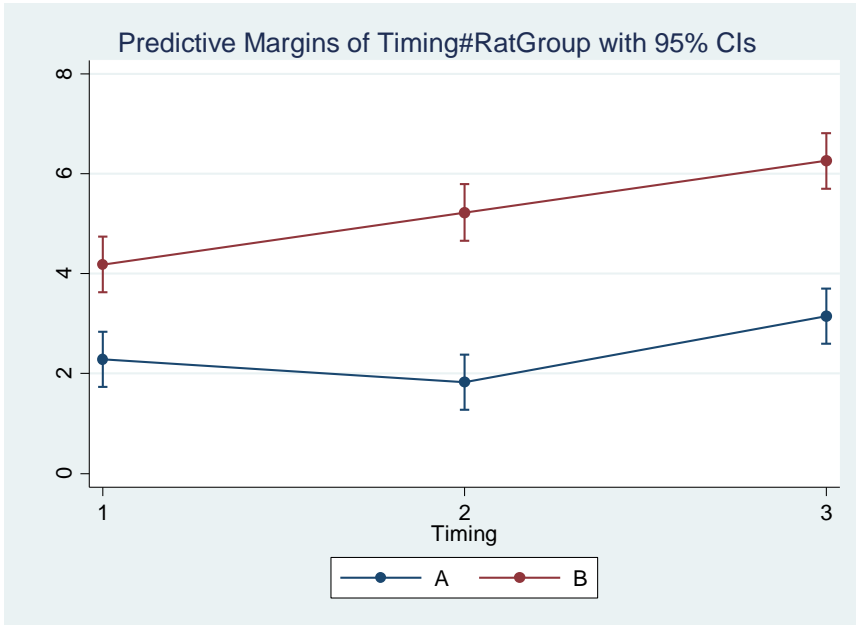
Figure 32. Estimated mean and 95% Confidence Interval per group and timing in Coronoid - Menton Initial

Figure 33. Estimated mean and 95% Confidence Interval per group and timing in Coronoid - Menton Final

Figure 34. Estimated mean change (Final – Initial) and 95% Confidence Interval per group and timing in Coronoid - Menton

Predictive Margins of Timing#RatGroup with 95% CIs

Figure 35. Estimated mean and 95% Confidence Interval per group and timing in Condylion/Go' - Menton Initial

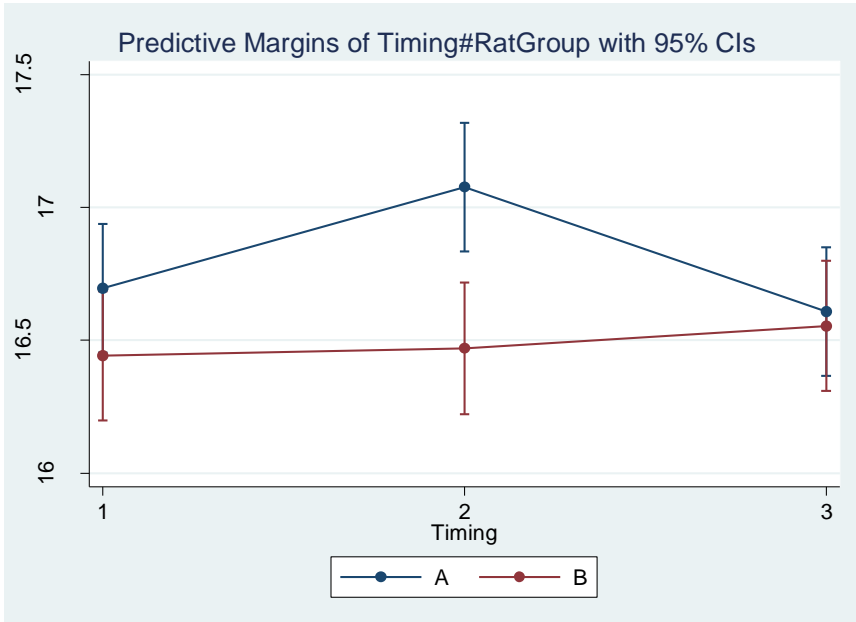Predictive Margins of Timing#RatGroup with 95% CIs

Figure 36. Estimated median and 95% Confidence Interval per group and timing in Condylion/Go' - Menton Final

Figure 37. Estimated mean change (Final – Initial) and 95% Confidence Interval per group and timing in Condylion/Go' - Menton
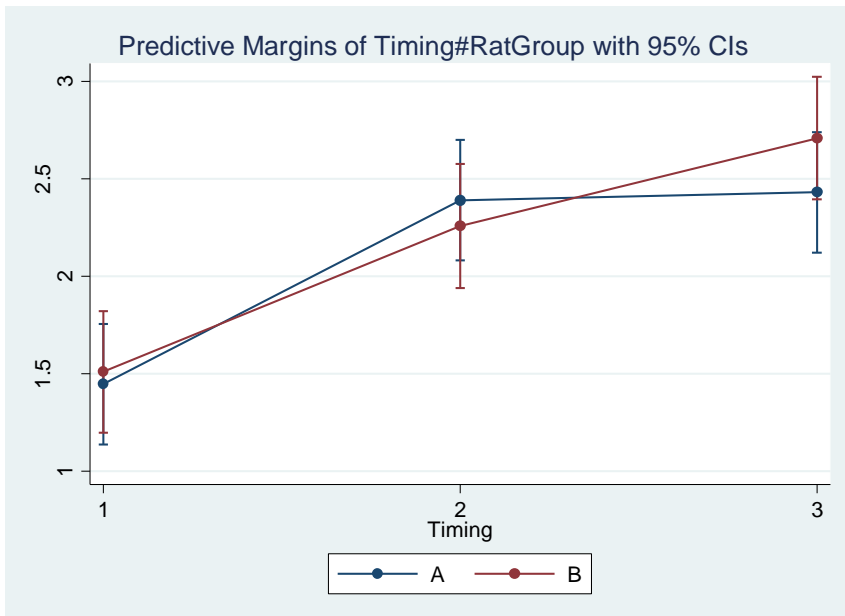
Figure 38. Estimated mean and 95% Confidence Interval per group and timing in Condylion - Go' Initial
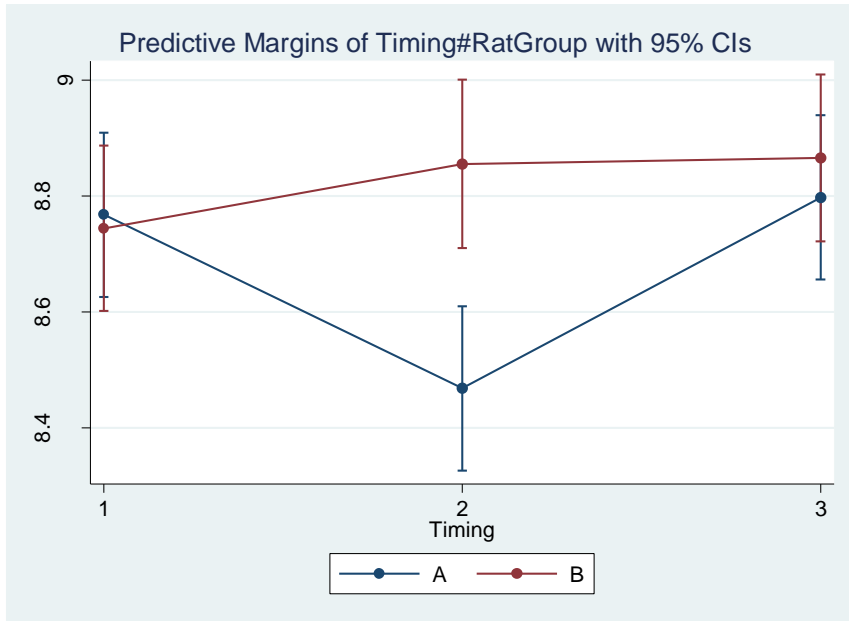
Figure 39. Estimated mean and 95% Confidence Interval per group and timing in Condylion - Go' Final
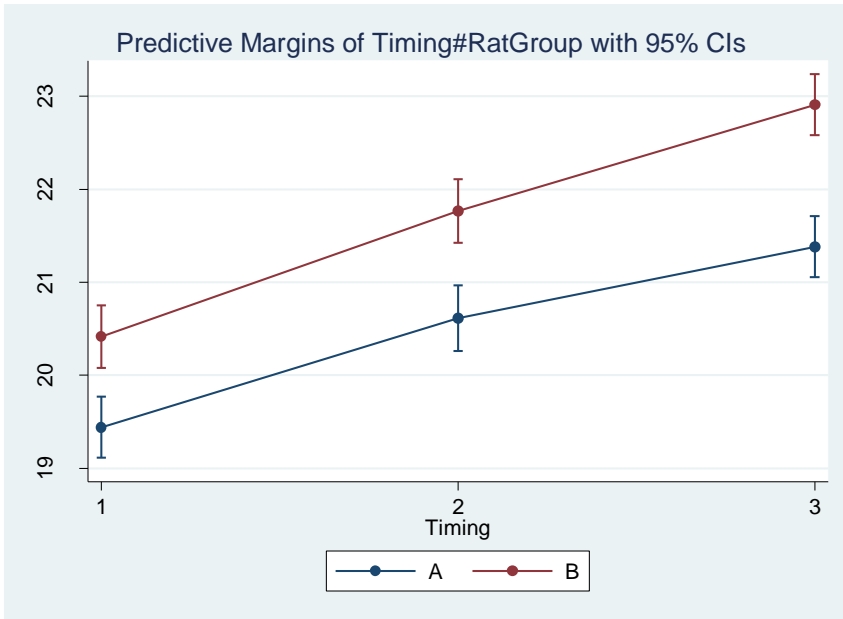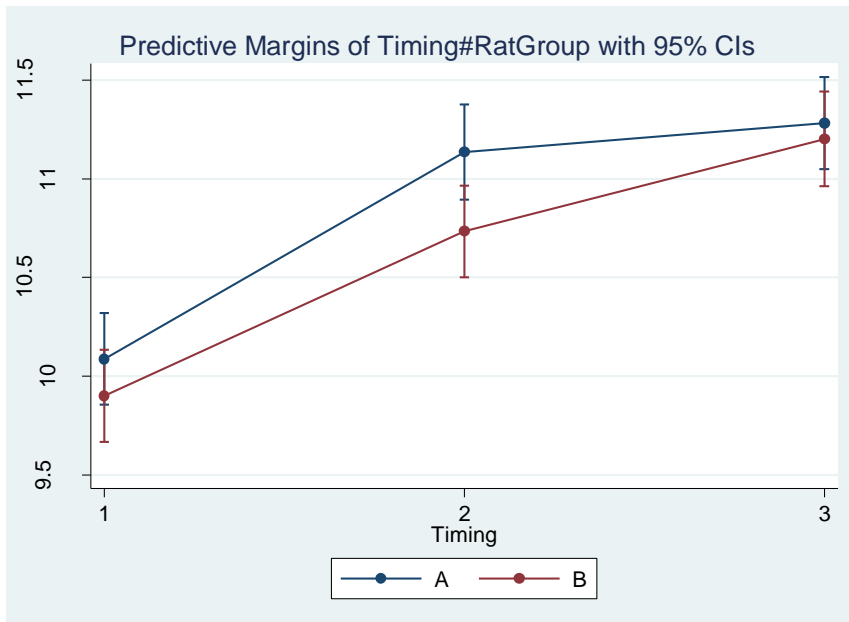
Figure 40. Estimated mean change (Final – Initial) and 95% Confidence Interval per group and timing in Condylion - Go'
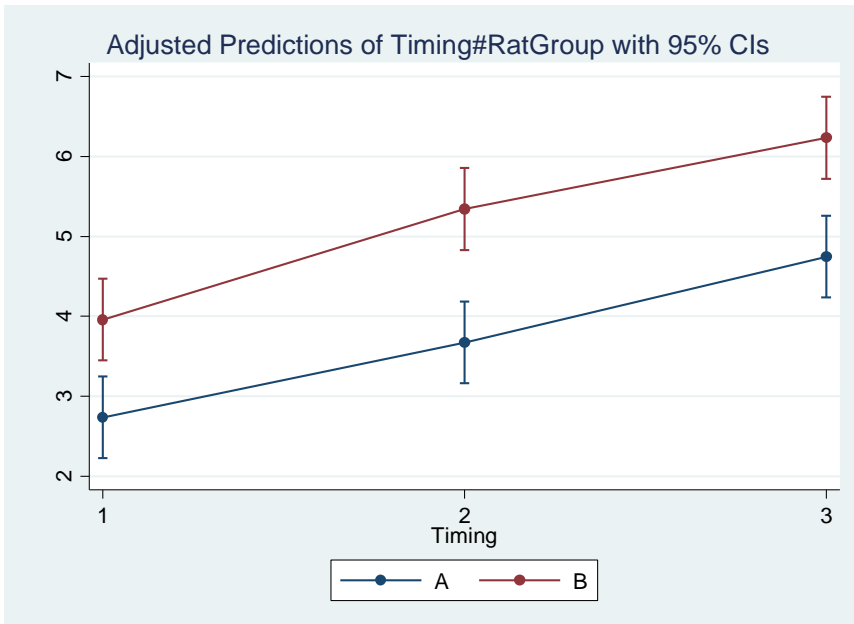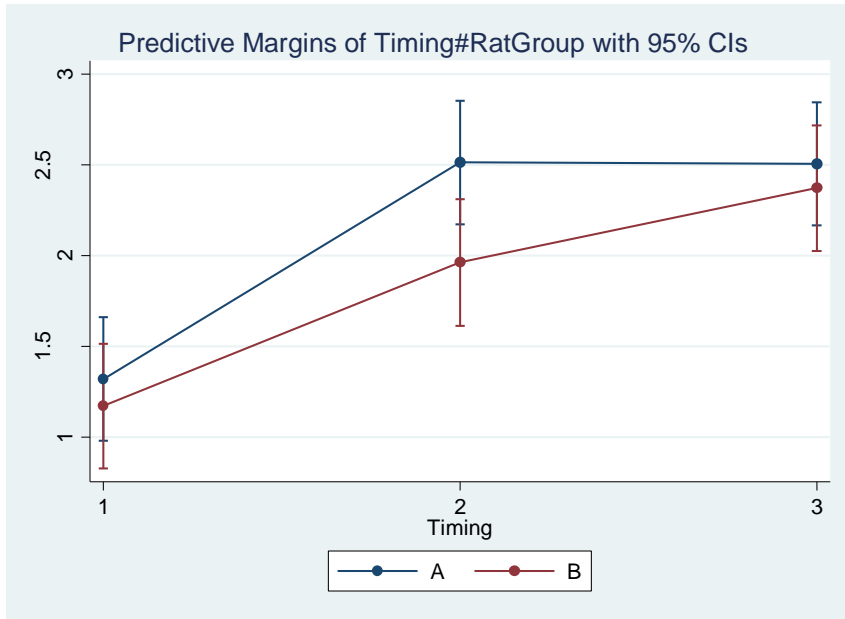

Figure 41. Estimated mean and 95% Confidence Interval per group and timing in Condylion - Menton Initial
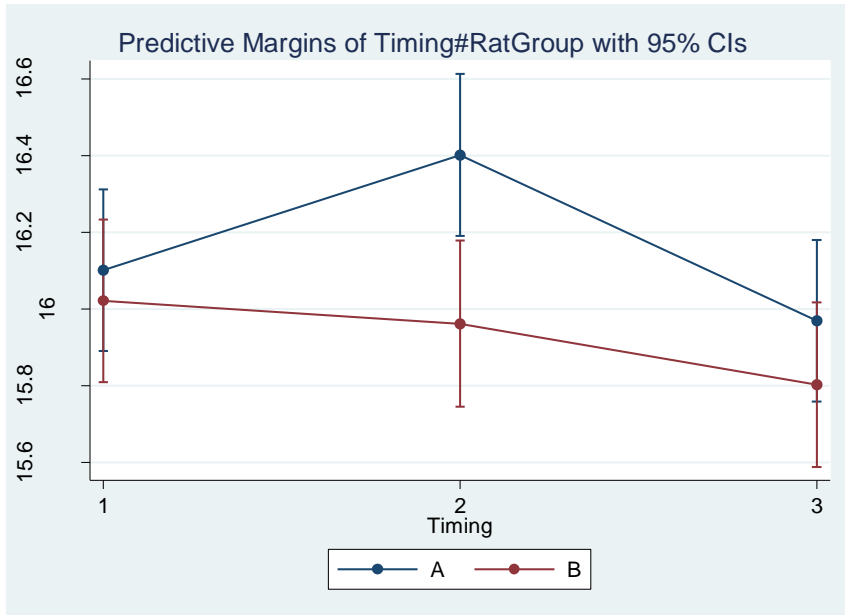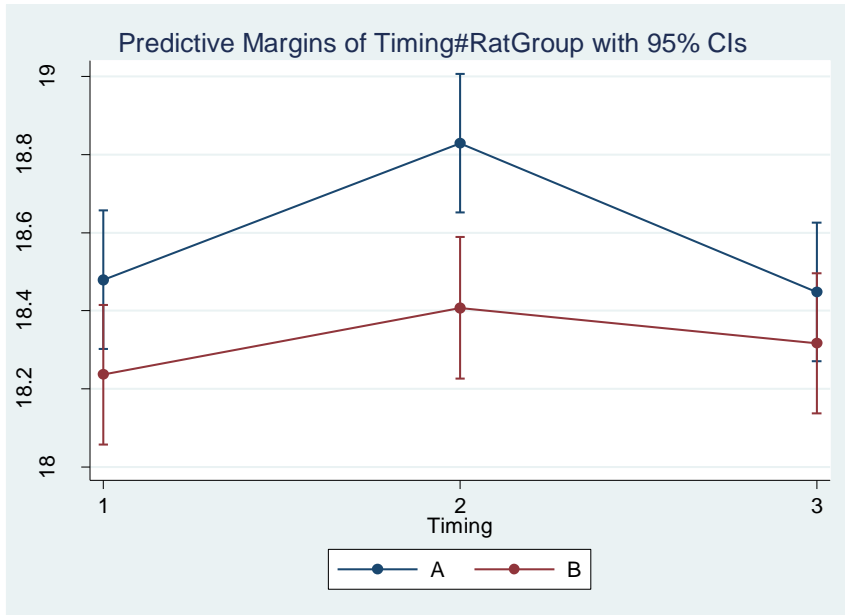
Figure 42. Estimated mean and 95% Confidence Interval per group and timing in Condylion - Menton Final
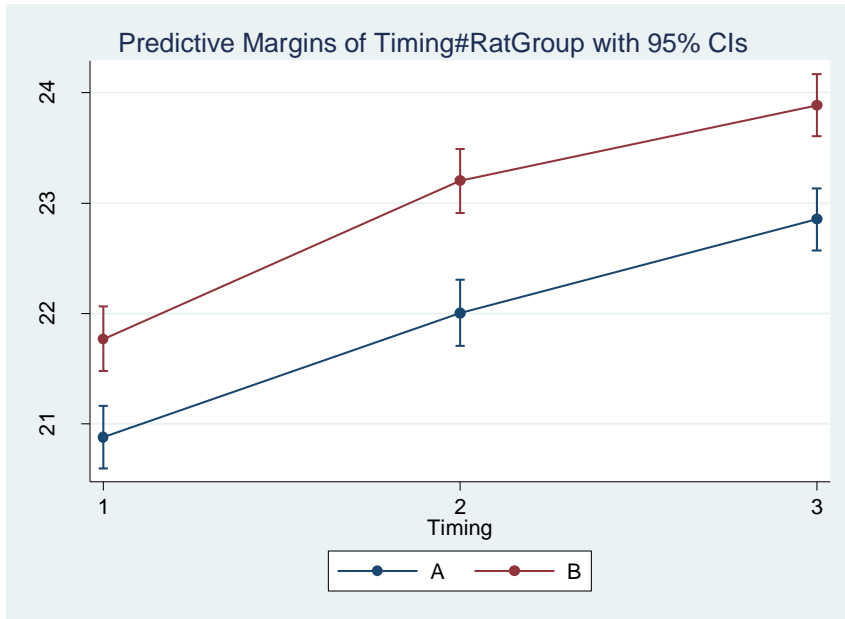
Figure 43. Estimated mean change (Final – Initial) and 95% Confidence Interval per group and timing in Condylion - Menton
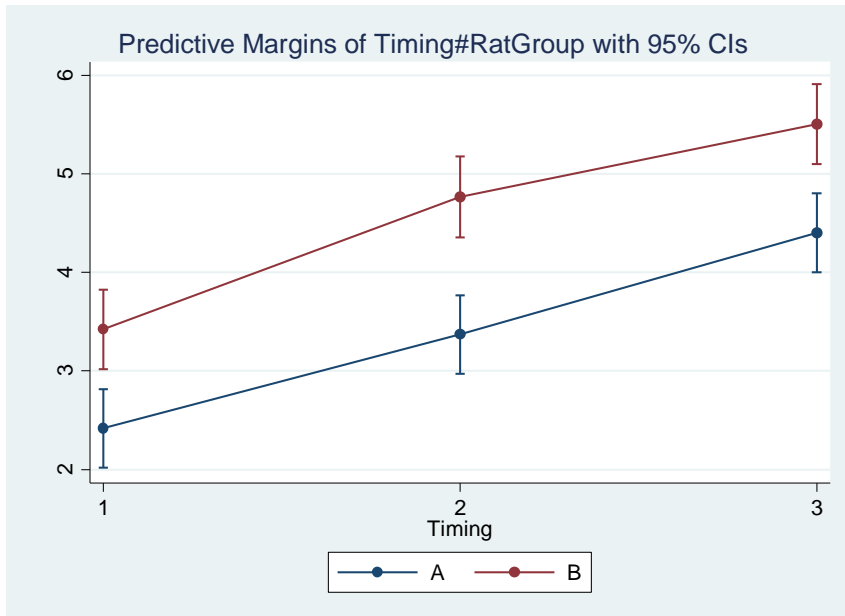
Figure 44. Estimated mean and 95% Confidence Interval per group and timing in Condylion - Id Initial

Predictive Margins of Timing#RatGroup with 95% CIs
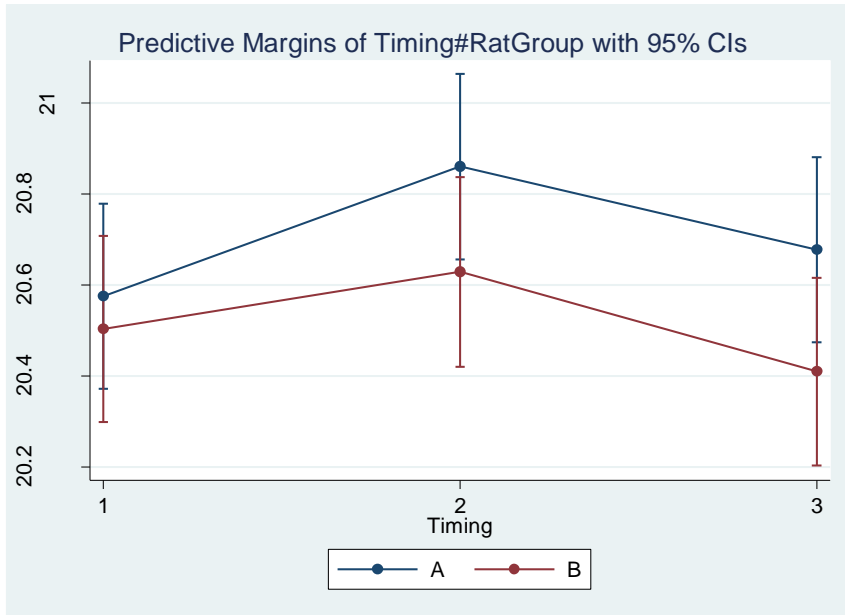
Figure 45. Estimated mean and 95% Confidence Interval per group and timing in Condylion - Id Final

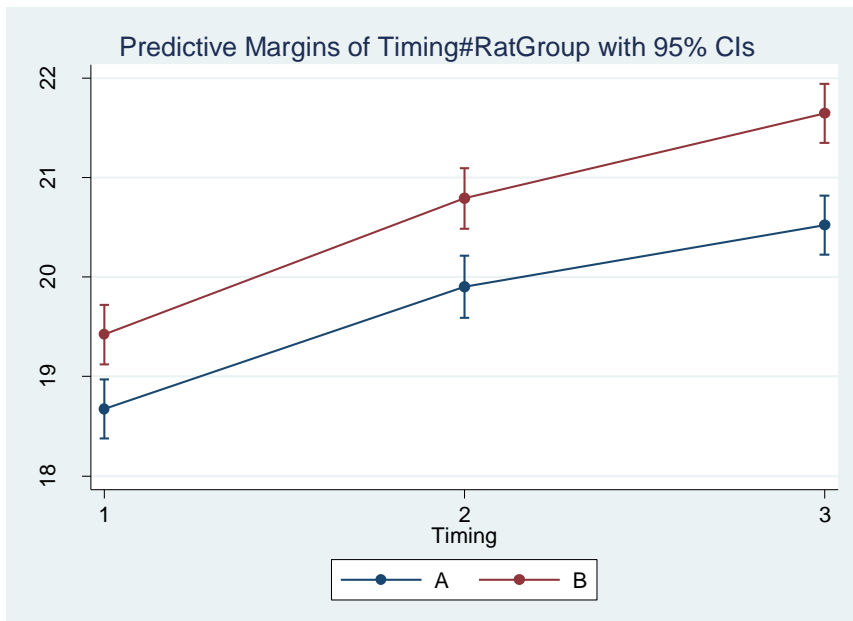Predictive Margins of Timing#RatGroup with 95% CIs
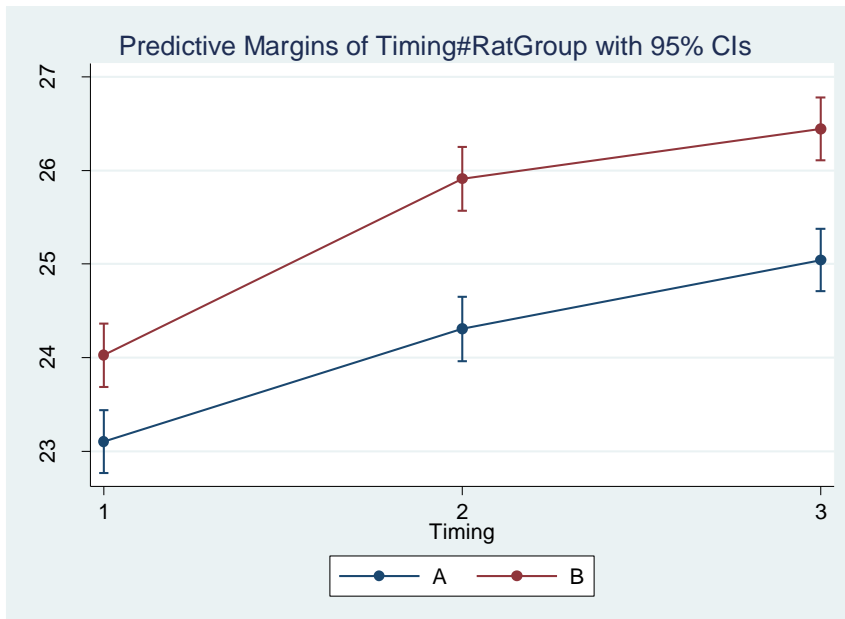
Figure 46. Estimated mean change (Final – Initial) and 95% Confidence Interval per group and timing in Condylion - Id
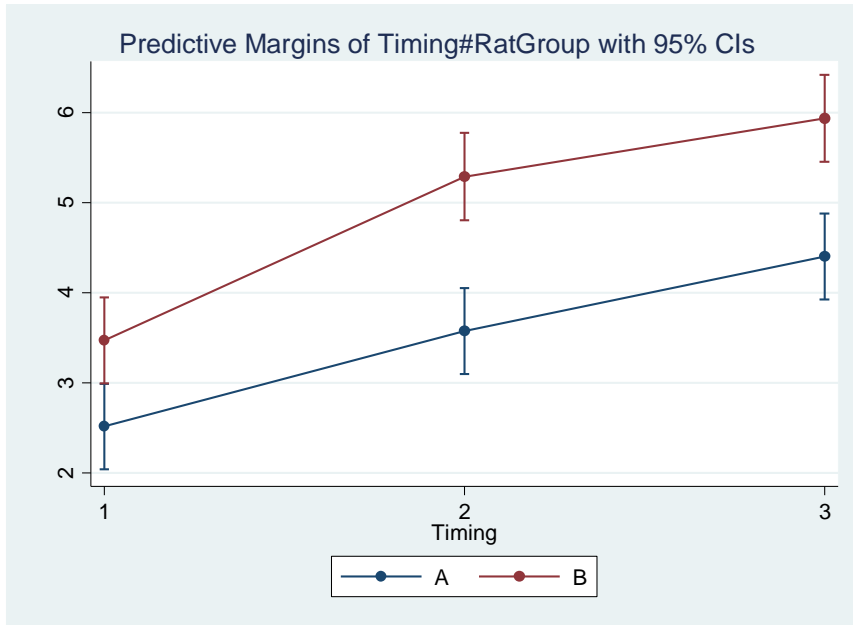
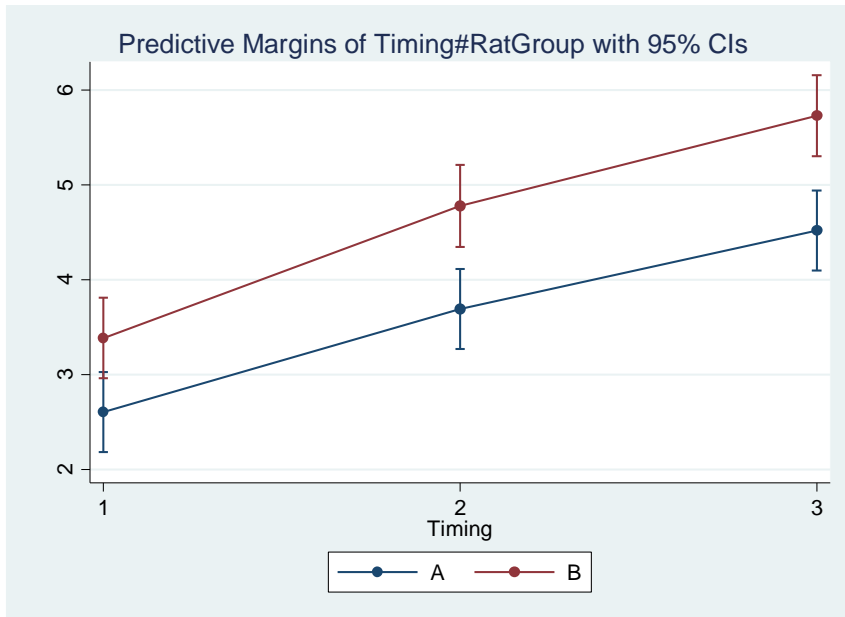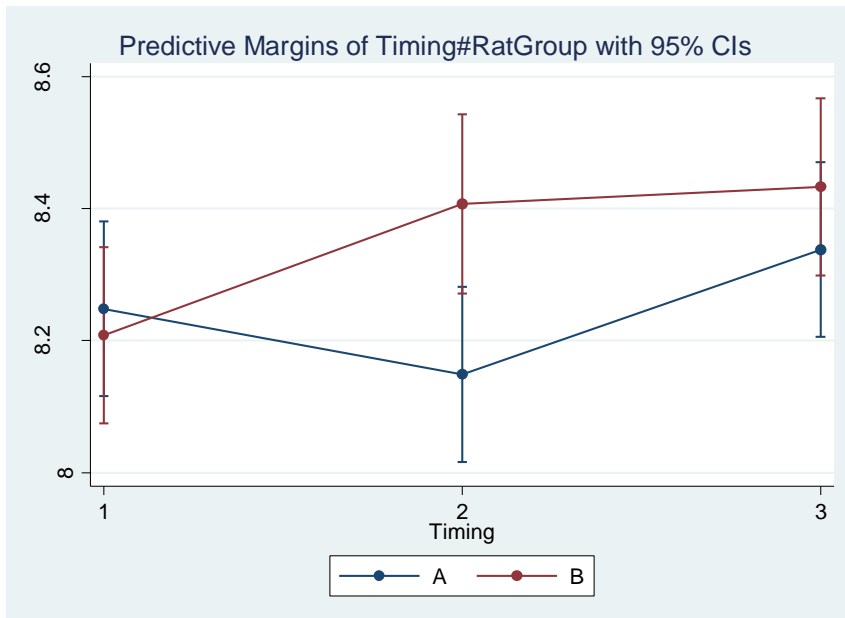**Figure 47. Estimated mean and 95% Confidence Interval per group and timing in Condylion - I' Initial**
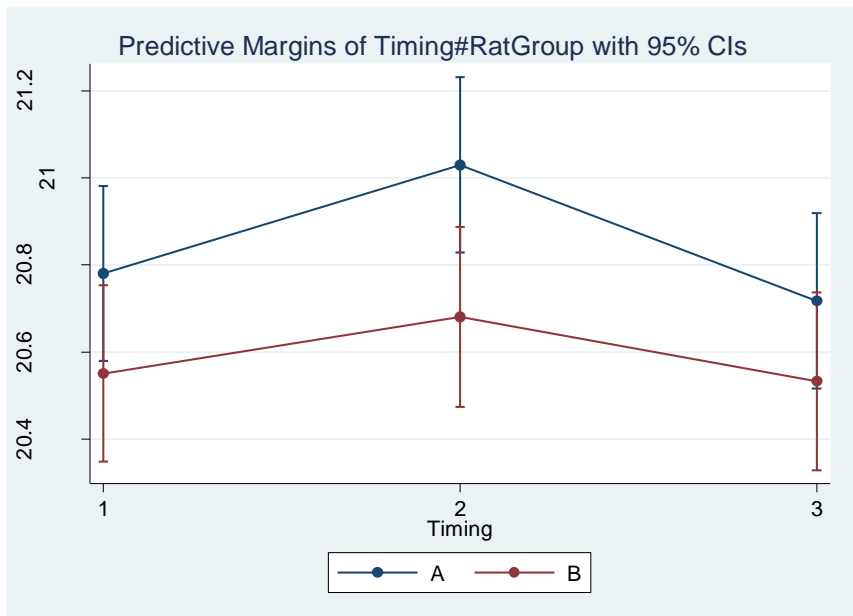
Figure 48. Estimated mean and 95% Confidence Interval per group and timing in Condylion - I' Final

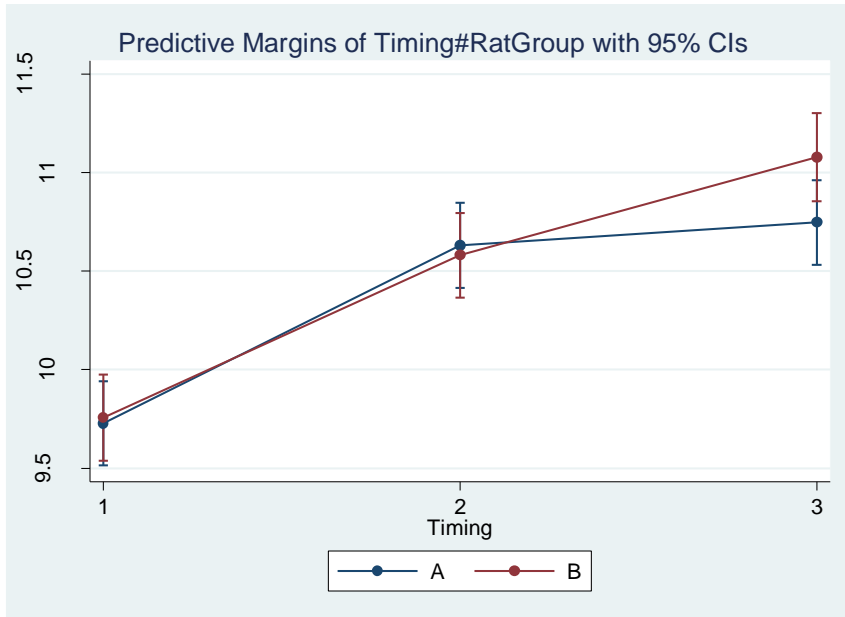Predictive Margins of Timing#RatGroup with 95% CIs
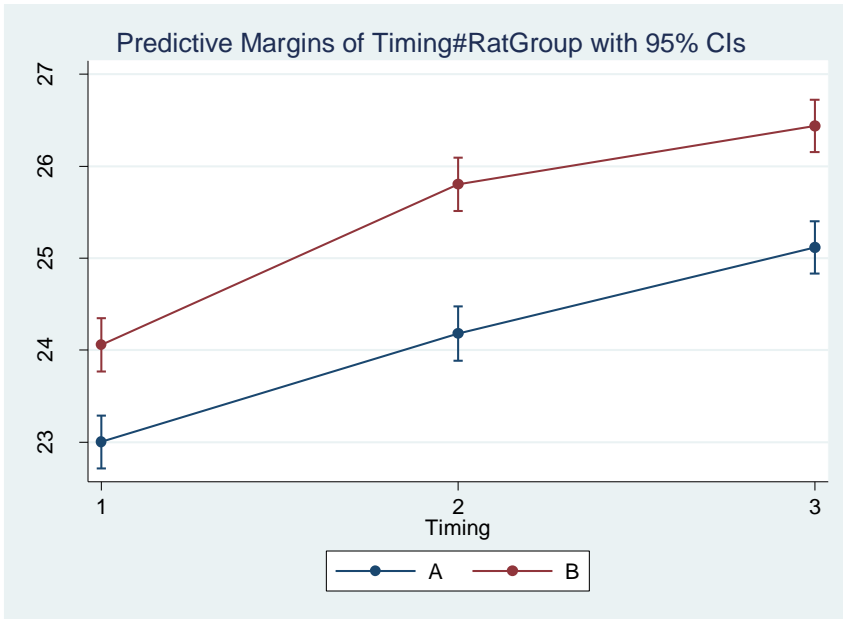
Figure 49. Estimated mean change (Final – Initial) and 95% Confidence Interval per group and timing in Condylion - I'

Predictive Margins of Timing#RatGroup with 95% CIs
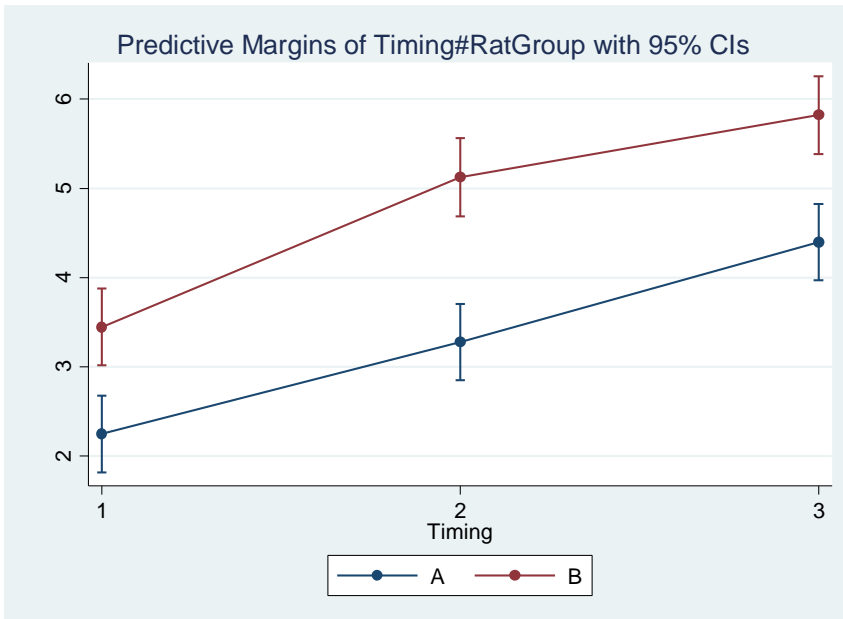
Figure 50. Estimated mean and 95% Confidence Interval per group and timing in Incisal - Id Initial

Predictive Margins of Timing#RatGroup with 95% CIs
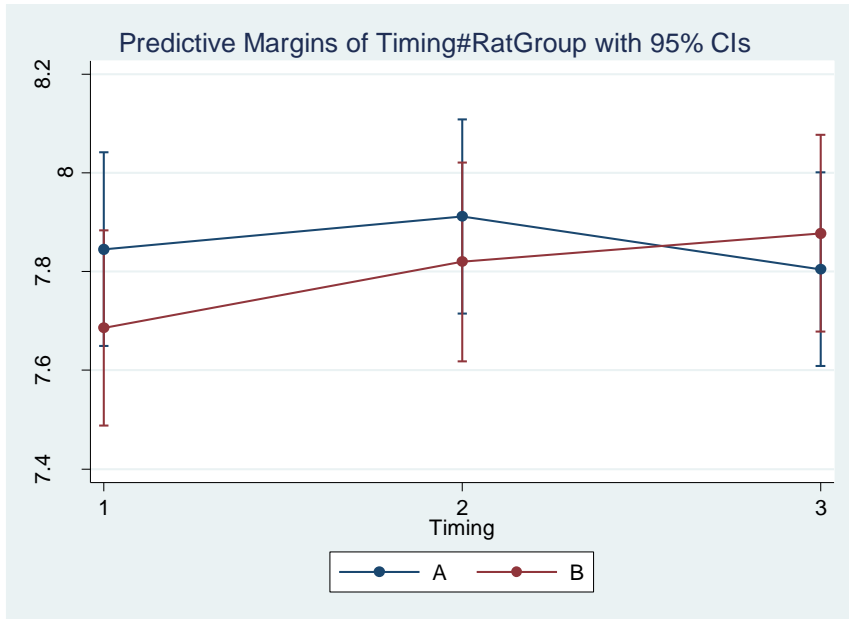
Figure 51. Estimated mean and 95% Confidence Interval per group and timing in Incisal - Id Final

Predictive Margins of Timing#RatGroup with 95% CIs
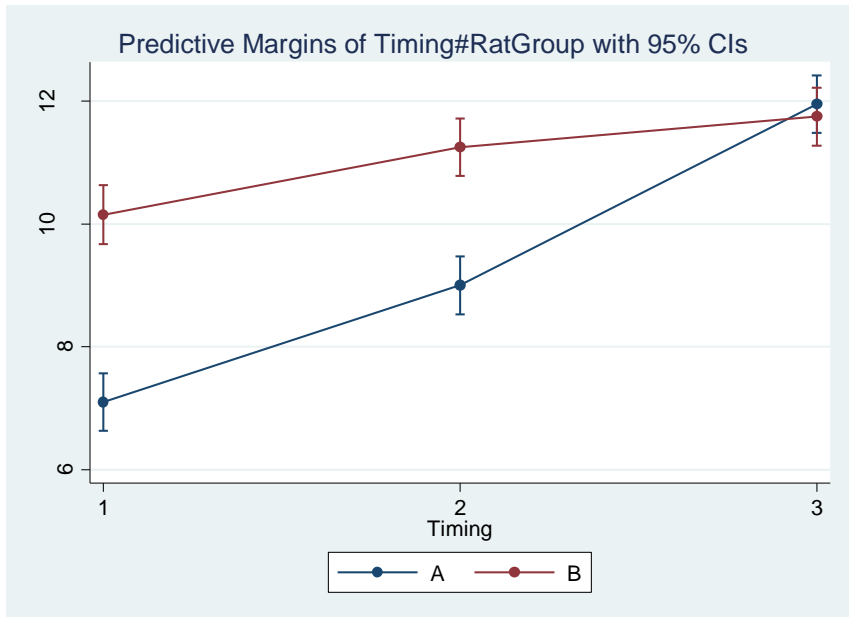
Figure 52. Estimated median change (Final – Initial) and 95% Confidence Interval per group and timing in Incisal - Id

Adjusted Predictions of Timing#RatGroup with 95% CIs
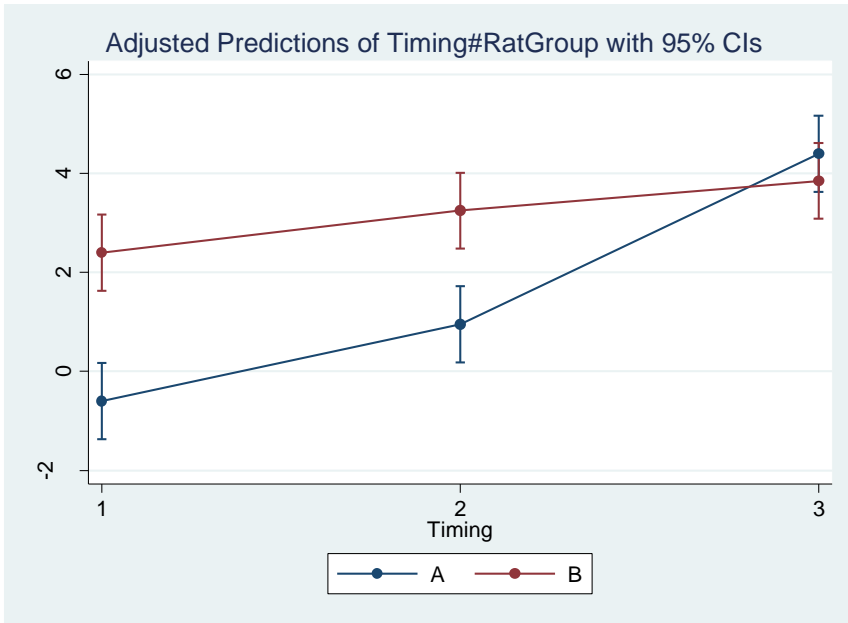
Figure 53. Estimated mean and 95% Confidence Interval per group and timing in Incisal - I' Initial

Predictive Margins of Timing#RatGroup with 95% CIs
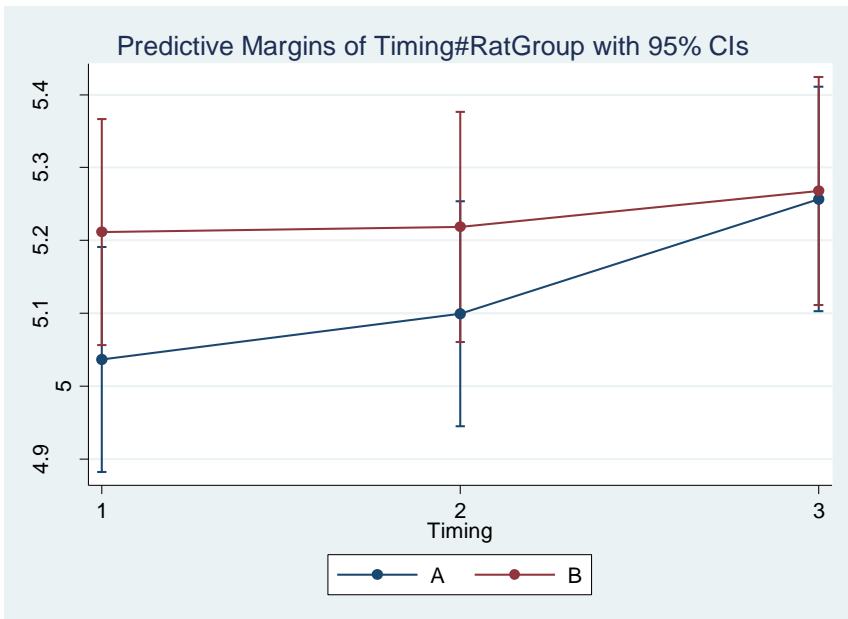
Figure 54. Estimated mean and 95% Confidence Interval per group and timing in Incisal - I' Final

Predictive Margins of Timing#RatGroup with 95% CIs
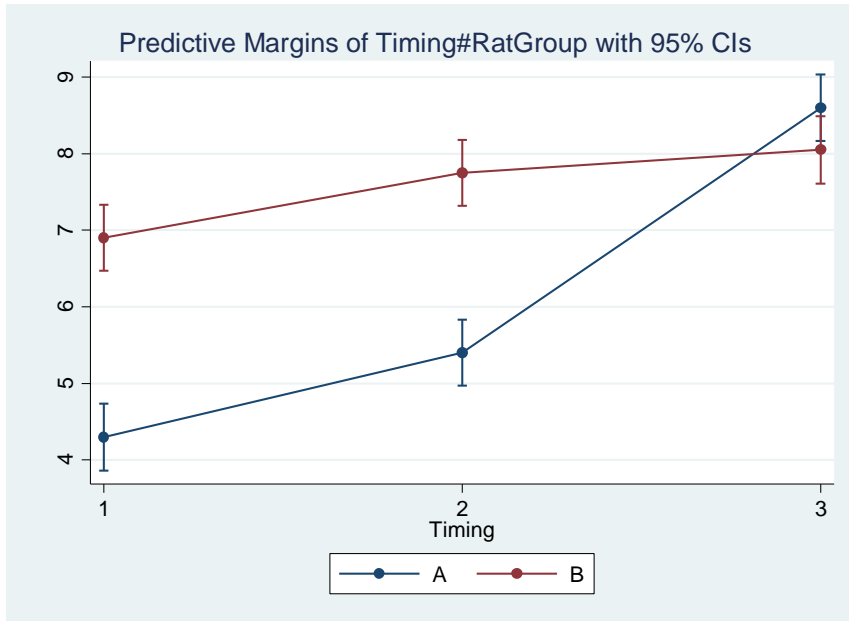
Figure 55. Estimated median change (Final – Initial) and 95% Confidence Interval per group and timing in Incisal - I'

Adjusted Predictions of Timing#RatGroup with 95% CIs
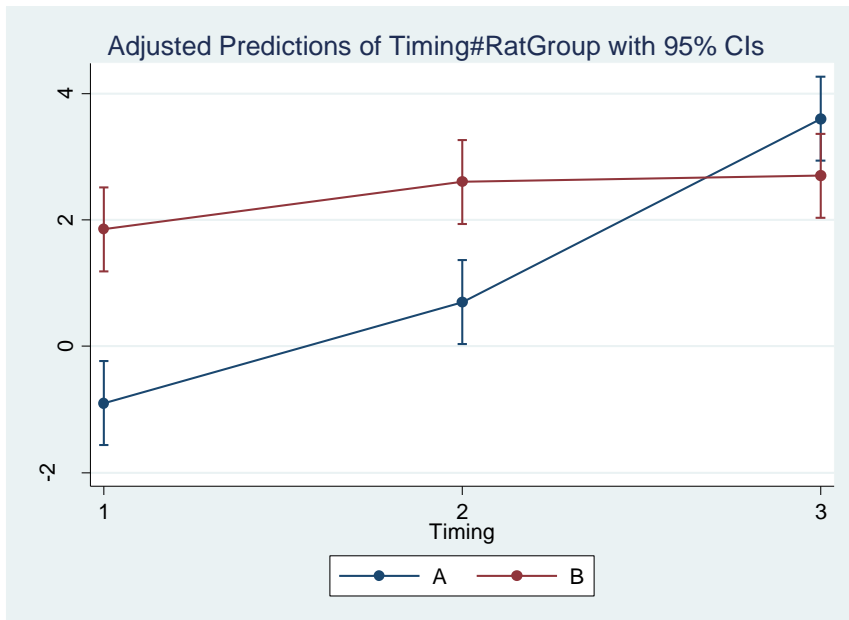
Figure 56. Estimated mean and 95% Confidence Interval per group and timing in Intercondylar Initial

Predictive Margins of Timing#RatGroup with 95% CIs
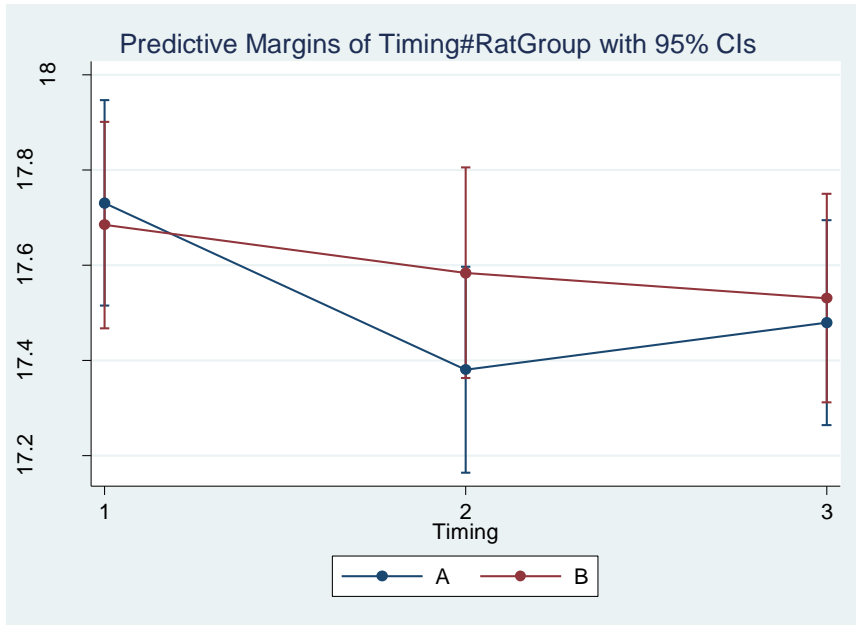
Figure 57. Estimated mean and 95% Confidence Interval per group and timing in Intercondylar Final

Predictive Margins of Timing#RatGroup with 95% CIs
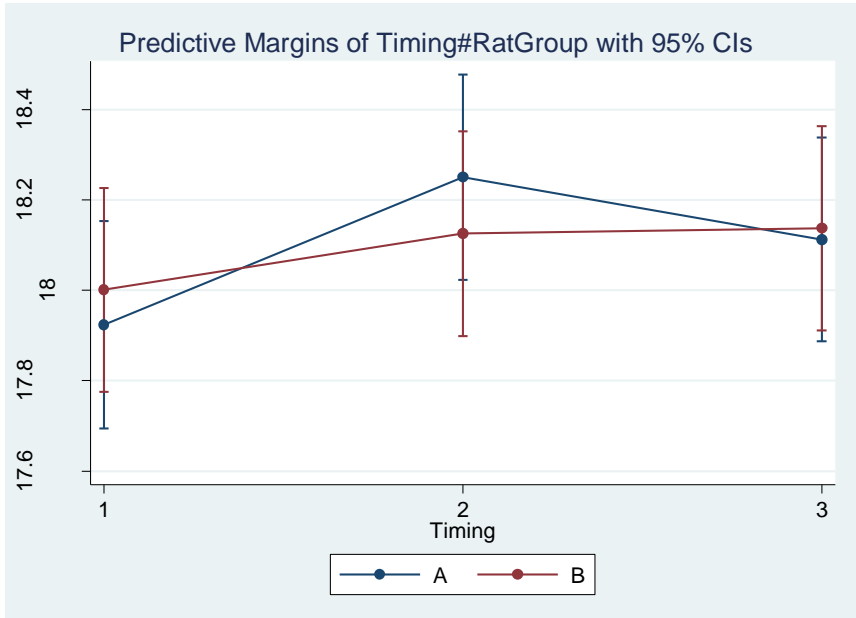
Figure 58. Estimated mean change (Final – Initial) and 95% Confidence Interval per group and timing in Intercondylar

Predictive Margins of Timing#RatGroup with 95% CIs
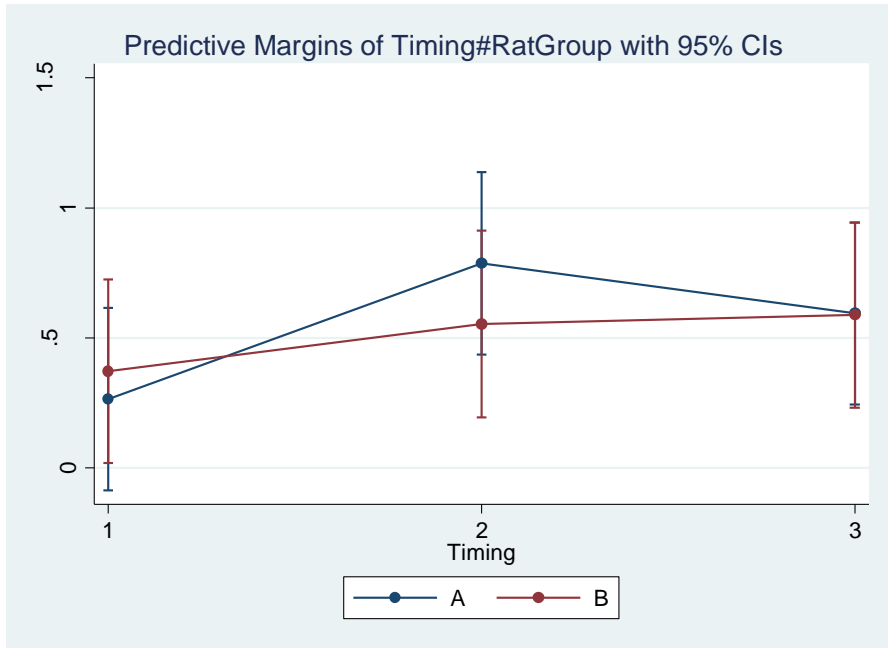
Biochemical analysis

Tables with descriptive statistics for each marker and graphs with estimated means changes (final – initial value) and 95% confidence intervals are provided (Tables 7-11, Figures 59-62).

Statistically significant increases were observed in OPG and OPG/RANKL ratio in both groups and all subgroups. Statistically significant decrease was observed in MCSF in subgroups B2 and B3. No statistically significant differences in changes by group or timing were found.

Table 7. Descriptive statistics (mean and standard deviation) for each measurement by subgroups and overall, for experimental group A.

Experimental Group A	Subgroups - Timing				
	A1 - 0d	A2 - 0d	A3 - 0d	Overall	
	Mean (SD)	Mean (SD)	Mean (SD)	Mean (SD)	p-value
RANKL (pg/ml)	275.45 (49.00)	289.02 (47.62)	285.12 (51.62)	283.20 (48.36)	0.788
OPG (pg/ml)	110.37 (46.96)	133.67 (65.64)	117.05 (27.81)	120.36 (48.88)	0.498
MCSF (pg/ml)	314.45 (173.31)	229.19 (111.97)	226.27 (103.80)	256.64 (135.97)	0.200
OPG/RANKL ratio	0.40 (0.17)	0.45 (0.17)	0.42 (0.13)	0.42 (0.15)	0.775
	A1 - 30d	A2 - 60d	A3 - 90d	Overall	
	Mean (SD)	Mean (SD)	Mean (SD)	Mean (SD)	p-value
RANKL (pg/ml)	293.64 (64.05)	303.22 (38.40)	306.50 (60.05)	301.12 (54.01)	0.840
OPG (pg/ml)	260.84 (183.64)	304.52 (121.77)	214.12 (123.69)	259.83 (146.52)	0.328
MCSF (pg/ml)	277.31 (126.81)	167.28 (69.35)	148.72 (83.99)	197.77 (109.98)	**0.005***
OPG/RANKL ratio	0.86 (0.57)	1.02 (0.42)	0.76 (0.51)	0.88 (0.50)	0.445

* A1vsA2 p=0.026, A1vsA3 p=0.008 (Bonferroni corrected)

Table 8. Descriptive statistics (mean and standard deviation) for each measurement by subgroups and overall, for control group B.

Control Group B	Subgroups - Timing				
	B1 - 0d	B2 - 0d	B3 - 0d	Overall	
	Mean (SD)	Mean (SD)	Mean (SD)	Mean (SD)	p-value
RANKL (pg/ml)	298.60 (38.86)	279.33 (25.34)	284.08 (43.13)	287.34 (36.47)	0.415
OPG (pg/ml)	121.94 (60.25)	97.29 (50.91)	112.28 (56.86)	110.50 (55.48)	0.561
MCSF (pg/ml)	335.30 (188.65)	276.49 (169.91)	279.70 (145.37)	297.16 (166.28)	0.635
OPG/RANKL ratio	0.41 (0.21)	0.35 (0.17)	0.39 (0.18)	0.38 (0.18)	0.711
	B1 - 30d	B2 - 60d	B3 - 90d	Overall	
	Mean (SD)	Mean (SD)	Mean (SD)	Mean (SD)	
RANKL (pg/ml)	305.90 (56.14)	285.00 (47.53)	304.79 (64.11)	298.56 (55.56)	0.597
OPG (pg/ml)	260.60 (120.48)	196.22 (69.83)	281.85 (139.57)	246.22 (116.53)	0.175
MCSF (pg/ml)	294.98 (176.11)	172.66 (134.08)	156.83 (69.58)	208.15 (144.36)	**0.032***
OPG/RANKL ratio	0.83 (0.28)	0.72 (0.29)	0.93 (0.43)	0.83 (0.34)	0.327

* B1vsB3 p=0.050 (Bonferroni corrected)

Table 9. Estimated mean changes (final – initial), 95% confidence Intervals and p-values (comparing with 0 i.e. no change) per group and timing.

Final - Initial	Mean Change	95% Confidence Interval	p-value
RANKL (pg/ml)			
A1	18.19	-15.47, 51.86	0.285
B1	7.30	-26.36, 40.97	0.666
A2	14.20	-19.47, 47.87	0.403
B2	5.67	-28.00, 39.34	0.738
A3	21.38	-12.29, 55.04	0.209
B3	20.71	-12.96, 54.38	0.224
OPG (pg/ml)			
A1	150.48	77.45, 223.51	**<0.001**
B1	138.66	65.63, 211.69	**<0.001**
A2	170.86	97.83, 243.89	**<0.001**
B2	98.94	25.91, 171.97	**0.009**
A3	97.07	24.04, 170.10	**0.010**
B3	169.57	96.54, 242.60	**<0.001**
MCSF (pg/ml)			
A1	-37.15	-119.23, 44.94	0.370
B1	-40.32	-122.41, 41.77	0.330
A2	-61.91	-144.00, 20.17	0.137
B2	-103.83	-185.92, -21.75	**0.014**
A3	-77.55	-159.64, 4.53	0.064
B3	-122.87	-204.95, -40.78	**0.004**
OPG/RANKL ratio			
A1	0.46	0.20, 0.72	**0.001**
B1	0.43	0.17, 0.68	**0.002**
A2	0.57	0.31, 0.83	**<0.001**
B2	0.37	0.11, 0.63	**0.006**
A3	0.34	0.08, 0.60	**0.011**
B3	0.54	0.28, 0.79	**<0.001**

Table 10. Pairwise group per timing mean changes (final – initial) comparisons' p-values derived from linear regression models, adjusted for multiple comparison (Bonferroni).

	p-values*		
Mean Change	**A1 vs B1**	**A2 vs B2**	**A3 vs B3**
RANKL	>0.999	>0.999	>0.999
OPG	>0.999	0.507	0.497
MCSF	>0.999	>0.999	>0.999
OPG/RANKL ratio	>0.999	0.802	0.857

Table 11. Pairwise timing per group mean changes (final – initial) comparisons' p-values derived from linear regression models, adjusted for multiple comparison (Bonferroni).

	p-values*					
Mean Change	**A1 vs A2**	**A1 vs A3**	**A2 vs A3**	**B1 vs B2**	**B1 vs B3**	**B2 vs B3**
RANKL	>0.999	>0.999	>0.999	>0.999	>0.999	>0.999
OPG	>0.999	>0.999	0.634	>0.999	>0.999	0.707
MCSF	>0.999	>0.999	>0.999	>0.999	0.642	>0.999
OPG/RANKL ratio	>0.999	>0.999	0.812	>0.999	>0.999	>0.999

Figure 59. Estimated mean change (final-initial) and 95% Confidence Interval per group and timing in RANKL

Adjusted Predictions of Timing#RatGroup with 95% CIs
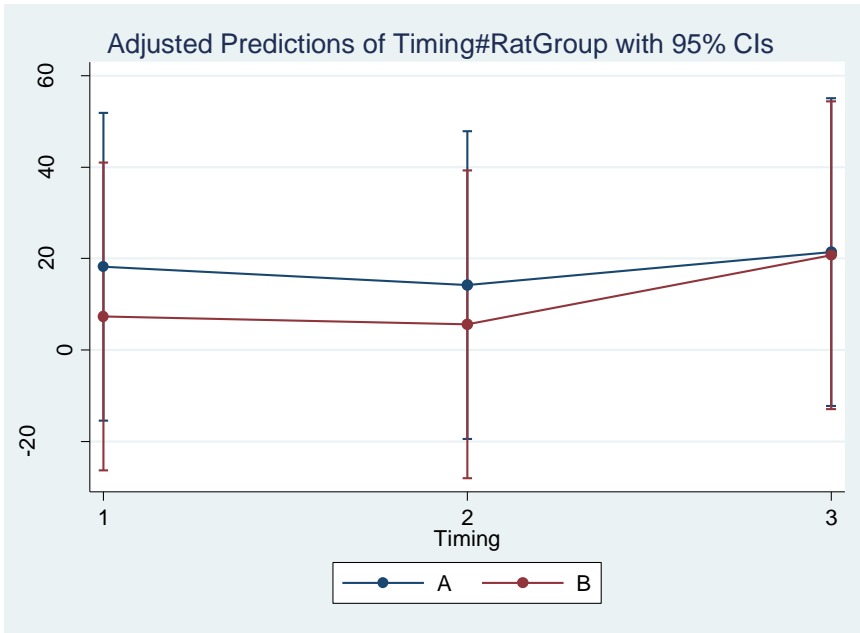
Figure 60. Estimated mean change (final-initial) and 95% Confidence Interval per group and timing in OPG

Adjusted Predictions of Timing#RatGroup with 95% CIs
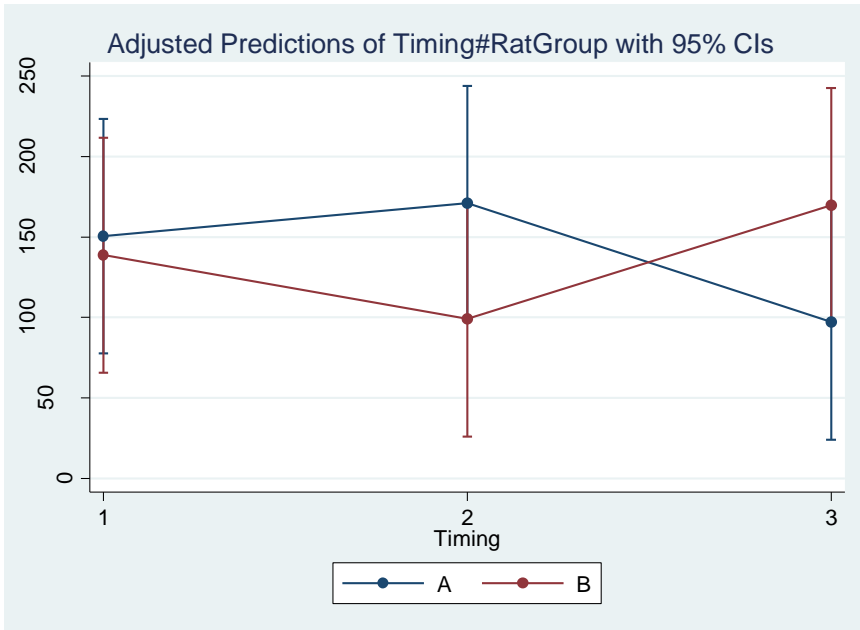
Figure 61. Estimated mean change (final-initial) and 95% Confidence Interval per group and timing in MCSF
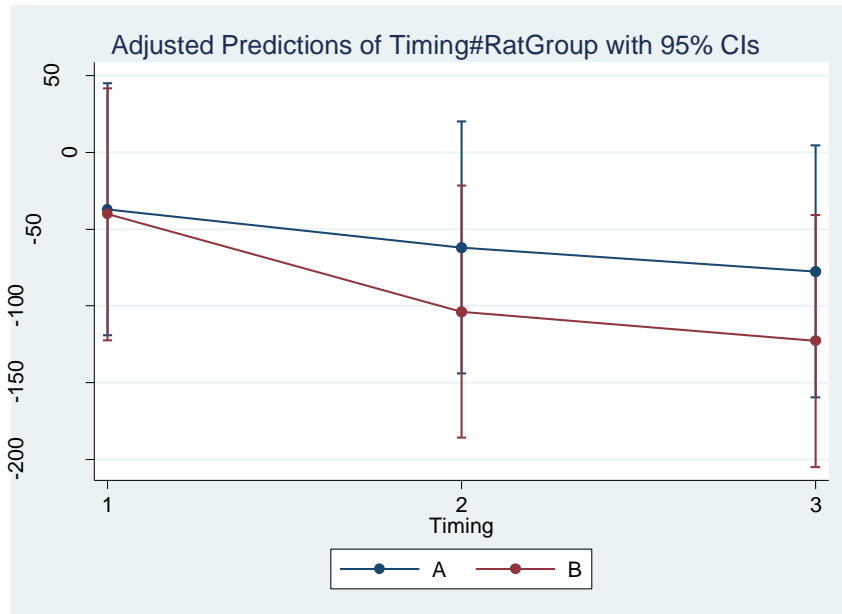
Figure 62. Estimated mean change (final-initial) and 95% Confidence Interval per group and timing in OPG/RANKL ratio
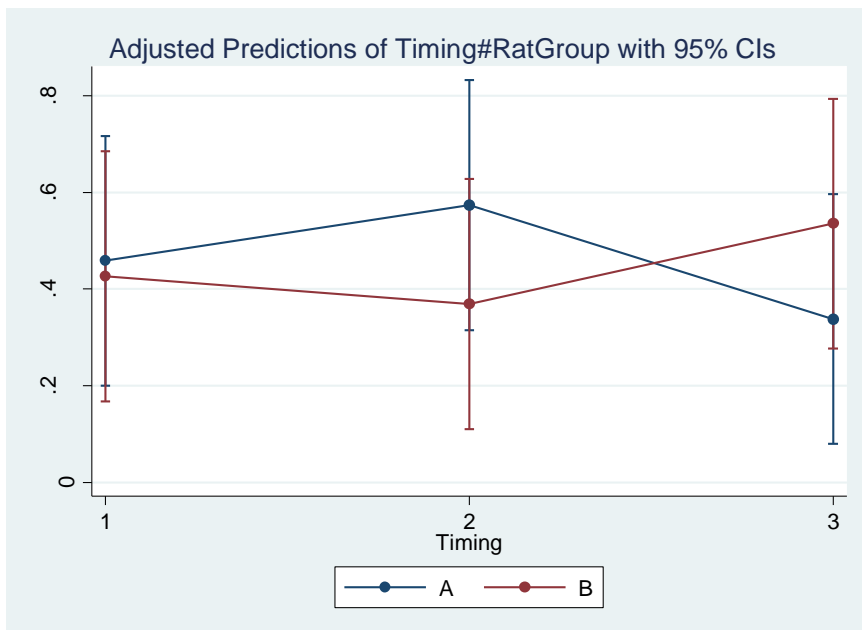
Histological observations

Descriptive statistics and estimated means (with 95% confidence intervals) are provided in Tables 12-14 and Figures 63-67.

The ratio Bone Surface/Total Surface (BS/TS) was found statistically significanty lower at both the anterior and posterior areas between A1 and B1 subgroups. In these subgroups, the Anterior Condylar Cartilage Thickness (Anterior CCT) measured statistically significantly greater.

In addition, statistically significantly lower values were evidenced between experimental and control groups, regarding the Posterior Condylar Cartilage Thickness (Posterior CCT) at 30, 60 and 90 days.

Table 12. Descriptive statistics (mean and standard deviation) for each measurement by Timing and overall, for group A and B

Experimental Group A	Subgroups - Timing				
	A1 - 30d	A2 - 60d	A3 - 90d	Overall	
	Mean (SD)	Mean (SD)	Mean (SD)	Mean (SD)	p-value*
Anterior Ratio BS/TS	69.45 (12.37)	75.52 (6.81)	76.30 (11.35)	73.76 (10.62)	0.342
Posterior Ratio BS/TS	78.31 (13.21)	88.99 (3.33)	89.86 (5.22)	85.72 (9.76)	0.079
Anterior CCT (µm)	252.52 (40.04)	202.96 (74.65)	202.27 (52.45)	219.25 (60.74)	0.186
Middle CCT (µm)	163.12 (68.22)	154.40 (58.46)	153.61 (42.75)	157.05 (55.95)	0.950
Posterior CCT (µm)	97.02 (24.66)	78.77 (30.65)	76.61 (17.56)	84.13 (25.87)	0.516
Weight final (grams)	256.08 (24.46)	320.67 (24.97)	337.00 (58.63)	304.58 (52.15)	<0.001
Control Group B	Subgroups - Timing				

	B1 - 30d	B2 - 60d	B3 - 90d	Overall	
	Mean (SD)	**Mean (SD)**	**Mean (SD)**	**Mean (SD)**	
Anterior Ratio BS/TS	80.32 (6.81)	83.37 (8.15)	84.20 (7.48)	82.63 (7.48)	0.862
Posterior Ratio BS/TS	90.45 (6.20)	93.51 (6.18)	94.15 (4.60)	92.70 (5.78)	0.576
Anterior CCT (μm)	196.78 (36.54)	166.33 (23.12)	165.78 (17.71)	176.30 (30.03)	0.708
Middle CCT (μm)	177.12 (54.72)	151.71 (29.35)	150.94 (29.06)	159.93 (40.37)	0.660
Posterior CCT (μm)	153.39 (35.55)	143.25 (14.65)	141.63 (14.34)	146.09 (23.60)	0.910
Weight final (grams)	282.25 (18.14)	365.25 (34.98)	430.17 (30.11)	359.22 (67.40)	<0.001

* derived from linear regression models; pairwise comparisons are provided in tables 13&14

Table 13. Pairwise group per timing measurements comparisons' p-values derived from linear regression models, adjusted for multiple comparison (Bonferroni)

	p-values*		
Measurements	**A1 vs B1**	**A2 vs B2**	**A3 vs B3**
Anterior Ratio BS/TS	**0.021**	0.196	0.416
Posterior Ratio BS/TS	**0.002**	>0.999	>0.999
Anterior CCT	**0.026**	0.468	>0.999
Middle CCT	>0.999	>0.999	>0.999
Posterior CCT	**<0.001**	**<0.001**	**<0.001**
Weight final	0.202	**0.007**	**<0.001**

* bold p-values indicate statistical significance at 5% level

Table 14. Pairwise timing per group measurements comparisons' p-values derived from linear regression models, adjusted for multiple comparison (Bonferroni)

	p-values*					
Measurements	**A1 vs A2**	**A1 vs A3**	**A2 vs A3**	**B1 vs B2**	**B1 vs B3**	**B2 vs B3**
Anterior Ratio BS/TS	0.788	0.705	>0.999	>0.999	>0.999	>0.999
Posterior Ratio BS/TS	0.141	0.198	>0.999	>0.999	>0.999	>0.999
Anterior CCT	0.321	0.496	>0.999	>0.999	>0.999	>0.999
Middle CCT	>0.999	>0.999	>0.999	>0.999	>0.999	>0.999
Posterior CCT	>0.999	>0.999	>0.999	>0.999	>0.999	>0.999
Weight final	**<0.001**	**<0.001**	0.999	**<0.001**	**<0.001**	**<0.001**

* bold p-values indicate statistical significance at 5% level

Figure 63. Estimated mean and 95% Confidence Interval per group and timing in Anterior Ratio Bone Surface/Total Surface

Predictive Margins of Timing#RatGroup with 95% CIs
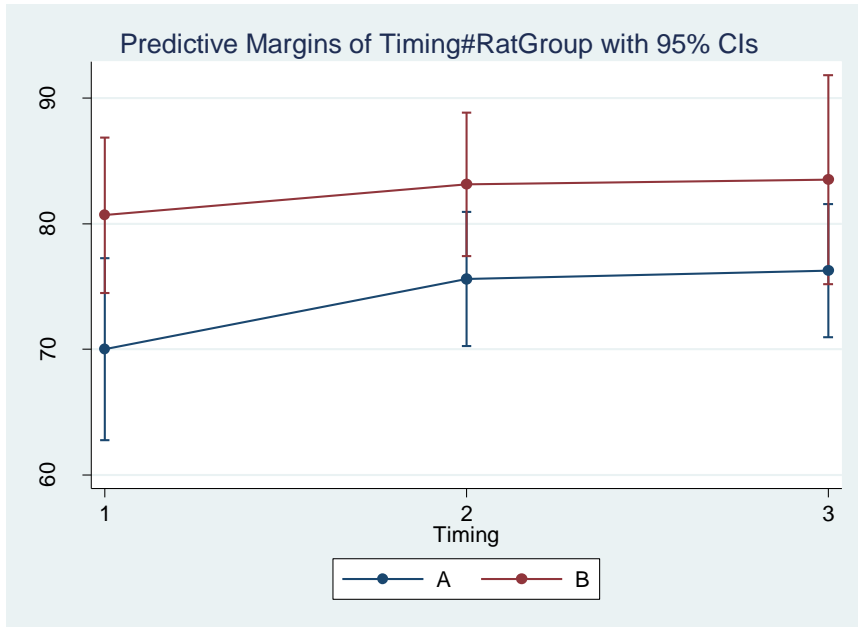
Figure 64. Estimated mean and 95% Confidence Interval per group and timing in Posterior Ratio Bone Surface/Total Surface

Predictive Margins of Timing#RatGroup with 95% CIs
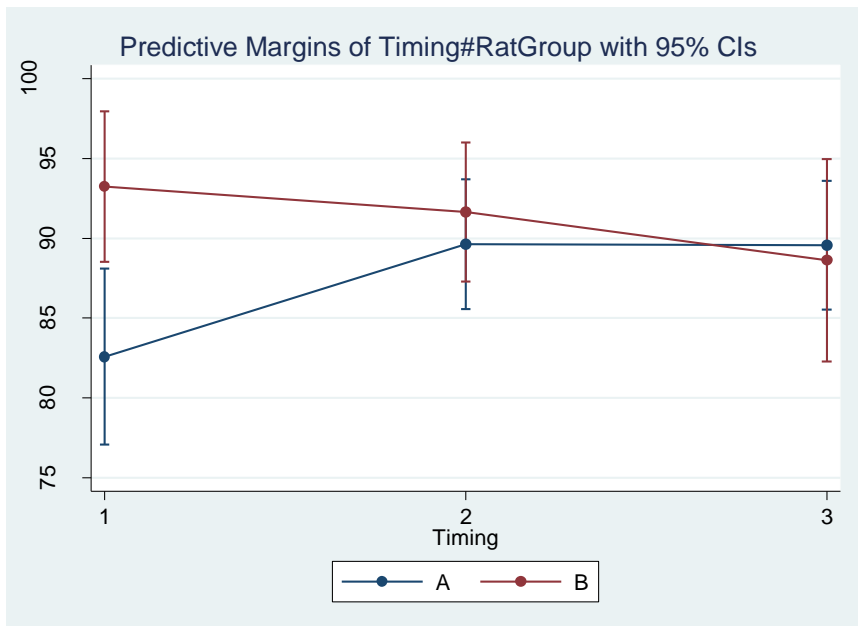
Figure 65. Estimated mean and 95% Confidence Interval per group and timing in Anterior Condylar Cartilage Thickness

Predictive Margins of Timing#RatGroup with 95% CIs
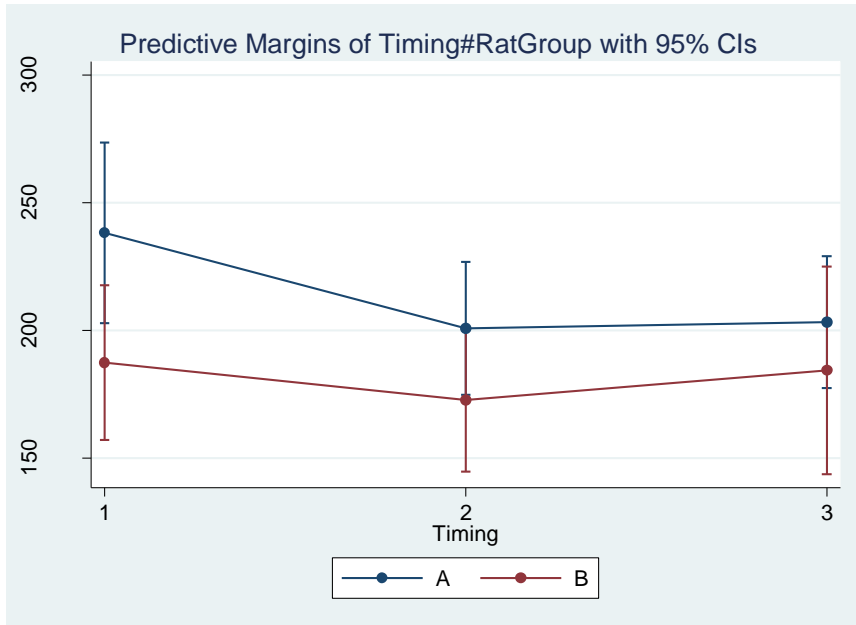
Figure 66. Estimated mean and 95% Confidence Interval per group and timing in Middle Condylar Cartilage Thickness

Predictive Margins of Timing#RatGroup with 95% CIs
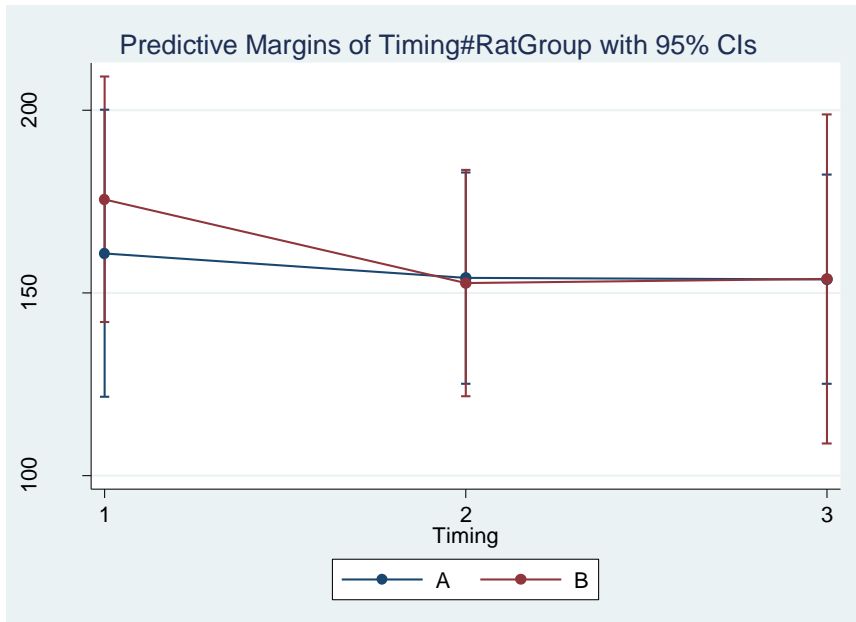
Figure 67. Estimated mean and 95% Confidence Interval per group and timing in Posterior Condylar Cartilage Thickness

Predictive Margins of Timing#RatGroup with 95% CIs
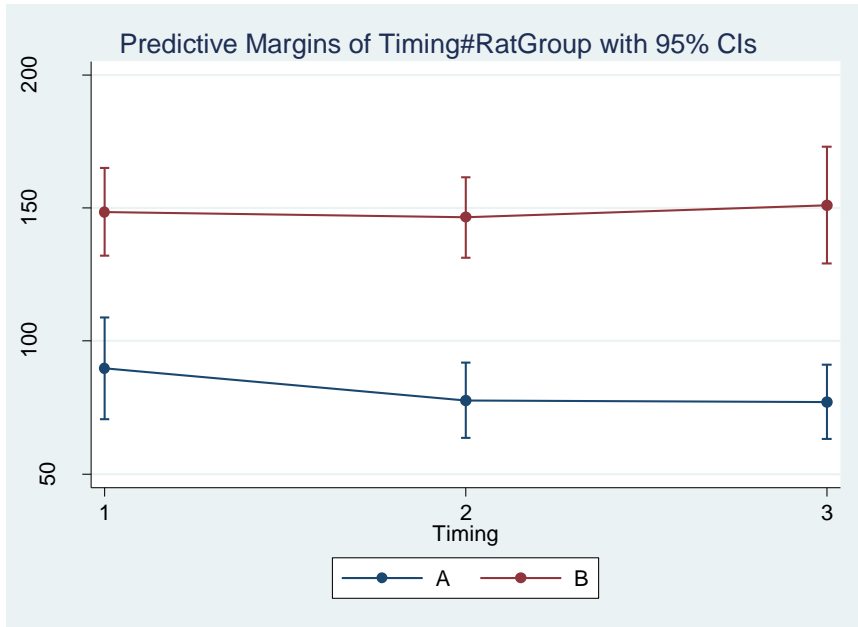
5. DISCUSSION

Orthodontics aims to study and guide the development of maxillofacial elements that contribute to normal appearance and basic function (de Oliveira Meira et al., 2020), namely mastication, swallowing and breathing (Ngiam and Cistulli, 2015; Nguee et al., 2020; Sommerlad, 1994). Thus, the ongoing interest in the growth of the maxilla and the mandible may not come as a surprise (Ghafari, 2015). Even conservative, non-surgical interventions may affect the functioning of the TMJ and facial appearance.

Knowledge on mandibular growth is acquired by longitudinal clinical studies in normal individuals as well as by experiments that use various animal models, mainly primates (Breitner, 1941; Janzen and Bluher, 1965), rodents and other mammals (Herring, 2003). The rationale for selecting the rodents has been a matter of debate. It is speculated that the existing anatomical differences with humans may lead to erroneous conclusions. On the other hand, higher financial costs seem to limit experimentation with non-human primates; additionally, current restrictions imposed by ethics prevent recruiting humans as experimental subjects in interventions that may culminate in irreversible or undesirable outcomes (Aersens et al., 1998). There has not been a definitive agreement on the mechanism (Mousoulea et al., 2016) and the possible side effects of the most common treatments of developmental deviations (Folke and Stallard, 1966; Figueroba et al., 2014; Zurfluh et al., 2015).

The present study investigates in rats an important feature of human face, the mandibular shape, which is a potential determinant of self-esteem (Lin et al., 2016; Alabdulrazaq, 2020), it affects the individual's social interaction (Sofer et al., 2015; Shen et al., 2016) and professional success (Frieze et al., 1991), and is important in the function of the orofacial complex (Rezaei et al., 2019; Lathrop-Marshall et al., 2021). Mandibular development is multifactorial and is regulated by genetic and environmental variables (Vieira, 2019; Kahn et al., 2020).

Currently, the rat is the most popular animal used in experimental studies involving anatomy and physiology (Sengupta, 2013; Logan, 2019; Sampaziotis, 2019; Rontogianni et al., 2022), despite the existing differences with humans (Herring, 2003; Suzuki and Iwata, 2016; Bolker, 2019). Thus, Wistar rats were used in the study with provision to eliminate potential confounding factors related to their characteristics. Therefore the animals were all male and had no significant differences in measurements that might be connected to the variables of interest. Although the majority of similar studies have also selected the rat, some past research has reported on rabbits (Tsolakis, 1981; Desai et al., 1996) and even monkeys (Baume et al., 1961; Janzen and Bluher, 1965).

Despite the randomization, some comparisons among subgroups, regarding initial measurements, were found to be statistically different. This might be attributed to the small size of each subgroup. Twelve rats were sufficient to detect the difference of interest, as was determined after power analysis, but they might not have been adequate to eliminate differences of initial characteristics. However, regression models concerning final measurements were adjusted for initial ones, when appropriate.

Every possible effort was made to breed the animals in a healthy, safe environment, to provide necessary nutrition and to treat them with dignity. Assessing the rat final weight, there was no difference between A1-B1, contrary to the observations between A2-B2 and A3-B3 (although the device in the A3 subgroup was removed on the 60th experimental day). It could be hypothesized that, initially, the appliance did not seem to have caused any important difference, but subsequently the animals might have faced trouble with feeding. Nevertheless, the rats continued growing as there were differences among A1, A2, A3 (Table 5).

Rats live for 3 years, on average. They develop rapidly and their adolescence ends by the end of the second month of ontogenesis. Thus, a rat at 2 months of age (60 days) is considered a young adult. The period of rapid growth allegedly ends by 5 weeks, whereas at the period from 8 to 16 weeks, growth slows down (Roach et al., 2003; Quinn, 2005; Andreollo et al., 2012; Klein and Romeo, 2013; Sengupta, 2013). The experimental period of the present study lasted for 90 days and the rat age in the last subgroup was 120 days. The differences between subgroups A3 and B3 remained statistically significant 30 days following the removal of the device (from A3 subgroup), meaning that the mandible did not exhibit any post-treatment catch-up growth.

Calculations were not performed separately for the left and right mandibular sides. By contrast, the mean values of the contralateral sides were used and are reported. The orthodontic device that was used in the present study was full – cast, intraoral, and was attached to the maxillary incisors. It was introduced by Desai et al. (1996) although their research did not report many details on osseous mandibular change, but focused mainly on the TMJ. It was adopted by Cholasueksa et al. (2004) and Farias-Neto et al. (2012), whereas Hua et al. (2012) and Wang et al. (2019) used modified upper/lower devices, unlike Asano (1986), Tsolakis (1981) and Teramoto et al. (2003) who selected extra-oral appliances. Teramoto et al., (2003) mention that the magnitude of the traction was excessive and, thus, ended up traumatic.

The mandibular distal displacement effected by the intraoral device that was cemented to the rat maxillary incisors caused a restriction in mandibular length and in mandibular body length (Lyros et al., 2022). Both Go and Go' landmarks were identified because the distal outline of the ramus in rodents appears to be particularly more concave related to human anatomy (Figure 12). Asano (1986) appears to have faced the same challenge to highlight similar landmarks. Such developmental restriction is in agreement with the observations of Desai et al. (1996), and

Cholasueksa et al. (2004), the conclusions of Asano (Asano, 1986), Farias-Neto et al. (2012), and Hua et al. (2012).

In addition, the oblique osseous and dento - osseous measurements as depicted by Coronoid - Menton, Condylion - Menton, Condylion - Id were also found significantly different. Interestingly, Hua et al. (2012) found their respective oblique measurement, namely the angle between the axis of the condylar process to the mandibular plane, also changing spatial orientation.

The present study also identified dental alterations, as manifested by the statistically significant differences between experimental and control animals regarding measurements Incisal - Id and Incisal - I' between A1 vs B1 and A2 vs B2 subgroups, respectively, albeit differences did not persist after 90 days of experiment (A3 vs B3 subgroups). Dental attrition of the lower incisors in subgroups A1, A2 during the first 60 days of the experiment due to their contact with the device discontinued after debonding the apparatus. Subsequently, lower incisors resumed eruption and so no difference appeared in the dental crown length between experimental and control subgroups.

Interestingly, the vertical component of the mandibular structure as expressed by the condylion height (Condylion/Go' - Menton) and ramus height (Condylion - Go') did not show significant difference across the experimental and control subgroups at the final records. The observation agrees with that of Farias-Neto et al., (2012) that did not observe any significant difference considering the ramus height. Asano's conclusions are similar regarding the condylar height, although he noticed also a thickening of the retromolar region and the condylar neck, potentially due to spatial remodelling (Asano, 1986). This leads to the conclusion that mandibular retrusion might not be expected to cause unwanted side - outcomes affecting facial appearance. Lastly, the Intercondylar distance remained

statistically unaffected, in agreement with the conclusions of Farias-Neto et al., (2012).

To increase accuracy in identifying anatomical landmarks (Nalçaci et al., 2010; Navarro et al., 2013; Sampaziotis, 2019) and for increased consistency in measuring dimensions (Lin et al., 2014), CBCT, a 3D reconstruction, was used in place of ordinary 2D lateral cephalometric radiography (Ghafari et al., 1998; Chadwick et al., 2009; Botticelli et al., 2011; American Academy of Oral and Maxillofacial Radiology, 2013; Durão et al., 2015). To our best knowledge this is the first study to use CBCT for comprehensive cephalometric evaluation in rats, although various digital radiographic techniques have been used (Farias-Neto et al., 2012; Wang et al., 2019).

Dental professionals treating patients would like to know whether a given treatment modality involving posterior mandibular dislocation has a stable effect on the net growth and consequently the facial dimensions, which affect the appearance (Hans et al., 2017). Nevertheless, the studies being reviewed here are heterogeneous regarding their outcomes and they predominantly report on histology and biochemistry despite the use of lateral radiography.

To our best knowledge, 3D radiography has not been previously used in research involving growing male Wistar rats during intermittent distal displacement of their mandibular condyle, effected by a modified functional, fully cast appliance with bands and inclined plane, permanently cemented on the upper incisors over a long period.

Because individuals featuring visible developmental aberrations pertaining to mandibular prognathism may have apparent need for treatment, it is of importance to ascertain the mechanism of action and to clarify the potential side effects of the intervention studied.

Concerning biochemical analysis, correlations among the subgroups, regarding concentrations of OPG, RANKL and MCSF in blood serum have not been found. The results indicate that concentrations of OPG, RANKL and MCSF in blood serum have no correlation between the subgroups. The data suggest that a localized intervention displacing posteriorly the mandible does not appear to affect the studied proteins in the systematic circulation.

The histological observations are in agreement with the results of previous studies. The statistically significant differences between subgroups A1 and B1 regarding the BS/TS Ratio confirm the findings of Kuroda et al. (2009). Moreover, reduction of condylar cartilage width at the posterior region has been identified by Teramoto et al. (2003), Cholasueksa et al. (2004) and Hua et al. (2012). Contrarily, Figueroba et al. (2014), observed that the four cartilage layers thickened in the middle region of the experimental condyles, undergoing functional posterior displacement, in comparison to controls, after 14 days of intervention. Hence, it might be concluded that the horizontal component of the chewing force is transmitted to the posterior condylar region.

The soft diet used in the present research may lead to a decrease in the thickness of condylar cartilage and the density of condylar subchondral bone (Tanaka et al, 2007; Chen et al, 2009) Nevertheless, one could still track the difference between experimental and control subgroups, as all the rats shared the same soft diet.

The present study was conducted in rodents with respect and complying with established legislature and regulations. Such experimentation would be off – limits in humans because the interventions might inflict irreversible changes. On the other hand, the existing differences between rodents and humans call for caution when interpreting the results. This study may be a contribution to evidence - based decision making in Orthodontics when treating skeletal Class III malocclusion, and

could intensify the call for further research on the long - lasting effects of such interventions aiming to alleviate facial deviations. A randomized controlled trial should be conducted to validate the present research.

6. CONCLUSIONS

1) Posterior mandibular displacement in growing rats alters the mandibular morphology and results in the development of a smaller mandible at a grown age. In the rat, it can be concluded that the effects of distal mandibular displacement follow a consistent temporal pattern and are statistically significant. The present study emphasized the long-term stability of the outcomes, revealing that the mandible does not show catch-up growth following treatment.

2) Localized intervention of posterior mandibular displacement is not found to affect the proteins of interest in the systematic circulation.

3) Histologically, posterior mandibular displacement has the potential to cause region-specific changes in the microarchitecture of the condylar bone and the cartilage thickness

7. ABSTRACTS

Greek abstract

Εισαγωγή - Επιστημονικό Υπόβαθρο

Οι μηχανικές φορτίσεις διαδραματίζουν θεμελιώδη ρόλο στις κυτταρικές διεργασίες κατά τη διάρκεια της μορφογένεσης των ιστών. Κατά την άσκηση της ορθοδοντικής και οδοντοπροσωπικής ορθοπεδικής εφαρμόζονται διάφορα θεραπευτικά σχήματα που ως στόχο έχουν τη συγκράτηση της αύξησης της κάτω γνάθου. Παρά το γεγονός, ότι οι προαναφερόμενες θεραπευτικές μέθοδοι χρησιμοποιούνται τουλάχιστον από τις αρχές του εικοστού αιώνα, δεν είναι ακόμη τεκμηριωμένες οι επιδράσεις τους στις κυτταρικές δομές της γναθοπροσωπικής περιοχής.

Κόνδυλος κάτω γνάθου

Πολλές μελέτες έχουν εξετάσει την αύξηση της κάτω γνάθου, και ειδικά τους κονδύλους της κάτω γνάθου. Η αύξηση του κονδύλου επηρεάζεται από κληρονομικούς και περιβαλλοντικούς παράγοντες, από ορμόνες και το μεταβολισμό, και είναι σημαντική για την αύξηση του κρανιοπροσωπικού συμπλέγματος. Η οδοντική σύγκλειση επηρεάζει την αύξηση των κονδύλων, καθώς οι κόνδυλοι δέχονται πιέσεις από μασητικές δυνάμεις που εφαρμόζονται στα δόντια. Συγκεκριμένα, οι συγκλεισιακές ανωμαλίες επηρεάζουν το μέγεθος του κονδύλου, το πάχος του χόνδρου και τον πολλαπλασιασμό των κυττάρων. Επίσης, επηρεάζουν τη θέση του κονδύλου, που φυσιολογικά θα πρέπει να βρίσκεται στο κέντρο της κροταφικής γλήνης, με αποτέλεσμα τη μη ομαλή μεταφορά των δυνάμεων στην Κροταφογναθική Διάρθρωση και τη δημιουργία δυσλειτουργιών. Ορισμένοι ερευνητές αναφέρονται στη συσχέτιση μεταξύ δυσλειτουργίας Κροταφογναθικής Διάρθρωσης και μεταβολής της θέσης του κονδύλου, ως αποτέλεσμα κακής σύγκλεισης.

Προηγούμενες μελέτες έχουν εξετάσει το χόνδρο του κονδύλου κάτω από διάφορες συνθήκες σε πειραματόζωα. Σε προηγούμενες μελέτες, η οπίσθια μετατόπιση του κονδύλου μείωσε τον αριθμό των αρχέγονων κυττάρων και εμπόδισε τον πολλαπλασιασμό των χονδροκυττάρων και μείωσε την ποσότητα της εξωκυττάριας θεμέλιας ουσίας. Διακοπτόμενη οπίσθια μετατόπιση του κονδύλου, λόγω ανωμαλίας σύγκλεισης, επιφέρει ακανόνιστη αναδιαμόρφωση του χόνδρου του κονδύλου και τραυματισμό των νεύρων. Ακανόνιστη φόρτιση της Κροταφογναθικής Διάρθρωσης μπορεί να είναι επιβλαβής για το χόνδρο του κονδύλου και για το σπογγώδες οστούν.

Ωστόσο, δεν υπάρχουν αρκετές μελέτες που να εξετάζουν τις αλλαγές στο οστούν μακροπρόθεσμα.

Σκοπός της μελέτης

Σκοπός της παρούσας έρευνας είναι η διερεύνηση σε ιστολογικό, απεικονιστικό και βιοχημικό επίπεδο των διεργασιών που συμβαίνουν στην Κροταφογναθική Διάρθρωση και συγκεκριμένα στον κόνδυλο και η μελέτη της αύξησης της κάτω γνάθου, κατά τη συγκράτηση της αύξησης της κάτω γνάθου, μέσω της άσκησης μηχανικής φόρτισης με ορθοδοντικούς/ορθοπεδικούς μηχανισμούς σε επίμυες.

Στόχοι της μελέτης

Η διερεύνηση των αλλαγών που συμβαίνουν στον κόνδυλο της κάτω γνάθου, ύστερα από οπίσθια μετατόπισή του και η μελέτη της αύξησης της κάτω γνάθου.

Συγκεκριμένα θα διερευνηθούν:
> **α)** Σε ιστολογικό επίπεδο, μέσω παρατήρησης σε οπτικό μικροσκόπιο οι ιστομορφολογικοί χαρακτήρες των εξεταζόμενων περιοχών, καθώς και η οστεοκλαστική δραστηριότητα.

β) Σε απεικονιστικό επίπεδο, η μελέτη της μορφολογίας και της δομής της κάτω γνάθου, σε τρισδιάστατο επίπεδο, μέσω μετρήσεων που θα προκύψουν από αξονική τομογραφία κωνικής δέσμης.

γ) Σε βιοχημικό επίπεδο, οι μεταβολές στο σύστημα Οστεοπροτεγερίνης (OPG) - Συνδέτη του ενεργοποιητή του υποδοχέα του πυρηνικού παράγοντα ΚΒ (RANKL) και στον Παράγοντα διέγερσης αποικιών μακροφάγων (MCSF).

Υλικά και Μέθοδος

Το πειραματικό πρωτόκολλο της μελέτης εγκρίθηκε από την Διεύθυνση Κτηνιατρικής της Περιφέρειας Αττικής και έλαβε αριθμό πρωτοκόλλου 598742/04-10-2019, με κωδικό καταχώρησης EL 25 BIO 05, σύμφωνα με την Ελληνική Εθνική νομοθεσία (ΠΔ 56/2013), την Ευρωπαϊκή Οδηγία 2010/63/ΕΕ και αυτή του Ευρωπαϊκού Συμβουλίου (276/33/20.10.2010) σχετικά με την προστασία των σπονδυλωτών ζώων που χρησιμοποιούνται σε πειράματα και για άλλους επιστημονικούς σκοπούς.

Σχεδιασμός Πειράματος

Στην παρούσα πειραματική μελέτη χρησιμοποιήθηκαν εβδομήντα δύο (72) αρσενικοί επίμυες Wistar ηλικίας τεσσάρων εβδομάδων. Μετά την αρχική τους ανατροφή τεσσάρων εβδομάδων στο Ελληνικό Ινστιτούτο Παστέρ, όλα τα ζώα μεταφέρθηκαν και στεγάστηκαν στο Εργαστήριο Πειραματικής Χειρουργικής και Χειρουργικής Έρευνας «Ν. Σ. Χρηστέας» στην Ιατρική Σχολή του Πανεπιστημίου Αθηνών. Η τυποποίηση σύμφωνα με την εθνική και ευρωπαϊκή νομοθεσία, καθόρισε επιλογή κλωβού (Tecniplast SPA, Ιταλία) και σταθερό κεντρικό αερισμό (15 αλλαγές αέρα/ώρα), περιβαλλοντικές συνθήκες σε 55% σχετική υγρασία, θερμοκρασία 20°C ± 2°C και

τεχνητό 12ωρο εύρος εναλλασσόμενων κύκλων φωτός-σκότους. Η πρόσβαση σε τροφή και νερό ήταν κατά βούληση.

Τα πειραματόζωα κατανεμήθηκαν τυχαία σε ίσες ομάδες, συγκεκριμένα τις ομάδες Α (πειραματική) και Β (ελέγχου), η καθεμία χωρίστηκε σε τρεις ίσου μεγέθους υποομάδες με δώδεκα επίμυες (Α1, Α2, Α3, Β1, Β2, Β3). Για την ομαδοποίηση χρησιμοποιήθηκε το διαδικτυακό εργαλείο Random Team Generator.

Τροποποιημένες ορθοδοντικές ενδοστοματικές συσκευές, που έχουν περιγραφεί σε προηγούμενες μελέτες, τοποθετήθηκαν στα πειραματόζωα και οδηγούσαν σε οπίσθια μετατόπιση της κάτω γνάθου. Οι πλήρως χυτές μεταλλικές ορθοδοντικές συσκευές κατασκευάστηκαν στο εργαστήριο, μετά από ψηφιακή ενδοστοματική σάρωση (TRIOS 3, 3Shape intraoral scanner) ενός ζώου που επιλέχτηκε τυχαία.

Οι τροποποιημένες συσκευές συγκολλήθηκαν στους τομείς της άνω γνάθου με οξυφωσφορική κονία (Harvard Cement Normal Setting; Harvard Dental International GmbH, 15366 Hoppegarten, Γερμανία). Κατά τη διάρκεια ολόκληρης της πειραματικής περιόδου, όλα τα πειραματόζωα (πειραματικά και ελέγχου) τρέφονταν με μαλακή τροφή, η οποία παρήχθη με ανάμειξη σφαιριδίων με νερό σε τυποποιημένες αναλογίες για να επιτευχθεί μαλακή σύσταση.

Συνολικά, η πειραματική περίοδος διήρκεσε 90 ημέρες. Τα πειραματόζωα θυσιάστηκαν στις 30 ημέρες (υποομάδες Α1, Β1), 60 ημέρες (υποομάδες Α2, Β2) και 90 ημέρες (υποομάδες Α3, Β3). Την 60ή ημέρα του πειράματος, οι ορθοδοντικές συσκευές αφαιρέθηκαν από την πειραματική υποομάδα Α3. Καθ' όλη τη διάρκεια της πειραματικής περιόδου, όλα τα πειραματόζωα παρακολουθούνταν στενά για φυσιολογική ανάπτυξη.

Τρισδιάστατη ακτινογραφική ανάλυση

Για τη μελέτη σε τρισδιάστατο επίπεδο της μορφολογίας της κάτω γνάθου, πραγματοποιήθηκαν αρχικές (ημέρα 1η του πειράματος) και τελικές (ημέρα θυσίας) υπολογιστικές τομογραφίες κωνικής δέσμης (CBCT) σε κάθε πειραματόζωο. Στους επίμυες έγινε αναισθησία με ενδομυϊκή ένεση με συνδυασμό κεταμίνης-ξυλαζίνης σε δοσολογία 0,2 ml/kg. Όλοι οι επίμυες σαρώθηκαν με την ίδια μονάδα CBCT (New Tom VGi, Cefla SC, Imola, Ιταλία) χρησιμοποιώντας το ίδιο οπτικό πεδίο (8X8 cm, υψηλής ανάλυσης, σάρωση οδοντοστοιχίας) με ρυθμίσεις έκθεσης 110 kV. Κάθε σάρωση διεξήχθη από Γναθοπροσωπικό Ακτινολόγο. Στις περιπτώσεις που κρίθηκε απαραίτητο, οι σαρώσεις επαναλήφθηκαν. Διενεργήθηκε τρισδιάστατη ανάλυση με γραμμικές μετρήσεις με χρήση του λογισμικού Viewbox (Viewbox© έκδοση 4.1.0.10, dHAL Software, Κηφισιά, Ελλάδα).

Βιοχημική ανάλυση

Για τη μέτρηση των επιπέδων των πρωτεϊνών OPG (Οστεοπρωτεγερίνη), RANKL (Συνδέτη του ενεργοποιητή του υποδοχέα του πυρηνικού παράγοντα ΚΒ) και MCSF (Παράγοντα διέγερσης αποικιών μακροφάγων), συλλέχθηκε ορός αίματος από τους επίμυες. Πιο συγκεκριμένα, έγινε αρχική (ημέρα 1η του πειράματος) και τελική (ημέρα θυσίας), αιμοληψία. Τα ζώα αναισθητοποιήθηκαν παροδικά σε θάλαμο αιθέρα. Στη συνέχεια, συλλέχθηκαν δείγματα αίματος, με τη χρήση λεπτής αποστειρωμένης εργαστηριακής πιπέτας, η οποία εισήχθη στην περιοχή των ματιών, πίσω από το βολβό του ματιού. Το αίμα τοποθετήθηκε σε δοκιμαστικούς σωλήνες που περιείχαν ηπαρίνη και φυγοκεντρήθηκε στις 13.000 rpm για 5 λεπτά σε θερμοκρασία δωματίου. Στη συνέχεια συλλέχθηκε ο ορός και αποθηκεύτηκε στους -20°C για περαιτέρω ανάλυση.

Τα επίπεδα πρωτεϊνών μετρήθηκαν χρησιμοποιώντας ELISA κιτ (Elabscience®, ΗΠΑ) σύμφωνα με το πρωτόκολλο του κατασκευαστή. Οι μετρήσεις πραγματοποιήθηκαν σε

φασματοφωτόμετρο ELISA (Thermo Scientific Multiskan GO Microplate Spectrophotometer).

Ιστολογική προετοιμασία

Πριν την ανάκτηση των αισθήσεών τους, τα πειραματόζωα θυσιάστηκαν. Τα παρασκευάσματα διατηρήθηκαν σε διάλυμα φορμαλίνης 10%. Στη συνέχεια, ο αριστερός κόνδυλος από κάθε κάτω γνάθο απομονώθηκε και επεξεργάστηκε περαιτέρω ιστολογικά. Οι τομές χρωματίστηκαν με αιματοξυλίνη και ηωσίνη (ΗΕ) για να παρατηρηθούν πιθανές ιστομορφολογικές αλλαγές, τόσο στο οστό του κονδύλου της κάτω γνάθου, όσο και στη χόνδρινη επιφάνειά του.

Στατιστική επεξεργασία

Ο αριθμός των πειραματόζωων, έπρεπε να είναι μεν μικρός για ηθικούς λόγου, ικανός όμως ώστε να εντοπιστούν αξιόπιστα στατιστικά αποτελέσματα. Ο υπολογισμός του δείγματος έγινε χρησιμοποιώντας ανάλυση ισχύος.

Ο αριθμός των 12 επίμυων ανά υποομάδα υπολογίστηκε χρησιμοποιώντας τυπικά στατιστικά κριτήρια (a = 0,05, b = 0,10), αποδίδοντας ισχύ 90% για την ανίχνευση διαφοράς 0,5 mm (26,5 έναντι 27,0 SD 0,37) για το πρωταρχικό αποτέλεσμα της μελέτης, δηλαδή το μήκος της κάτω γνάθο (Condylion - I'). Ως εκ τούτου, χρησιμοποιήθηκαν συνολικά 72 επίμυες, κατανεμημένοι ισόρροπα σε πειραματική και ομάδα ελέγχου.

Διενεργήθηκαν γραμμικά μοντέλα παλινδρόμησης, ANOVA ή Kruskal-Wallis στατιστικές δοκιμασίες. Ορίστηκε α = 5% επίπεδο στατιστικής σημαντικότητας (p − value < 0.05). Τα δεδομένα επεξεργάστηκαν και αναλύθηκαν χρησιμοποιώντας το λογισμικό στατιστικής Stata ver.14 (Stata Statistical Software: Release 14. College Station, TX: StataCorp LP.)

Αποτελέσματα

Από την τρισδιάστατη ακτινογραφική ανάλυση, οι μετρήσεις αποκάλυψαν σημαντικές διαφορές στις προσθιοπίσθιες διαστάσεις μεταξύ των πειραματικών υποομάδων και των υποομάδων ελέγχου. Ωστόσο, οι παρατηρούμενες αλλαγές στις κατακόρυφες μετρήσεις, Condylion/Go' - Menton και στη διακονδυλική απόσταση, δε βρέθηκαν στατιστικά σημαντικές. Επίσης, δεν εντοπίστηκαν στατιστικά σημαντικές διαφορές στη βιοχημική ανάλυση, αναφορικά στα επίπεδα OPG, RANKL και MCSF. Επιπλέον, από την ιστολογική ανάλυση, προέκυψαν σημαντικές ιστομορφομετρικές διαφορές στον κόνδυλο της κάτω γνάθου. Συγκεκριμένα, η αναλογία Bone Surface/Total Surface (Επιφάνεια οστού/Συνολική επιφάνεια) βρέθηκε μειωμένη τόσο στην πρόσθια, όσο και στην οπίσθια περιοχή του κονδύλου. Σημαντική μείωση, ανιχνεύτηκε και στο πάχος της οπίσθιας χόνδρινης επιφάνειας του κονδύλου.

Συμπεράσματα

1. Η οπίσθια μετατόπιση της κάτω γνάθου σε αναπτυσσόμενους επίμυες αλλάζει τη μορφολογία της κάτω γνάθου και έχει ως αποτέλεσμα την ανάπτυξη μικρότερης κάτω γνάθου στην ενηλικίωση.
2. Η συγκεκριμένη ορθοδοντική/ορθοπεδική παρέμβαση δεν αποδείχτηκε να επηρεάζει τα επίπεδα των μελετηϊνών πρωτεϊνών στη συστηματική κυκλοφορία.
3. Ιστολογικά, βρέθηκε να τροποποιείται η αρχιτεκτονική του οστού του κονδύλου και της χόνδρινης επιφάνειάς του.

Η παρούσα πειραματική μελέτη έδωσε έμφαση στη μακροπρόθεσμη σταθερότητα των αποτελεσμάτων, αποκαλύπτοντας ότι η κάτω γνάθος δεν εμφανίζει υποτροπή αύξησης μετά τη θεραπεία.

Λέξεις – κλειδιά: Αύξηση κάτω γνάθου, οπίσθα μετατόπιση κάτω γνάθου, μήκος κάτω γνάθου, αύξηση κονδύλου, τρισδιάστατη ανάλυση, επίμυες, IIIη Σκελετική τάξη, ορθοδοντική θεραπεία, Οστεοπρωτεγερίνη, Συνδέτης του ενεργοποιητή του υποδοχέα του πυρηνικού παράγοντα ΚΒ, Παράγοντα διέγερσης αποικιών μακροφάγων, πάχος χόνδρινης επιφάνειας κονδύλου

English abstract

Objective

To investigate changes (radiographic, biochemical, histological) of the condyle and the mandible in rats that have undergone restriction in the mandibular growth through mechanical loading with an orthodontic / orthopedic device compared to rats without using the device.

Materials and Methods

Seventy-two Wistar rats were used in this study. They were divided into two equal groups of thirty-six rats each. Each group consisted of 3 subgroups. For each experimental subgroup there was a corresponding control subgroup, with no devices. The first day of the experiment was the 30th day of life of the experimental animals and the total duration of the experiment was 90 days. The animals were sacrificed after 30 (subgroups A1, B1), 60 (subgroups A2, B2) and 90 (subgroups A3, B3) days from the start of the experiment. On the 60th day of the experiment the orthodontic devices were removed from the remaining animals. During the whole experimental period, rats were fed mashed food. Cone beam computed tomographies were used for three-dimensional analysis. Blood samples were collected from the eye. Enzyme-linked immunosorbent assay (ELISA) kits were used to determine OPG, RANKL and MCSF levels in the blood serum. After sacrifice, the mandibular condyles were isolated and examined histologically.

Results

From the three-dimensional radiographic analysis, measurements revealed significant differences in the anteroposterior dimensions between experimental and control subgroups. However, the observed changes in the vertical dimensions, Condylion/Go' - Menton and the Intercondylar

distance proved insignificant. No statistically significant changes by group or timing were found in the levels of OPG, RANKL and MCSF. Histological analysis revealed significant histomorphometric differences in the mandibular condyle. In particular, the Bone Surface / Total Surface ratio was found diminished in both the anterior and posterior condylar regions. Lastly, a significant reduction was found in the Posterior Condylar Cartilage Thickness.

Conclusions

1. Posterior mandibular displacement of the mandible in growing rats affects the morphology of the mandible and culminates in the development of a smaller mandible at a grown age.
2. The orthodontic / orthopedic intervention was not shown to affecting the levels of the studied proteins in the systemic circulation.
3. Posterior mandibular displacement can cause histological region-specific changes in the microarchitecture of the condylar bone and cartilage thickness.

The present study emphasized the long-term stability of the outcomes, revealing that the mandible does not show catch-up growth following treatment.

Key – words:, mandibular growth; mandibular posterior displacement; mandibular length; condylar growth; rat; class III malocclusion; orthodontic treatment, Osteoprotegerin, RANKL, MCSF, condylar cartilage thickness

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9. APPENDIX

Supplementary Table 1. Lin's concordance correlation coefficient (rc), Mean difference and 95% LOA (Limits of Agreement) Bland–Altman between 1st και 2nd measurement of 1st observer regarding the initial measurements of the experimental group.

	rc	p-value	Mean diff.	95% LOA
Go' - Menton Initial R A	0.994	<0.001	-0.014	(-0.177, 0.149)
Go - Menton Initial R A	0.992	<0.001	-0.022	(-0.156, 0.111)
Go' - Menton Initial L A	0.996	<0.001	-0.008	(-0.136, 0.119)
Go - Menton Initial L A	0.988	<0.001	0.019	(-0.142, 0.181)
Coronoid - Menton Initial R A	0.991	<0.001	0.017	(-0.120, 0.153)
Coronoid - Menton Initial L A	0.994	<0.001	0.019	(-0.094, 0.132)
Condylion/Go' - Menton Initial R A	0.944	<0.001	-0.006	(-0.249, 0.238)
Condylion/Go' - Menton Initial L A	0.948	<0.001	0.031	(-0.188, 0.249)
Condylion - Go' Initial R A	0.963	<0.001	-0.008	(-0.204, 0.187)
Condylion - Go' Initial L A	0.970	<0.001	0.017	(-0.162, 0.195)
Condylion - Menton Initial R A	0.934	<0.001	-0.050	(-0.383, 0.283)
Condylion - Menton Initial L A	0.979	<0.001	-0.008	(-0.192, 0.175)
Condylion - Id Initial R A	0.873	<0.001	-0.061	(-0.521, 0.399)
Condylion - Id Initial L A	0.987	<0.001	-0.003	(-0.147, 0.142)
Condylion - I' Initial R A	0.909	<0.001	-0.042	(-0.456, 0.373)
Condylion - I' Initial L A	0.989	<0.001	0.000	(-0.133, 0.133)
Incisal - Id Initial R A	0.989	<0.001	0.008	(-0.090, 0.106)
Incisal - Id Initial L A	0.988	<0.001	-0.017	(-0.127, 0.093)
Incisal - I' Initial R A	0.989	<0.001	0.011	(-0.067, 0.089)
Incisal - I' Initial L A	0.997	<0.001	-0.006	(-0.051, 0.040)
Intercondylar Initial A	0.736	<0.001	-0.15	(-0.666, 0.366)

*R=Right, L=Left, A=Group A

Supplementary Table 2. Lin's concordance correlation coefficient (rc), Mean difference and 95% LOA (Limits of Agreement) Bland–Altman between 1st και 2nd measurement of 1st observer regarding the final measurements of the experimental group.

	rc	p-value	Mean diff.	95% LOA
Go' - Menton Final R A	0.998	<0.001	-0.006	(-0.120, 0.109)
Go - Menton Final R A	0.997	<0.001	0.008	(-0.135, 0.152)
Go' - Menton Final L A	0.995	<0.001	-0.006	(-0.167, 0.156)
Go - Menton Final L A	0.998	<0.001	0.011	(-0.111, 0.133)
Coronoid - Menton Final R A	0.997	<0.001	0.022	(-0.127, 0.171)
Coronoid - Menton Final L A	0.996	<0.001	0.056	(-0.096, 0.207)
Condylion/Go' - Menton Final R A	0.981	<0.001	0.036	(-0.158, 0.230)
Condylion/Go' - Menton Final L A	0.973	<0.001	0.044	(-0.242, 0.331)
Condylion - Go' Final R A	0.986	<0.001	0.050	(-0.116, 0.216)
Condylion - Go' Final L A	0.982	<0.001	0.064	(-0.193, 0.321)
Condylion - Menton Final R A	0.997	<0.001	-0.003	(-0.162, 0.156)
Condylion - Menton Final L A	0.997	<0.001	0.017	(-0.135, 0.168)
Condylion - Id Final R A	0.998	<0.001	0.011	(-0.111, 0.133)
Condylion - Id Final L A	0.998	<0.001	0.006	(-0.142, 0.153)
Condylion - I' Final R A	0.998	<0.001	0.019	(-0.094, 0.132)
Condylion - I' Final L A	0.996	<0.001	0.039	(-0.118, 0.196)
Incisal - Id Final R A	1.000	<0.001	0.006	(-0.060, 0.071)
Incisal - Id Final L A	1.000	<0.001	-0.014	(-0.130, 0.102)
Incisal - I' Final R A	1.000	<0.001	-0.003	(-0.060, 0.054)
Incisal - I' Final L A	1.000	<0.001	0.000	(0.000, 0.000)
Intercondylar Final A	0.816	<0.001	-0.183	(-0.529, 0.163)

*R=Right, L=Left, A=Group A

Supplementary Table 3. Lin's concordance correlation coefficient (rc), Mean difference and 95% LOA (Limits of Agreement) Bland–Altman between 1st και 2nd measurement of 1st observer regarding the initial measurements of the control group.

	rc	p-value	Mean diff.	95% LOA
Go' - Menton Initial R B	0.970	<0.001	-0.011	(-0.299, 0.277)
Go - Menton Initial R B	0.980	<0.001	0.011	(-0.217, 0.240)
Go' - Menton Initial L B	0.985	<0.001	-0.022	(-0.204, 0.160)
Go - Menton Initial L B	0.984	<0.001	-0.011	(-0.209, 0.186)
Coronoid - Menton Initial R B	0.991	<0.001	0.014	(-0.149, 0.177)
Coronoid - Menton Initial L B	0.989	<0.001	0.014	(-0.149, 0.177)
Condylion/Go' - Menton Initial R B	0.985	<0.001	0.019	(-0.120, 0.159)
Condylion/Go' - Menton Initial L B	0.981	<0.001	0.011	(-0.150, 0.172)
Condylion - Go' Initial R B	0.982	<0.001	0.022	(-0.119, 0.164)
Condylion - Go' Initial L B	0.992	<0.001	0.011	(-0.091, 0.114)
Condylion - Menton Initial R B	0.982	<0.001	-0.003	(-0.220, 0.214)
Condylion - Menton Initial L B	0.986	<0.001	-0.014	(-0.208, 0.180)
Condylion - Id Initial R B	0.996	<0.001	-0.003	(-0.122, 0.117)
Condylion - Id Initial L B	0.994	<0.001	-0.014	(-0.170, 0.143)
Condylion - I' Initial R B	0.993	<0.001	0.006	(-0.142, 0.153)
Condylion - I' Initial L B	0.990	<0.001	0.017	(-0.162, 0.195)
Incisal - Id Initial R B	0.983	<0.001	-0.017	(-0.161, 0.128)
Incisal - Id Initial L B	0.990	<0.001	0.006	(-0.109, 0.120)
Incisal - I' Initial R B	0.991	<0.001	-0.011	(-0.102, 0.080)
Incisal - I' Initial L B	0.992	<0.001	-0.011	(-0.102, 0.080)
Intercondylar Initial B	0.931	<0.001	-0.014	(-0.401, 0.373)

*R=Right, L=Left, B=Group B

Supplementary Table 4. Lin's concordance correlation coefficient (rc), Mean difference and 95% LOA (Limits of Agreement) Bland–Altman between 1st και 2nd measurement of 1st observer regarding the final measurements of the control group.

	rc	p-value	Mean diff.	95% LOA
Go' - Menton Final R B	0.996	<0.001	0.039	(-0.156, 0.234)
Go - Menton Final R B	0.998	<0.001	0.003	(-0.125, 0.131)
Go' - Menton Final L B	0.997	<0.001	0.011	(-0.181, 0.203)
Go - Menton Final L B	0.997	<0.001	-0.011	(-0.225, 0.202)
Coronoid - Menton Final R B	0.998	<0.001	0.022	(-0.084, 0.128)
Coronoid - Menton Final L B	0.998	<0.001	0.028	(-0.101, 0.157)
Condylion/Go' - Menton Final R B	0.974	<0.001	-0.053	(-0.351, 0.245)
Condylion/Go' - Menton Final L B	0.976	<0.001	0.011	(-0.375, 0.397)
Condylion - Go' Final R B	0.965	<0.001	-0.061	(-0.393, 0.271)
Condylion - Go' Final L B	0.977	<0.001	0.017	(-0.345, 0.378)
Condylion - Menton Final R B	0.999	<0.001	-0.008	(-0.106, 0.090)
Condylion - Menton Final L B	0.995	<0.001	-0.006	(-0.204, 0.193)
Condylion - Id Final R B	0.999	<0.001	-0.003	(-0.113, 0.107)
Condylion - Id Final L B	0.998	<0.001	-0.017	(-0.153, 0.120)
Condylion - I' Final R B	0.999	<0.001	0.003	(-0.117, 0.122)
Condylion - I' Final L B	0.995	<0.001	-0.044	(-0.261, 0.173)
Incisal - Id Final R B	0.995	<0.001	-0.017	(-0.168, 0.135)
Incisal - Id Final L B	0.997	<0.001	0.006	(-0.118, 0.129)
Incisal - I' Final R B	0.996	<0.001	-0.014	(-0.109, 0.082)
Incisal - I' Final L B	0.995	<0.001	0.008	(-0.100, 0.117)
Intercondylar Final B	0.913	<0.001	-0.111	(-0.421, 0.199)

*R=Right, L=Left, B=Group B

Supplementary Table 5. Lin's concordance correlation coefficient (rc), Mean difference and 95% LOA (Limits of Agreement) Bland–Altman between 1st και 2nd measurement of 2nd observer regarding the initial measurements of the experimental group.

	rc	p-value	Mean diff.	95% LOA
Go' - Menton Initial R A	0.965	<0.001	-0.017	(-0.399, 0.365)
Go - Menton Initial R A	0.979	<0.001	0.028	(-0.190, 0.246)
Go' - Menton Initial L A	0.986	<0.001	0.008	(-0.241, 0.258)
Go - Menton Initial L A	0.979	<0.001	0.006	(-0.209, 0.220)
Coronoid - Menton Initial R A	0.985	<0.001	0.017	(-0.162, 0.195)
Coronoid - Menton Initial L A	0.982	<0.001	0.006	(-0.193, 0.204)
Condylion/Go' - Menton Initial R A	0.962	<0.001	0.000	(-0.193, 0.193)
Condylion/Go' - Menton Initial L A	0.907	<0.001	-0.078	(-0.331, 0.175)
Condylion - Go' Initial R A	0.966	<0.001	0.031	(-0.137, 0.198)
Condylion - Go' Initial L A	0.952	<0.001	-0.058	(-0.254, 0.137)
Condylion - Menton Initial R A	0.961	<0.001	0.025	(-0.237, 0.287)
Condylion - Menton Initial L A	0.973	<0.001	0.019	(-0.184, 0.223)
Condylion - Id Initial R A	0.984	<0.001	0.006	(-0.156, 0.167)
Condylion - Id Initial L A	0.979	<0.001	0.033	(-0.148, 0.215)
Condylion - I' Initial R A	0.977	<0.001	-0.031	(-0.228, 0.167)
Condylion - I' Initial L A	0.972	<0.001	0.031	(-0.183, 0.244)
Incisal - Id Initial R A	0.974	<0.001	-0.003	(-0.162, 0.156)
Incisal - Id Initial L A	0.989	<0.001	0.014	(-0.092, 0.120)
Incisal - I' Initial R A	0.991	<0.001	0.003	(-0.071, 0.077)
Incisal - I' Initial L A	0.980	<0.001	-0.003	(-0.113, 0.107)
Intercondylar Initial A	0.843	<0.001	-0.006	(-0.432, 0.421)

*R=Right, L=Left, A=Group A

Supplementary Table 6. Lin's concordance correlation coefficient (rc), Mean difference and 95% LOA (Limits of Agreement) Bland–Altman between 1st και 2nd measurement of 2nd observer regarding the final measurements of the experimental group.

	rc	p-value	Mean diff.	95% LOA
Go' - Menton Final R A	0.992	<0.001	0.022	(-0.198, 0.243)
Go - Menton Final R A	0.994	<0.001	0.028	(-0.185, 0.240)
Go' - Menton Final L A	0.989	<0.001	0.044	(-0.173, 0.261)
Go - Menton Final L A	0.993	<0.001	0.031	(-0.193, 0.254)
Coronoid - Menton Final R A	0.993	<0.001	0.039	(-0.177, 0.255)
Coronoid - Menton Final L A	0.994	<0.001	0.044	(-0.151, 0.240)
Condylion/Go' - Menton Final R A	0.985	<0.001	-0.050	(-0.202, 0.102)
Condylion/Go' - Menton Final L A	0.981	<0.001	-0.064	(-0.274, 0.146)
Condylion - Go' Final R A	0.987	<0.001	-0.056	(-0.207, 0.096)
Condylion - Go' Final L A	0.985	<0.001	-0.078	(-0.272, 0.116)
Condylion - Menton Final R A	0.993	<0.001	0.008	(-0.228, 0.244)
Condylion - Menton Final L A	0.994	<0.001	0.006	(-0.204, 0.215)
Condylion - Id Final R A	0.996	<0.001	-0.033	(-0.221, 0.154)
Condylion - Id Final L A	0.996	<0.001	-0.014	(-0.214, 0.186)
Condylion - I' Final R A	0.997	<0.001	-0.022	(-0.185, 0.141)
Condylion - I' Final L A	0.997	<0.001	-0.014	(-0.163, 0.135)
Incisal - Id Final R A	0.999	<0.001	0.019	(-0.173, 0.212)
Incisal - Id Final L A	1.000	<0.001	0.025	(-0.093, 0.143)
Incisal - I' Final R A	1.000	<0.001	0.014	(-0.111, 0.139)
Incisal - I' Final L A	1.000	<0.001	0.000	(-0.081, 0.081)
Intercondylar Final A	0.907	<0.001	0.050	(-0.293, 0.393)

*R=Right, L=Left, A=Group A

Supplementary Table 7. Lin's concordance correlation coefficient (rc), Mean difference and 95% LOA (Limits of Agreement) Bland–Altman between 1st και 2nd measurement of 2nd observer regarding the initial measurements of the control group.

	rc	p-value	Mean diff.	95% LOA
Go' - Menton Initial R B	0.992	<0.001	0.003	(-0.142, 0.147)
Go - Menton Initial R B	0.987	<0.001	0.000	(-0.193, 0.193)
Go' - Menton Initial L B	0.981	<0.001	-0.006	(-0.215, 0.204)
Go - Menton Initial L B	0.973	<0.001	0.014	(-0.243, 0.271)
Coronoid - Menton Initial R B	0.989	<0.001	0.006	(-0.176, 0.187)
Coronoid - Menton Initial L B	0.986	<0.001	-0.003	(-0.193, 0.187)
Condylion/Go' - Menton Initial R B	0.966	<0.001	-0.019	(-0.238, 0.199)
Condylion/Go' - Menton Initial L B	0.957	<0.001	-0.028	(-0.269, 0.214)
Condylion - Go' Initial R B	0.979	<0.001	0.000	(-0.169, 0.169)
Condylion - Go' Initial L B	0.976	<0.001	-0.031	(-0.205, 0.144)
Condylion - Menton Initial R B	0.988	<0.001	0.019	(-0.161, 0.200)
Condylion - Menton Initial L B	0.976	<0.001	0.008	(-0.250, 0.267)
Condylion - Id Initial R B	0.988	<0.001	0.011	(-0.192, 0.214)
Condylion - Id Initial L B	0.989	<0.001	0.011	(-0.186, 0.209)
Condylion - I' Initial R B	0.980	<0.001	0.033	(-0.201, 0.268)
Condylion - I' Initial L B	0.978	<0.001	0.050	(-0.196, 0.296)
Incisal - Id Initial R B	0.965	<0.001	0.036	(-0.164, 0.236)
Incisal - Id Initial L B	0.975	<0.001	0.019	(-0.161, 0.200)
Incisal - I' Initial R B	0.989	<0.001	0.014	(-0.082, 0.109)
Incisal - I' Initial L B	0.993	<0.001	-0.003	(-0.090, 0.085)
Intercondylar Initial B	0.839	<0.001	0.050	(-0.493, 0.593)

*R=Right, L=Left, B=Group B

Supplementary Table 8. Lin's concordance correlation coefficient (rc), Mean difference and 95% LOA (Limits of Agreement) Bland–Altman between 1st και 2nd measurement of 2nd observer regarding the final measurements of the control group.

	rc	p-value	Mean diff.	95% LOA
Go' - Menton Final R B	0.995	<0.001	0.003	(-0.214, 0.220)
Go - Menton Final R B	0.994	<0.001	0.003	(-0.243, 0.248)
Go' - Menton Final L B	0.998	<0.001	-0.036	(-0.193, 0.120)
Go - Menton Final L B	0.998	<0.001	-0.022	(-0.192, 0.147)
Coronoid - Menton Final R B	0.996	<0.001	0.011	(-0.181, 0.203)
Coronoid - Menton Final L B	0.997	<0.001	-0.031	(-0.185, 0.124)
Condylion/Go' - Menton Final R B	0.986	<0.001	-0.036	(-0.266, 0.194)
Condylion/Go' - Menton Final L B	0.986	<0.001	-0.039	(-0.329, 0.251)
Condylion - Go' Final R B	0.985	<0.001	-0.033	(-0.272, 0.206)
Condylion - Go' Final L B	0.987	<0.001	-0.031	(-0.299, 0.238)
Condylion - Menton Final R B	0.996	<0.001	0.003	(-0.187, 0.193)
Condylion - Menton Final L B	0.991	<0.001	-0.050	(-0.313, 0.213)
Condylion - Id Final R B	0.998	<0.001	-0.008	(-0.173, 0.156)
Condylion - Id Final L B	0.995	<0.001	-0.011	(-0.235, 0.212)
Condylion - I' Final R B	0.997	<0.001	0.031	(-0.131, 0.192)
Condylion - I' Final L B	0.995	<0.001	-0.014	(-0.244, 0.216)
Incisal - Id Final R B	0.995	<0.001	-0.003	(-0.175, 0.169)
Incisal - Id Final L B	0.999	<0.001	-0.011	(-0.089, 0.067)
Incisal - I' Final R B	0.990	<0.001	-0.017	(-0.176, 0.142)
Incisal - I' Final L B	0.997	<0.001	0.000	(-0.094, 0.094)
Intercondylar Final B	0.775	<0.001	-0.083	(-0.711, 0.544)

*R=Right, L=Left, B=Group B

Supplementary Table 9. Lin's concordance correlation coefficient (rc), Mean difference and 95% LOA (Limits of Agreement) Bland–Altman between 1st και 2nd observer regarding the initial measurements of the experimental group.

	rc	p-value	Mean diff.	95% LOA
Go' - Menton Initial R A	0.991	<0.001	-0.042	(-0.225, 0.142)
Go - Menton Initial R A	0.981	<0.001	-0.061	(-0.238, 0.116)
Go' - Menton Initial L A	0.991	<0.001	-0.022	(-0.227, 0.183)
Go - Menton Initial L A	0.988	<0.001	-0.011	(-0.172, 0.150)
Coronoid - Menton Initial R A	0.988	<0.001	-0.039	(-0.182, 0.104)
Coronoid - Menton Initial L A	0.992	<0.001	0.006	(-0.127, 0.138)
Condylion/Go' - Menton Initial R A	0.954	<0.001	-0.044	(-0.246, 0.157)
Condylion/Go' - Menton Initial L A	0.936	<0.001	0.006	(-0.238, 0.249)
Condylion - Go' Initial R A	0.959	<0.001	-0.039	(-0.228, 0.150)
Condylion - Go' Initial L A	0.971	<0.001	0.003	(-0.176, 0.181)
Condylion - Menton Initial R A	0.967	<0.001	-0.047	(-0.279, 0.185)
Condylion - Menton Initial L A	0.984	<0.001	-0.014	(-0.170, 0.143)
Condylion - Id Initial R A	0.980	<0.001	-0.011	(-0.197, 0.175)
Condylion - Id Initial L A	0.986	<0.001	-0.011	(-0.165, 0.143)
Condylion - I' Initial R A	0.976	<0.001	0.031	(-0.178, 0.239)
Condylion - I' Initial L A	0.987	<0.001	-0.006	(-0.153, 0.142)
Incisal - Id Initial R A	0.979	<0.001	0.022	(-0.111, 0.156)
Incisal - Id Initial L A	0.989	<0.001	-0.014	(-0.120, 0.092)
Incisal - I' Initial R A	0.993	<0.001	0.000	(-0.066, 0.066)
Incisal - I' Initial L A	0.997	<0.001	-0.006	(-0.051, 0.040)
Intercondylar Initial A	0.786	<0.001	-0.025	(-0.539, 0.489)

*R=Right, L=Left, A=Group A

Supplementary Table 10. Lin's concordance correlation coefficient (rc), Mean difference and 95% LOA (Limits of Agreement) Bland–Altman between 1ˢᵗ και 2ⁿᵈ observer regarding the final measurements of the experimental group.

	rc	p-value	Mean diff.	95% LOA
Go' - Menton Final R A	0.994	<0.001	-0.011	(-0.214, 0.192)
Go - Menton Final R A	0.995	<0.001	-0.006	(-0.198, 0.187)
Go' - Menton Final L A	0.998	<0.001	0.003	(-0.096, 0.102)
Go - Menton Final L A	0.996	<0.001	0.011	(-0.163, 0.185)
Coronoid - Menton Final R A	0.995	<0.001	-0.011	(-0.209, 0.186)
Coronoid - Menton Final L A	0.997	<0.001	0.028	(-0.125, 0.180)
Condylion/Go' - Menton Final R A	0.980	0	0.053	(-0.132, 0.237)
Condylion/Go' - Menton Final L A	0.986	0	0.039	(-0.161, 0.239)
Condylion - Go' Final R A	0.986	0	0.053	(-0.113, 0.218)
Condylion - Go' Final L A	0.992	0	0.047	(-0.118, 0.213)
Condylion - Menton Final R A	0.995	<0.001	-0.017	(-0.218, 0.185)
Condylion - Menton Final L A	0.996	<0.001	0.014	(-0.162, 0.190)
Condylion - Id Final R A	0.997	<0.001	0.022	(-0.141, 0.185)
Condylion - Id Final L A	0.998	<0.001	0.017	(-0.128, 0.161)
Condylion - I' Final R A	0.998	<0.001	0.031	(-0.100, 0.162)
Condylion - I' Final L A	0.996	<0.001	0.053	(-0.106, 0.212)
Incisal - Id Final R A	0.999	<0.001	-0.011	(-0.165, 0.143)
Incisal - Id Final L A	1.000	<0.001	-0.019	(-0.132, 0.094)
Incisal - I' Final R A	1.000	<0.001	-0.008	(-0.094, 0.078)
Incisal - I' Final L A	1.000	<0.001	-0.008	(-0.081, 0.064)
Intercondylar Final A	0.821	<0.001	-0.189	(-0.529, 0.151)

*R=Right, L=Left, A=Group A

Supplementary Table 11. Lin's concordance correlation coefficient (rc), Mean difference and 95% LOA (Limits of Agreement) Bland–Altman between 1st και 2nd observer regarding the initial measurements of the control group.

	rc	p-value	Mean diff.	95% LOA
Go' - Menton Initial R B	0.997	<0.001	-0.006	(-0.099, 0.087)
Go - Menton Initial R B	0.991	<0.001	0.008	(-0.150, 0.166)
Go' - Menton Initial L B	0.991	<0.001	0.000	(-0.148, 0.148)
Go - Menton Initial L B	0.988	<0.001	0.014	(-0.149, 0.177)
Coronoid - Menton Initial R B	0.993	<0.001	0.011	(-0.128, 0.150)
Coronoid - Menton Initial L B	0.992	<0.001	0.042	(-0.077, 0.160)
Condylion/Go' - Menton Initial R B	0.979	<0.001	-0.011	(-0.185, 0.163)
Condylion/Go' - Menton Initial L B	0.984	<0.001	0.044	(-0.083, 0.172)
Condylion - Go' Initial R B	0.984	<0.001	-0.014	(-0.156, 0.128)
Condylion - Go' Initial L B	0.987	<0.001	0.033	(-0.091, 0.157)
Condylion - Menton Initial R B	0.990	<0.001	0.000	(-0.162, 0.162)
Condylion - Menton Initial L B	0.990	<0.001	0.017	(-0.149, 0.182)
Condylion - Id Initial R B	0.991	<0.001	0.006	(-0.169, 0.181)
Condylion - Id Initial L B	0.993	<0.001	0.003	(-0.156, 0.162)
Condylion - I' Initial R B	0.991	<0.001	0.014	(-0.143, 0.170)
Condylion - I' Initial L B	0.991	<0.001	0.000	(-0.169, 0.169)
Incisal - Id Initial R B	0.980	<0.001	-0.022	(-0.178, 0.134)
Incisal - Id Initial L B	0.969	<0.001	-0.031	(-0.223, 0.162)
Incisal - I' Initial R B	0.998	<0.001	-0.006	(-0.051, 0.040)
Incisal - I' Initial L B	0.999	<0.001	-0.003	(-0.035, 0.030)
Intercondylar Initial B	0.925	<0.001	-0.075	(-0.420, 0.270)

*R=Right, L=Left, B=Group B

Supplementary Table 12. Lin's concordance correlation coefficient (rc), Mean difference and 95% LOA (Limits of Agreement) Bland–Altman between 1st και 2nd observer regarding the final measurements of the control group.

	rc	p-value	Mean diff.	95% LOA
Go' - Menton Final R B	0.996	<0.001	-0.058	(-0.223, 0.106)
Go - Menton Final R B	0.997	<0.001	-0.044	(-0.196, 0.107)
Go' - Menton Final L B	0.999	<0.001	-0.006	(-0.110, 0.099)
Go - Menton Final L B	0.999	<0.001	0.006	(-0.135, 0.146)
Coronoid - Menton Final R B	0.998	<0.001	-0.014	(-0.156, 0.128)
Coronoid - Menton Final L B	0.997	<0.001	0.014	(-0.143, 0.170)
Condylion/Go' - Menton Final R B	0.992	<0.001	-0.033	(-0.209, 0.142)
Condylion/Go' - Menton Final L B	0.989	<0.001	0.025	(-0.233, 0.283)
Condylion - Go' Final R B	0.992	<0.001	-0.022	(-0.192, 0.147)
Condylion - Go' Final L B	0.992	<0.001	0.022	(-0.198, 0.243)
Condylion - Menton Final R B	0.996	<0.001	-0.039	(-0.203, 0.125)
Condylion - Menton Final L B	0.992	<0.001	0.017	(-0.242, 0.275)
Condylion - Id Final R B	0.997	<0.001	-0.019	(-0.200, 0.161)
Condylion - Id Final L B	0.996	<0.001	0.008	(-0.203, 0.220)
Condylion - I' Final R B	0.998	<0.001	-0.008	(-0.136, 0.119)
Condylion - I' Final L B	0.996	<0.001	0.017	(-0.190, 0.224)
Incisal - Id Final R B	0.996	<0.001	0.000	(-0.155, 0.155)
Incisal - Id Final L B	0.999	<0.001	0.006	(-0.075, 0.086)
Incisal - I' Final R B	0.995	<0.001	0.011	(-0.102, 0.124)
Incisal - I' Final L B	1.000	<0.001	-0.003	(-0.035, 0.030)
Intercondylar Final B	0.825	<0.001	-0.078	(-0.634, 0.479)

*R=Right, L=Left, B=Group B

Veterinary Directorate approval form

a.

ΕΛΛΗΝΙΚΗ ΔΗΜΟΚΡΑΤΙΑ
ΠΕΡΙΦΕΡΕΙΑ ΑΤΤΙΚΗΣ
ΓΕΝΙΚΗ ΔΙΕΥΘΥΝΣΗ ΑΓΡΟΤΙΚΗΣ
ΟΙΚΟΝΟΜΙΑΣ, ΚΤΗΝΙΑΤΡΙΚΗΣ &
ΑΛΙΕΙΑΣ
Δ/ΝΣΗ ΑΓΡΟΤΙΚΗΣ &
ΚΤΗΝΙΑΤΡΙΚΗΣ ΠΟΛΙΤΙΚΗΣ
ΤΜΗΜΑ: ΚΑΦΕ
ΣΥΓΓΡΟΥ 80-88, 117 41 Αθήνα
ΠΛΗΡΟΦΟΡΙΕΣ: Χ. Φλώτσιος
Τηλέφωνο: 213 2065 751
Fax: 213 2065 020
e-mail: pandriopoulos@patt.gov.gr

Αθήνα, 4/10/2019

ΘΕΜΑ: «Χορήγηση άδειας πρωτοκόλλου διαδικασιών σε ζώα»

ΑΠΟΦΑΣΗ

Έχοντας υπ' όψη:

Α) Τις διατάξεις:

1. Του Ν.3852/2010 «Νέα Αρχιτεκτονική της Αυτοδιοίκησης και της Αποκεντρωμένης Διοίκησης- Πρόγραμμα Καλλικράτης» (ΦΕΚ 87/τ.Α'/7-6-2010)
2. Της υπ' αριθμ: 37419/13479/08-05-2018 Απόφασης του Συντονιστή της Αποκεντρωμένης Διοίκησης Αττικής, με την οποία εγκρίθηκε η υπ' αριθμ: 121/2018 Απόφαση του Περιφερειακού Συμβουλίου Περιφέρειας Αττικής (ΦΕΚ 1661 / τ.Β' / 11.05.2018), περί τροποποίησης – επικαιροποίησης του Οργανισμού Εσωτερικής Υπηρεσίας της Περιφέρειας Αττικής.
3. Του Ν. 1197/81 «Περί προστασίας ζώων» και συγκεκριμένα το άρθρο 4.
4. Του Ν. 2015/92 «Περί Κυρώσεως της Ευρωπαϊκής Σύμβασης για την Προστασία των σπονδυλωτών ζώων που χρησιμοποιούνται για πειραματικούς ή άλλους επιστημονικούς σκοπούς».
5. Του Π.Δ. 56/2013 Προσαρμογή της Ελληνικής νομοθεσίας στην Οδηγία 2010/63/ΕΕ του Ευρωπαϊκού Κοινοβουλίου και του Συμβουλίου της 22ας Σεπτεμβρίου 2010(276/33/20.10.2010) «σχετικά με την προστασία των ζώων που χρησιμοποιούνται για επιστημονικούς σκοπούς»

Β) Την με αριθ. πρωτ. **590801/2-10-2019** αίτηση του **κου Απόστολου Τσολάκη** (χειρούργου οδοντιάτρου) για πραγματοποίηση Πρωτοκόλλου διαδικασιών σε ζώα (**επίμυες**) με τον τίτλο: **«Ιστολογικές, απεικονιστικές και βιοχημικές μεταβολές της Κροταφογναθικής Διάρθρωσης σε επίμυες μετά από συμπιεστική φόρτιση στην κάτω γνάθο.»**, που θα διεξαχθεί στο **Εργαστήριο Πειραματικής Χειρουργικής και Χειρουργικής Έρευνας «Ν.Σ Χρηστέας»**, επί της οδού Αγίου Θωμά 15Β, Τ.Κ.11527, Αθήνα, Αττική, με κωδικό καταχώρησης **EL 25 ΒΙΟ 05.**

Γ) Την θετική γνωμάτευση της Επιτροπής Αξιολόγησης Πρωτοκόλλων.

ΑΠΟΦΑΣΙΖΟΥΜΕ

Χορηγούμε άδεια για την πραγματοποίηση του συγκεκριμένου Πρωτοκόλλου διαδικασιών σε ζώα από τον **κο Απόστολο Τσολάκη** (χειρούργο οδοντίατρο) ως **Υπεύθυνο του Πρωτοκόλλου** και τον **κο Ιωάννη Λύρο** (χειρούργο οδοντίατρο) ως **Υπεύθυνο Εκτέλεσης/Υλοποίησης του Πρωτοκόλλου** και ως **Υπεύθυνο Συμμόρφωσης προς την αδειοδότηση του Πρωτοκόλλου**, καθόσον πληρούνται οι προϋποθέσεις της σχετικής, για την Προστασία των ζώων, νομοθεσίας. **Λοιποί συμμετέχοντες και Υπεύθυνοι για την συνολική υλοποίηση του Πρωτοκόλλου** θα είναι: ο **κος Δημήτριος Χαλαζωνίτης** (Ορθοδοντικός) και η **κα Δέσποινα Περρέα** (Βιοχημικός)

Διανομή μέσω 'ΙΡΙΔΑ' με UID: 5d96f752425ebb5a50d61fcc στις 04/10/19 11:36

b.

Η άδεια αυτή ισχύει **για τρία χρόνια** από την ημερομηνία εκδόσεώς της.

Οποιαδήποτε μεταβολή, που αφορά τους υπευθύνους του πρωτοκόλλου, όπως αυτοί αναφέρονται παραπάνω, θα πρέπει να δηλώνονται στην υπηρεσία μας.

Ο υπεύθυνος του Πρωτοκόλλου **υποχρεούται σε αναδρομική αξιολόγηση** μετά το τέλος του πειραματισμού.

Οι χρησιμοποιούμενες φαρμακευτικές ή και λοιπές ουσίες για την διεξαγωγή του πρωτοκόλλου υπόκεινται, όσον αφορά την εισαγωγή και εν γένει κυκλοφορία τους, στις σχετικές κείμενες διατάξεις.

ΑΚΡΙΒΕΣ ΑΝΤΙΓΡΑΦΟ

ΜΙΧΑΛΟΠΟΝΗΣ ΑΘΑΝΑΣΙΟΣ

Ο Προϊστάμενος της Δ/νσης

Στυλιανός Μάργαρης

ΚΟΙΝΟΠΟΙΗΣΗ:

1. Υπουργείο Αγροτικής Ανάπτυξης & Τροφίμων

Γεν. Δ/νση Βιώσιμης Ζωικής Παραγωγής & Κτηνιατρικής

Δ/νση Προστασίας των Ζώων, Φαρμάκων και Κτηνιατρικών Εφαρμογών

Βερανζέρου 46, Τ.Κ.10438 Αθήνα

2. Δ/νση Αγρ. Οικονομίας & Κτηνιατρικής Περιφερειακής Ενότητας Κεντρικού Τομέα Αθηνών

Συγγρού 80-88 , Αθήνα, Τ.Κ.: 117 41

3. Επιτροπή Αξιολόγησης Πρωτοκόλλων του Εργαστηρίου Πειραματικής Χειρουργικής και Χειρουργικής Έρευνας «Ν.Σ Χρηστέας»

Αγίου Θωμά 15Β, Τ.Κ.11527, Αθήνα, Αττική

4. Επιτροπή Παρακολούθησης και Γνωμοδότησης για την Ευζωία των Ζώων του Εργαστήριου Πειραματικής Χειρουργικής και Χειρουργικής Έρευνας «Ν.Σ Χρηστέας»

Αγίου Θωμά 15Β, Τ.Κ.11527, Αθήνα, Αττική

5. α) κο Απόστολο Τσολάκη

β) κο Ιωάννη Λύρο

γ) κο Δημήτριο Χαλαζωνίτη

δ) κα Δέσποινα Περρέα

δια του Εργαστήριου Πειραματικής Χειρουργικής και Χειρουργικής Έρευνας «Ν.Σ Χρηστέας»

Αγίου Θωμά 15Β, Τ.Κ.11527, Αθήνα, Αττική

Διανομή μέσω 'ΙΡΙΔΑ' με UID: 5d96f752425ebb5a50d61fcc στις 04/10/19 11:36

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