

# **Temporomandibular joint disk displacement and subsequent adverse mandibular growth**

**A radiographic, histologic and biomolecular experimental study**

Fredrik Bryndahl



Department of Odontology, Oral and Maxillofacial Radiology  
Umeå University  
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Department of Odontology, Oral and Maxillofacial Radiology  
Umeå University  
SE-901 87 Umeå, Sweden

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*"A dwarf sees farther than the giant,  
when he has the giant's shoulder  
to mount on."*

*Coleridge (1772-1834)*

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# PREFACE

The thesis is based on the following papers which will be referred to in the text by their Roman numerals.

- I** Bryndahl F, Legrell PE, Eriksson L, Isberg A (2004). Titanium screw implants in optimization of radiographic evaluation of facial growth in longitudinal animal studies. *Angle Orthod* 74:610-617.
- II** Bryndahl F, Eriksson L, Legrell PE, Isberg A (2006). Bilateral TMJ disk displacement induces mandibular retrognathia. *J Dent Res* 85:1118-1123.
- III** Bryndahl F, Warfvinge G, Eriksson L, Isberg A (2008). Cartilage changes link retrognathic growth to TMJ disk displacement. *J Dent Res* In review.
- IV** Bryndahl F, Warfvinge G, Rabie ABM, Isberg A (2008). Subchondral bone resorption induced by TMJ disk displacement explains subsequent adverse mandibular growth. *Manuscript*.

Papers I and II are reproduced with kind permission of The Angle Orthodontist and Journal of Dental Research.

## ABSTRACT

The mandibular condyles represent important growth sites within the facial skeleton. Condylar growth is not a pacemaker of mandibular development, but it provides regional adaptive growth that is of considerable clinical significance, as the condyle's upward and backward growth movement regulates the anteriorly and inferiorly directed displacement of the mandible as a whole.

Orthopedic problems of the temporomandibular joint (TMJ), such as displacement of the TMJ disk, are common in the adolescent population. Clinical studies of mandibular asymmetry and mandibular retrognathia in adults as well as in children and adolescents, have reported an association with coexisting non-reducing displacement of the TMJ disk without identifying the cause and effect. Through experimental studies causality has been established, and unilateral affliction during growth has been shown to retard ipsilateral mandibular development with facial asymmetry as the sequel. It was hypothesized that bilateral non-reducing TMJ disk displacement during growth would impair mandibular development bilaterally, resulting in mandibular retrognathia. TMJ disk displacement has repeatedly been demonstrated to induce histological reactions of the condylar cartilage. An additional assumption was therefore that a non-deranged TMJ disk function is crucial for the maintenance of the growing condyle's biophysical environment, and that a connection ought to exist between the amount of condylar cartilage changes caused by TMJ disk displacement and the amount of subsequent adverse mandibular growth. It was also hypothesized that non-reducing displacement of the TMJ disk in growing individuals would result in qualitative and quantitative changes of the condylar subchondral bone.

An improved experimental cephalometric method was developed in order to optimize the reliability of longitudinal radiographic evaluation of fast growing small animals. Bilateral non-reducing TMJ disk displacement was surgically created in ten growing New Zealand White rabbits, with ten additional rabbits serving as a sham operated control group. The amount and direction of craniofacial growth was followed over time in serial cephalograms, aided by tantalum implants in the jaws. The study period was chosen to correspond to childhood and adolescence in man. The assessed growth of each side of the mandible was correlated to the histological feature of ipsilateral condylar cartilage at the end of the growth period. The amount and composition of subchondral bone from three

regions of interest in the condyle, and the expression of local growth factors in the adjacent condylar cartilage was evaluated.

The results verified that bilateral non-reducing TMJ disk displacement retarded mandibular growth bilaterally; the extent corresponding to mandibular retrognathia in man. Displacement of the TMJ disk during the growth period induced condylar cartilage adaptive reactions that were associated with both an adverse amount and direction of mandibular growth, manifesting in a retrognathic mandibular growth pattern. Growth impairment fluctuated over time, with the most striking retardation occurring during periods of increased general growth, implying a local growth reduction explicitly counteracting general hormonal growth acceleration. A significant decrease of the total amount of subchondral bone, in spite of a general increase of new bone formation in the experimental condyles, pointed to a reparative compensation for an extensive resorption of subchondral bone due to displacement of the TMJ disk, but not to the extent that normal growth would be maintained. These results constitute an explanation for the adverse mandibular development following non-reducing TMJ disk displacement in growing individuals.

This project has shown that non-reducing displacement of the TMJ disk during growth has significant consequences on facial development. The findings strongly advocate early and accurate diagnosis and treatment of TMJ disk displacement in the adolescent population, thereby presumably reducing the need for future orthodontic and surgical craniofacial corrective therapy. The results furthermore enhance the need for full appraisal of TMJ disk function in the adolescent population during orthodontic functional therapy, as the condylar cartilage and subchondral bone reactions to a concomitantly displaced non-reducing TMJ disk must be expected to interfere with the intended growth stimulating treatment. The findings of intact articular layers in spite of gross histological and morphological soft and hard tissue changes as a sequel to TMJ disk displacement in growing individuals, implicate a clinical risk of false positive radiographic diagnosis of degenerative changes of the TMJ in children and adolescents.

## DEFINITIONS AND ABBREVIATIONS

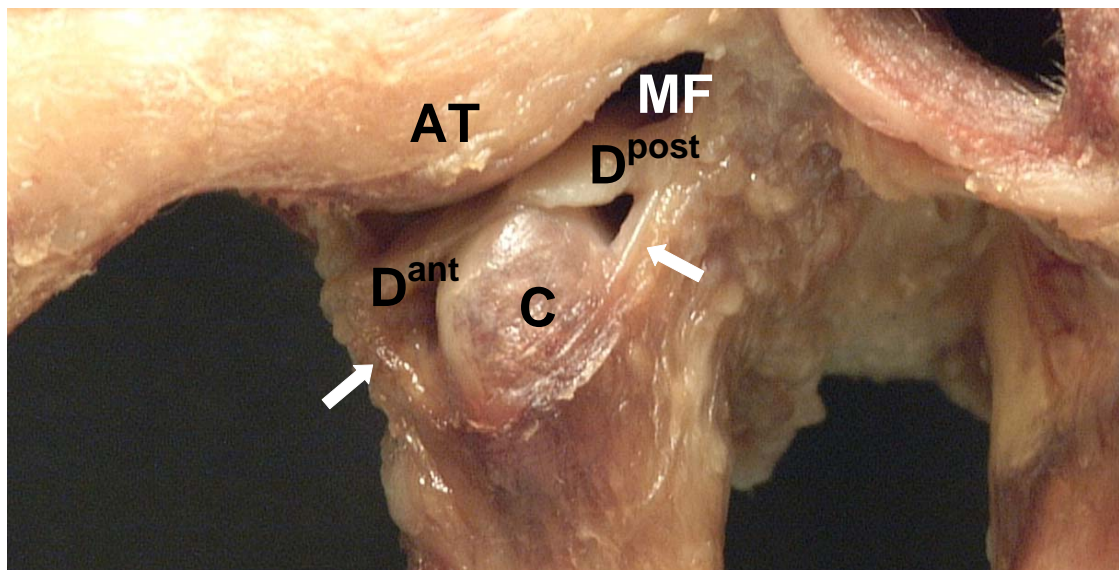
TMJ	Temporomandibular joint. The articulation between the mandible and the temporal bone of the facial skeleton.
MR imaging	Magnetic resonance imaging. Computerized radiographic technique that provides high definition tomographic soft tissue depiction without the use of ionizing radiation.
Cephalostat	Head fixation devise used to achieve congruent positioning of the patient when exposing serial skull radiographs.
Cephalogram	A skull radiograph achieved by aid of a cephalostat.
Cephalometry	Metric analyzes made on a cephalogram.
Superimposition	A technique where two radiographs are placed on top of each other for comparison.
Tantalum implants	Small metallic objects that, when inserted in the skeleton, can be used as easily identified landmarks in radiographs.
Mandibular asymmetry	When the two sides of the mandible are unequal in size. Often seen as a midline shift of the chin to the smaller side.
Mandibular retrognathia	When the mandible is too small in size to harmonize with the rest of the facial skeleton. Often seen as a development of a "bird face" with or without a sagittal overbite.
Functional appliances	Orthodontic devise intended to stimulate the growth of the mandible by protruding the mandible and displacing the condyle out of the mandibular fossa.
Condylar cartilage	The secondary fibrocartilage covering the bone surface of the condylar process of the mandible.
Subchondral bone	Bone beneath articular cartilage.
Endochondral bone formation	The formation of new bone deriving from growth activity in cartilage.
Appositional growth	Growth gained from new bone formation on top of already existing bone.



# INTRODUCTION

## ***The temporomandibular joint***

The craniomandibular articulation is unique as it involves two separate synovial joints that function in unison. The two facial bones that compose the temporomandibular joint (TMJ) are the mandible and the temporal bone, with the condylar process of the mandible articulating against the mandibular fossa of the temporal bone. The joint is defined by a capsule, consisting of an outer fibrous layer and an inner layer of synovial tissue. The articulating surfaces of the joint are covered with fibrocartilage and the joint has an intracapsular disk that divides the synovial cavity into two separate compartments (Petrén and Carlsöö, 1983; Isberg, 2001) (Fig. 1). In an adequately functioning joint, the disk fills the space between the two convex bony joint components during all functional jaw movements. The articular load is thereby dispersed over a surface, the size of the disk, with the viscoelastic behavior of the disk protecting the articular surfaces in areas susceptible to load concentrations (Koolstra and van Eijden, 2007).

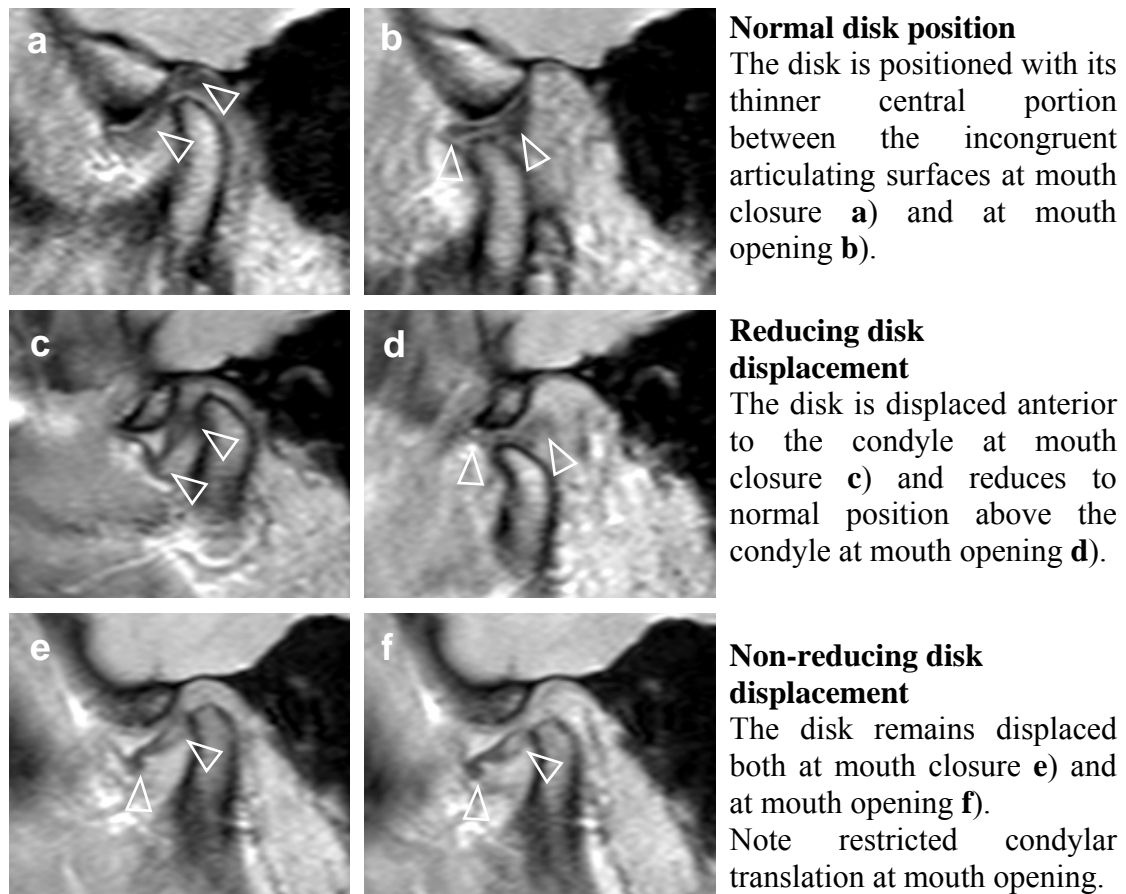


**Figure 1**

TMJ. Lateral parts of capsule and disk are removed. Arrows point to anterior and posterior capsule. (MF); mandibular fossa. Mandibular condyle (C) at protrusive translation beneath the articular tubercle (AT). The thick anterior (D<sup>ant</sup>) and posterior (D<sup>post</sup>) parts of the disk in normal functional position, with the thin central part interposed between the incongruous articulating bony joint components, the disk position thereby increasing the contact surface between the joint components.

## TMJ disk displacement

Orthopedic terminology defines internal derangement as interference of the normal smooth action of a joint by intra-articular tissue. By far the most common cause of TMJ internal derangement is displacement of the TMJ disk (Isacsson *et al.*, 1986; Paesani *et al.*, 1992). Displacement of the TMJ disk may occur in different directions, and may be either partial or complete, with the most common displacement being anterior or anterolateral (Tasaki *et al.*, 1996). If the displaced disk returns to a normal position relative to the condyle during jaw opening, the disk displacement is reducing in nature. If it remains in a faulty position during all jaw movements, the displacement is non-reducing (Isberg, 2001) (Fig. 2). Non-reducing TMJ disk displacement is often associated with impaired mouth opening ability, inflammatory and degenerative reactions in the joint, and is frequently associated with pain and dysfunction of the masticatory apparatus (Isacsson *et al.*, 1989; Larheim *et al.*, 2001a; 2001b; Tomas *et al.*, 2007).



**Figure 2**

MR-images illustrating TMJ disk displacement. Arrowheads mark the TMJ disk.

Displacement of the TMJ disk is common in the adolescent population. MR-imaging studies have reported a prevalence of non-reducing TMJ disk displacement amounting to approximately 4% in asymptomatic and about 60% in symptomatic children and adolescents (Ribeiro *et al.*, 1997). Bilateral affliction is common and reported in 50% of both these groups (Sanchez-Woodworth *et al.*, 1988; Ribeiro *et al.*, 1997). The onset of symptomatic TMJ disk displacement peaks during puberty in both sexes but there is a four times higher incidence among girls (Isberg *et al.*, 1998). More than 10% of the girls in a general pre-orthodontic population had non-reducing disk displacement verified with MR-imaging (Nebbe and Major, 2000).

### ***Craniofacial growth***

”Growth” is a general term implying that something changes in magnitude. However, it does not explain how it happens. Therefore the more descriptive term ”development” is often used to focus on the actual biological mechanisms that account for growth. A fundamental principle of craniofacial development is that the individual growth of each anatomical and morphological component of the face constantly interacts, working towards a functional and structural balance. If growth is disturbed or altered in any part of the craniofacial complex, the physiological equilibrium inevitably changes as well (Enlow and Hans, 1996).

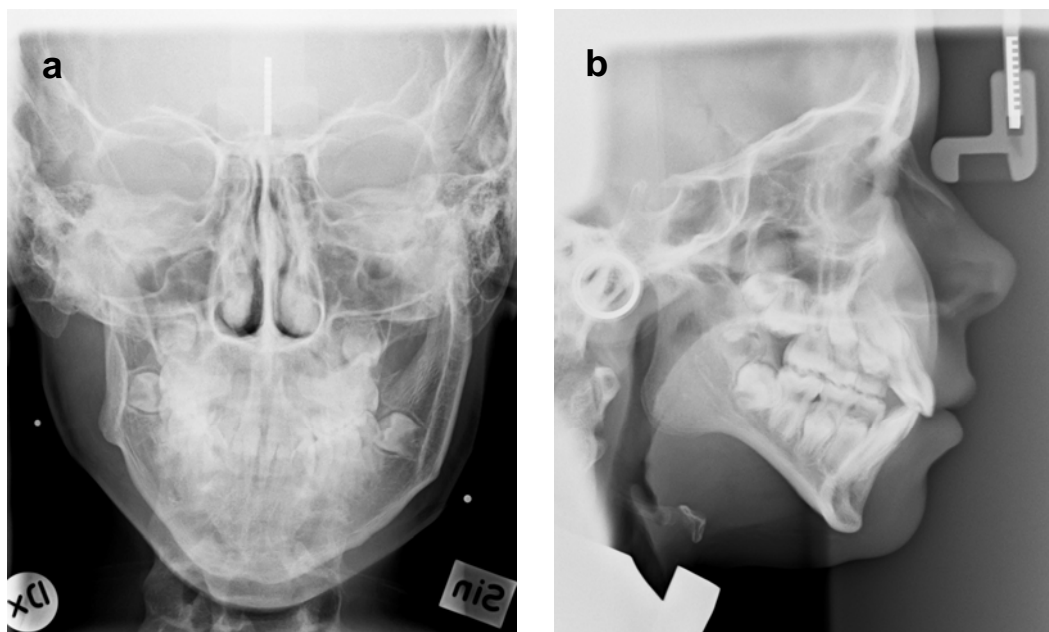
### ***Mandibular condylar growth***

The mandibular condyles represent important growth sites within the facial skeleton. Condylar growth does not set the pace of mandibular growth, but it provides regional adaptive growth that is of considerable clinical significance, as the condyle’s upward and backward growth movement, regulates the anteriorly and inferiorly directed displacement of the mandible as a whole (Enlow and Hans, 1996).

Unlike epiphyseal growth cartilage of the long bones, the mandibular condylar growth cartilage is a biologically unique articular cartilage with an exceptional capacity for adaptive modeling in response to external stimuli, both during and after natural growth (Shen and Darendeliler, 2005). The condylar cartilage is a secondary fibrocartilage derived from the periosteum of the membranous mandibular bone as a result of the functional demands of the evolutionary development of an articular process among mammals. The cellular activity of the cartilage is regulated by local growth factors (Leung *et al.*, 2004; Ng *et al.*, 2006), such as Vascular Endothelial Growth Factor (VEGF) and Indian Hedgehog (Ihh), and changes in the cartilage’s biophysical environment, trigger or impair their endogenous expression, leading to increased or decreased condylar growth (Chayanupatkul *et al.*, 2003; Shen

and Darendeliler, 2005). Due to its evolutionary origin, the endochondral growth of the condyle is appositional, and the adaptive growth capacity of the condylar cartilage is highly dependent on the articular function of the joint (Enlow and Hans, 1996; Meikle, 2007).

Because the functional condition of the joint is essential for the differentiation and maintenance of the condylar cartilage, trauma to the face and jaw during childhood or adolescence has been described as a disturbance of condylar growth and as a sequel also causes impairment of the overall mandibular development (Schellhas *et al.*, 1990; Skolnik *et al.*, 1994). Intra-articular disorders such as inflammatory diseases of the TMJ or degenerative bone changes are other afflictions of the TMJ known to result in adverse mandibular growth (Stabrun *et al.* 1988; Schellhas *et al.*, 1993; Kjellberg *et al.*, 1995). If one joint is affected, asymmetry of the mandibular dimensions can result in an underdeveloped mandible on the afflicted side. When both joints are affected, mandibular retrognathia can be expected (Fig.3).



**Figure 3**

Cephalograms illustrating mandibular asymmetry and mandibular retrognathia associated with TMJ affliction in growing individuals.

- a)** Unilateral non-reducing disk displacement in the right joint and lateral shift of the mandible towards the afflicted side (Dx).
- b)** Bilateral affliction of JIA (Juvenile Idiopathic Arthritis) and a retrognathic mandible.

## ***TMJ disk displacement and mandibular growth***

The orthopedic problem of a displaced TMJ disk without reduction is another intra-articular affliction shown to affect the growth of the mandible. Clinical studies of craniofacial asymmetry have reported an association with coexisting unilateral non-reducing TMJ disk displacement (Yamada *et al.*, 1999; Isberg and Legrell, 2000; Nakagawa *et al.*, 2002; Gidarakou *et al.*, 2003). Whether the adverse craniofacial growth predisposed for displacement of the TMJ disk or *vice versa*, remained unclear until the cause and effect was established in longitudinal experimental studies, verifying that non-reducing TMJ disk displacement with onset during the growth period induced subsequent ipsilateral impairment of mandibular growth (Legrell and Isberg, 1998; 1999).

## ***Hypothesis***

The fact that unilateral non-reducing TMJ disk displacement is shown to impair ipsilateral mandibular growth, with facial asymmetry as the consequence, made it likely that bilateral non-reducing TMJ disk displacement would affect the growth of the mandible on both sides and induce mandibular retrognathia. Several clinical studies of mandibular retrognathia support this assumption by reporting an association with coexisting bilateral non-reducing TMJ disk displacement in adults as well as in children and adolescents (Schellhas *et al.*, 1993; Nebbe *et al.*, 1998; Yamada *et al.*, 1999; Gidarakou *et al.*, 2004).

It has repeatedly been demonstrated that the altered articulating biomechanics following a faulty functioning TMJ disk causes histological reactions of the mandibular condylar cartilage (Ali and Sharawy, 1995; Legrell *et al.*, 1999; Long and Li, 2000). Any disk displacement results in histological reaction of the cartilage and there is a positive correlation between the amount of displacement and the degree of cartilage response (Berteretche *et al.*, 2001).

Based on the above issues, the fundamental basis of this project was that bilateral non-reducing displacement of the TMJ disk during growth would have significant consequences on facial development and mandibular growth in particular. It was hypothesized that the observed clinical association between mandibular retrognathia and bilateral non-reducing displacement of the TMJ disk would be connected through the subsequent histological reaction of the condylar cartilage. It was furthermore assumed that non-reducing displacement of the TMJ disk during the growth period would induce qualitative and quantitative changes of the condylar subchondral bone, offering an explanation to the adverse mandibular development following non-reducing displacement of the TMJ disk.

## **Experimental method**

### **Animal model**

In medicine, the conversion of data and conclusions from experimental studies to man is fundamental. In the evaluation of adverse or beneficial effects on craniofacial growth induced by pathological conditions or different treatment modalities, the use of animal models is commonly indispensable (Sarnat, 1997). This can be exemplified by the large number of longitudinal radiographic experimental studies evaluating facial growth in the monkey (McNamara and Graber, 1975; Carlson and Ellis, 1988; Isberg *et al.*, 1990; Isacsson *et al.*, 1993). However, with concern for ethics, accessibility and cost-benefit, alternative animal models have been explored (Losken *et al.*, 1992). Publications from various research groups focusing on growth effects on the facial skeleton due to temporomandibular disorders have been performed as radiographic evaluations in rabbits (Hatala *et al.*, 1996; Legrell and Isberg, 1998; 1999; Qadan *et al.*, 1999). The rabbit is the experimental model of choice in these cases because of the suitability of the rabbit TMJ for studying afflictions of this joint (Tallents *et al.*, 1990; Mills *et al.*, 1994).

The rabbit is, however, a small animal that shows low relative change in mandibular size from juvenility through adolescence (Masoud *et al.*, 1986). Metric analysis of deviations in growth by longitudinal radiographic evaluation in a fast growing small animal, such as the rabbit, therefore stresses the need for a highly reliable method to be used for growth analysis.

### **Radiography**

If the growth deviations to be studied are small, superimposition of serial radiographs of the skull and facial skeleton during the growth period is generally the method of choice (Baumrind *et al.*, 1976). Such radiographic evaluation, must consider two basic objectives, *i.e.* (i) correct repositioning of the object relative to the film-focus assembly to avoid image distortion due to deviation in placement (Ahlqvist *et al.*, 1986), and (ii) the possibility of identification of reference points, planes, or areas, as a prerequisite for correct orientation of the radiographs relative to each other at superimpositioning (Brodie, 1949).

In dental practice and in research that includes human beings, repositioning of the patient at repeated radiographic examinations, as well as superimposition of the subsequent radiographs, are well known issues and usually not a problem. When fast-growing small animals are to be studied difficulties arise. Limitations due to the positioning of an

anaesthetized animal at repeated radiographic examinations as well as the difficulty of translating human anatomical landmarks to a small animal, entails the risk of flawed results (Alberius *et al.*, 1990).

To minimize the problem of repositioning, specially designed animal cephalostats have been constructed (Sarnat, 1997; Masoud *et al.*, 1986; Mooney *et al.*, 1993; Legrell and Isberg, 1998), however, the smaller the experimental animal, the more difficult it is to accomplish accurate repositioning. A study design that avoids the problems of repositioning is one that sacrifices the animals at different ages prior to the radiographic examination (Kantonmaa, 1984; Monje *et al.*, 1994). Such a longitudinal investigation fails to follow each animal over time and intra-individual differences in growth cannot be studied. To overcome the difficulties in translation and identification of anatomical landmarks, metal indicators have successfully been utilized in the study of single bones (Sarnat, 1968; Alberius *et al.*, 1990; Mooney *et al.*, 1993; Sinsel *et al.*, 1995; Legrell and Isberg, 1999; Tavakkoli-Jou *et al.*, 1999). When studying the combined maxillary and mandibular growth and the effect on the intra-maxillary relationship it is moreover fundamental to find stable fixed points for superimposition outside the facial skeleton (Baumrind *et al.*, 1976).

In humans, accurate superimposition is commonly made on the best anatomic fit of the anterior cranial base (Björk and Skieller, 1983; Ghafari *et al.*, 1998). While this area is considered to be a stable configuration from juvenility through adolescence in humans, the anterior cranial base of the rabbit displays a substantial amount of growth during the corresponding period (Putz *et al.*, 2002). Superimposition on constructed reference planes as analogues to the anterior cranial base is moreover doubtful, as the identification of stable and adequate anatomical landmarks in the neural cranium may be difficult or impossible in the growing rabbit (Alberius *et al.*, 1990).

Although the rabbit model is especially suited for studies of the TMJ, the difficulty of finding a reliable and stable reference area representing the anterior cranial base for longitudinal growth studies is a major disadvantage. Furthermore the use of conventional cephalometric methods, including superimposition of serial radiographs, is a problem of the model. Therefore, in order to test the hypotheses of the thesis, an experimental method that would optimize the reliability of longitudinal radiographic evaluation of craniofacial growth in fast growing small animals, such as the rabbit, had to be developed

## AIM

The overall aim of this project was to investigate how bilateral non-reducing displacement of the TMJ disk, with onset during the growth period, influences facial growth and the mandibular growth in particular, and to build up new knowledge about the biological mechanisms that account for adverse mandibular development following non-reducing displacement of the TMJ disk in growing individuals.

The specific aims of the studies presented in the theses were:

- to develop a method that, by admitting congruent positioning at repeated radiographic examination and by introducing readily identified reference structures usable at superimposition, would optimize the reliability of longitudinal radiographic evaluation of fast growing small animals, such as the rabbit (I).
- to experimentally test the hypothesis that bilateral non-reducing TMJ disk displacement during the growth period impairs craniofacial growth to an extent corresponding to mandibular retrognathia in man, and to elucidate the impact on the mandibular and maxillary growth pattern over time (II).
- to evaluate the histological response of the mandibular condylar cartilage following non-reducing TMJ disk displacement and to correlate the histological feature of the condylar cartilage at the end of the growth period to mandibular growth on the ipsilateral side (III).
- to assess the amount and composition of condylar subchondral bone and the expression of local growth factors in condylar cartilage following non-reducing TMJ disk displacement, in order to find an explanation for adverse mandibular growth following non-reducing TMJ disk displacement (IV).



## MATERIAL AND METHODS

This experimental study was reviewed and approved by the Ethics committee on animal experiments, Umeå University, Sweden (Registration Nr. A 128-00).

### **Animals**

In the methodological study (I) the material was comprised of ten male New Zealand white rabbits (*Oryctolagus cuniculus*). The animals were ten weeks old at the beginning of the study and were allowed to grow for 91 to 101 days, with a mean study period of 98.6 days.

In the following experimental series (II-IV) the material was comprised of twenty additional New Zealand White rabbits for growth evaluation. The animals were randomized into two study groups:

- An experimental group (n = 10) in which bilateral non-reducing TMJ disk displacement was surgically created.
- A sham operated control group (n = 10) in which the same surgical procedure was performed but without any manipulation of the TMJ disk.

The animals were ten weeks old at the beginning of the study and were allowed to grow for a mean period of 96 days; range 93-98 days. The rabbit's growth period (Bang and Enlow, 1967; Masoud *et al.*, 1986) thereby approximated childhood and adolescence in man (Losken *et al.*, 1992).

The inclusion of a third non-operated control group in the experimental series was not considered ethically justified, because the sham operation, as performed in this material, had previously been shown not to influence craniofacial growth (Legrell and Isberg 1998; 1999).

### **General growth**

To detect signs of malnutrition that could presumably affect growth, the animal's body weight was registered at study inception and throughout the three month study period (II).

## **Surgery**

The animals underwent surgery that was conducted at study inception according to the following protocol.

### **Titanium screw surgery**

A two cm long sagittal incision was made centrally on the scalp and the sagittal crest of the parietal bone was identified. Two, square-fit head, self tapping, 1.2 x 3 mm titanium alloy screws were placed in holes drilled approximately two and 12 mm posterior to the *sutura coronalis*, respectively (Fig. 4). The incision was then closed with a continuous 4-0 silk suture.



**Figure 4**

Two titanium screws were placed in the midline of the animal's skull. The anterior screw served as an attachment for a steel pin inserted in the square screw head hole of the screw

The steel pin was aimed to secure the fixation of the rabbit's head to a specially designed animal cephalostat in order to achieve congruent positioning of the animal's skull during the repeated radiographic examinations.

For repeated radiographic examinations after one and two months, re-incisions were made on the scalp in order to uncover the anterior screw for fixation to a cephalostat (I-III).

### **Implant surgery**

To allow identification of sides in subsequent radiographic images, tantalum spheres ( $\varnothing = 0.5$  mm) were inserted on the left side, and tantalum pins ( $\varnothing = 0.37$  mm) on the right side, of both the mandible and the maxilla.

At insertion of the implants a minor incision was made in the alveolar mucosa, and an implant insertion instrument was used (I-III).

## **TMJ surgery**

Bilateral non-reducing TMJ disk displacement was created in each experimental animal (Ali *et al.*, 1993; Legrell and Isberg, 1998) (II-IV). The TMJ was approached through a skin incision followed by blunt dissection until the joint capsule was disclosed. The capsule was incised and the disk exposed. The medial, anterior, and lateral disk attachments were detached, and the disk was pulled anteriorly, placing the intact posterior disk attachment above the condyle. A ligature, looped through a hole drilled in the anterior zygomatic arch, anchored the displaced disk anteriorly. Maintenance of the incorrect disk position was checked, the surgical area was flushed with saline solution, and the wound was closed in layers.

The sham operation followed the same surgical procedure until the disk was exposed. The wound was then flushed with saline and closed without any further manipulation of the disk.

## **Anesthesia**

TMJ surgery in the experimental series (II-IV), and the insertion of screws and implants (I-III) was performed under general anesthesia with 0.4 ml Dormicum® per kilogram of body weight, intraperitoneally administered and 0.2 to 0.3 ml of Hypnorm® per kilogram of body weight, intramuscularly injected. Subcutaneous injections of 0.3 to 0.5 ml Citanest Octapressin® was made prior to incisions in the scalp, the TMJ area, and the alveolar mucosa to achieve local anesthesia.

Following the surgical procedures, the animal was given 0.1 ml Temgesic® subcutaneously per kilogram of body weight for analgesia, and approximately 15 ml of saline per kilogram of body weight to prevent dehydration.

At radiographic examination after one and two months, respectively, the animal was anesthetized intramuscularly with Hypnorm® according to the previously described procedure.

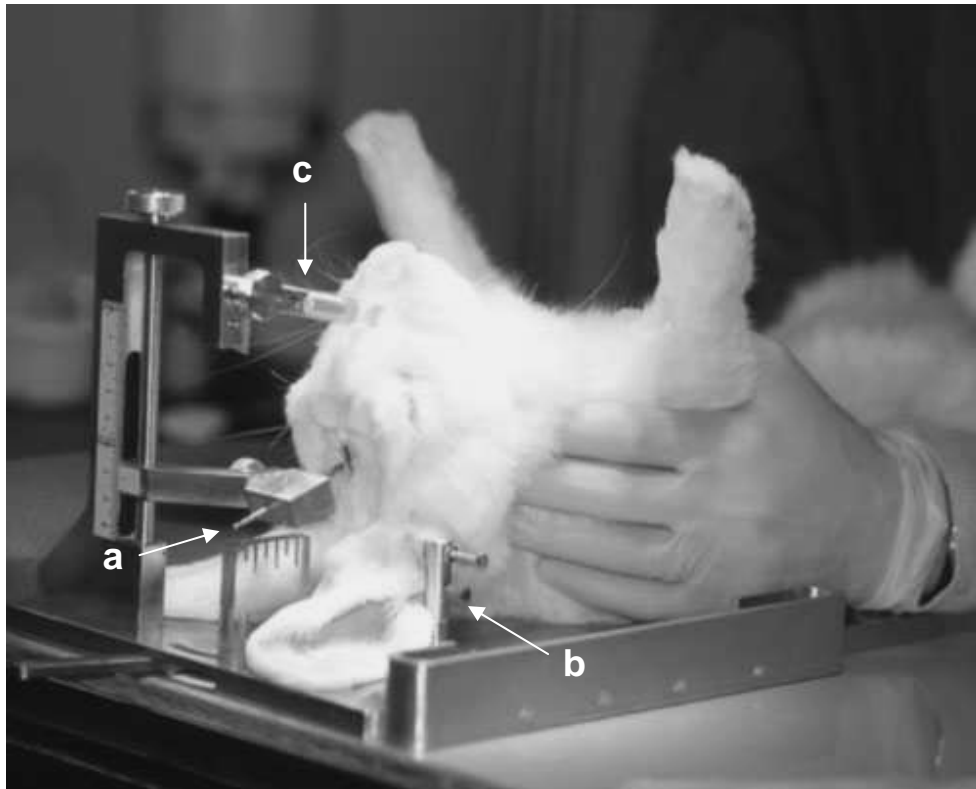
## **Euthanasia**

At the final radiographic examination after three months, the animal was sacrificed by an intravenous injection of approximately 1.2 ml Pentotal® per kilogram of body weight.

## ***Radiographic examinations and analysis***

### **Skull radiographs**

A specially designed animal cephalostat was constructed for fixation of the animal's head during exposure of lateral cephalograms (I) and was utilized in the experimental series (II-III) (Fig. 5).



**Figure 5**

Rabbit placed in the animal cephalostat. During the examinations, the animal was placed in a supine position with the left side of the head facing the radiographic focus. The head was fixed in the cephalostat by attaching a steel pin **a**) to the anterior screw in the calvarium, with ear pins **b**) bilaterally in the external auditory canals, and with a nose fork **c**) on the anterior aspect of the nose.

Lateral cephalograms were exposed of each animal at the beginning of the study (T1), after one month (T2), after two months (T3), and after three months (T4). Hence, a total of four radiographs were taken of each animal. At the first examination, the angulation of the steel pin in degrees, and the position of its holder in mm were registered with the aid of a protractor and

a ruler mounted on the cephalostat. These data were then used at each subsequent examination for individual positioning.

The cephalograms were exposed using a Philips Practix dental X-ray unit. The film-screen combination was DuPont Cronex Hi-plus ZJ screens and CEA blue sensitive film, chosen to allow for detailed measurements of the cephalograms.

All cephalograms were digitized in an Agfa Arcus II scanner. The software used in handling the digitized cephalograms was Adobe Photoshop v. 5.0.2.

### Superimposition tests

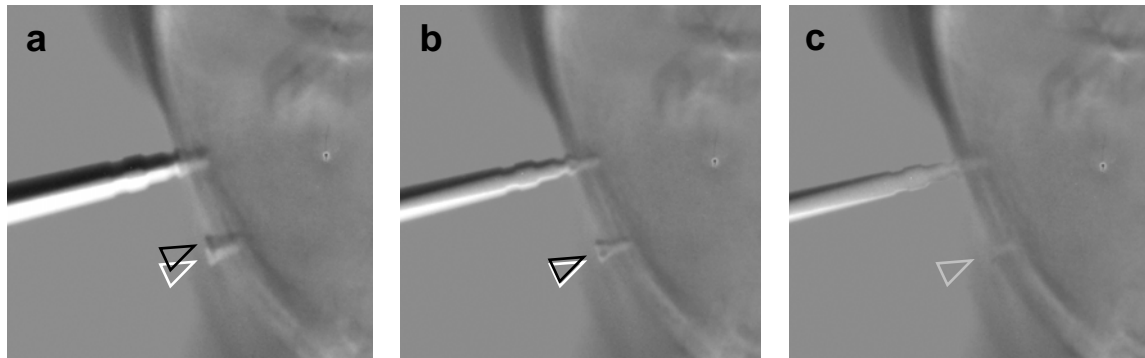
Three tests were made to evaluate if the screws in the calvarium could be used as reliable reference structures at radiographic superimposition (I).

The first test was to evaluate if the geometric structure of a titanium-alloy screw is complex enough to be used for high precision superimposition. Ten cephalograms were randomly chosen, one from each animal, and duplicated in the computer. One of the paired images was inverted regarding gray-scale, reduced to approximately 50% transparency and randomly rotated and displaced relative to the other. The structures surrounding the screw were masked and the paired images were superimposed with only the aid of the visible posterior screw. After superimposition, the masking was removed and the discrepancy in position of the left tantalum implant in the maxilla was measured in each pair of cephalograms.

The second and third tests were to evaluate the reproducibility of superimposition of serial cephalograms, during longitudinal studies. In the second test the posterior titanium alloy screw in the calvarium was used as a single reference structure, while the third test utilized both titanium alloy screws in the calvarium as paired structures. The images of examinations two, three, and four were superimposed on the image of examination one in each of the ten animals. Hence, a total of 30 different superimpositions were performed in each test. Every superimposition was then repeated after three to eight weeks.

Precision in the two longitudinal tests was calculated by measuring the discrepancy in position of the tantalum implant in the anterior part of the maxilla at each of the repeated superimpositions.

For illustration of the superimposition technique see Figure 6.



**Figure 6**

Detailed illustration of superimposition of two serial cephalograms exposed 3 months apart, one inverted in grayscale with 50 % transparency. Example shows superimposition made on the posterior screw in the calvarium (black and white arrowheads). Matching of screw is increasingly accurate from picture **a**) to **c**).

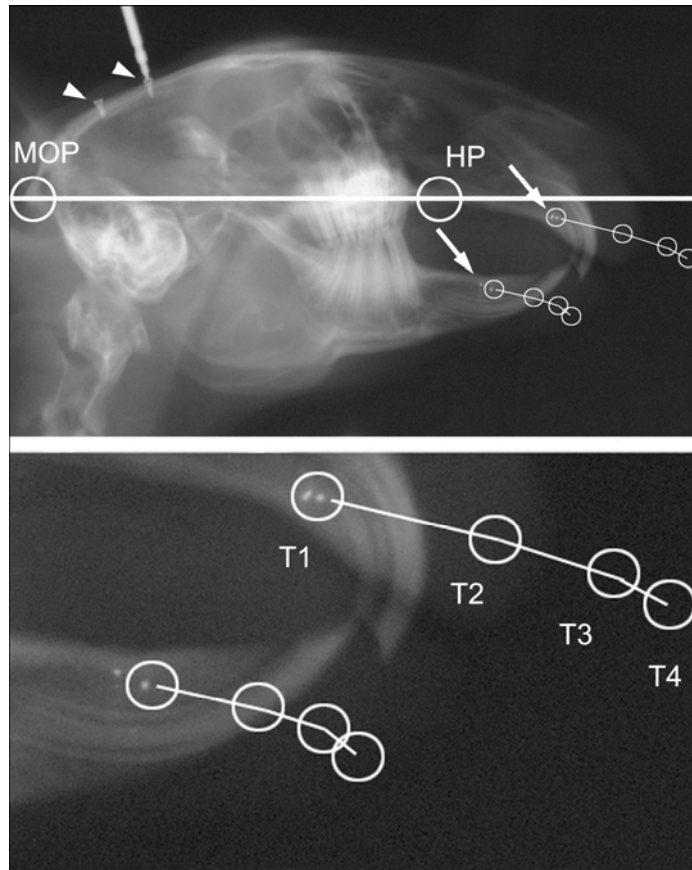
Note extinction of matching structures when correct superimposition is achieved **c**).

## Assessment of craniofacial growth

Lateral cephalograms of each of the 20 animals comprising the material utilized for growth studies were exposed on four occasions *i.e.* at study inception (T1), and after one (T2), after two (T3), and after three (T4) months. For each animal, the T2, T3, and T4 cephalograms were superimposed on the T1 cephalogram, using the titanium alloy screws in the calvarium as reference structures according to the procedure described in paper I.

A horizontal reference plane was constructed in the T1 cephalogram by inserting a line through the maximum occipital point (MOP), running as a tangent to the hard palate anterior to the upper molars (HP) (Fig. 7). This individual reference plane was then carried forward in the subsequent superimpositions. The position of each tantalum implant in the jaw was digitally plotted in each cephalogram, giving it x- and y-coordinates to the nearest tenth of a millimeter. The position in the T1 cephalogram was defined as the implant's origin, and the magnitude and direction of growth in relation to the constructed horizontal reference plane could be followed over time (II-III) (Fig. 7).

All measured values of maxillary and mandibular growth were corrected for the radiographic magnification in the cephalograms prior to statistical analysis. The measurements of craniofacial growth were assessed differently in papers II and III.



**Figure 7**

Lateral cephalogram of rabbit skull showing titanium screws in the calvarium (arrowheads), tantalum implants in the maxilla and the mandible (arrows).

A horizontal reference plane (white line) was constructed in the inception cephalogram as a straight line running through the maximum occipital point (MOP) and the most superior point of the hard palate anterior to the molars (HP).

Both the magnitude and direction of mandibular and maxillary growth could be followed for each month throughout the three-month study period (T1-T4).

### *Maxillary and mandibular growth*

The horizontal and vertical aspects of craniofacial growth were calculated as a mean of the values from the left and right side of both jaws, mimicking the clinical situation in the evaluation of growing humans. Measurements from three mandibular tantalum implants were discarded because of interference with the lower incisors. Therefore, unilateral mandibular values were achieved in one control and two experimental animals (II).

### *Unilateral mandibular growth*

The amount of mandibular growth and its horizontal and vertical growth vectors were assessed separately for the left and right side of each animal. This was carried out in order to correlate the cartilage features of each condyle to the intra-individual ipsilateral growth of the mandible. Because the mandibular implant interfered in three of the animals, these unilateral measurements were discarded from the study, as were the corresponding ipsilateral joint specimens, resulting in a total number of 37 TMJ specimens with matching mandibular growth measurements (III).

## ***Histological preparation and analyses***

To study the screw-to-tissue contact, a parietal bone block containing the paired titanium alloy screws was removed from the skull after sacrifice of four of the animals comprising the material in the methodological study, and kept in formaldehyde solution until preparation (I). Utilizing a cutting grinding technique, a section of the selected screws and surrounding bone was obtained with a thickness of 30  $\mu\text{m}$ . The sections were stained with Stevenel's blue for histological analysis of the bone surrounding the anterior screw, which served as attachment for the steel pin during the radiographic examinations.

After sacrifice of animals in the experimental series, the rabbit was decapitated and the skull was skinned (II-IV). The jaws were maintained in normal intercuspal position by intermaxillary fixation with a wire, and the skull was kept in buffered formalin until each TMJ-sample was removed *en bloc*, approximately 4 x 2 x 2 cm in size.

Following decalcification, the specimen was dehydrated, incubated and immersed in three graded methyl salicylate/paraffin mixtures followed by pure paraffin for two days. The paraffin embedded specimen was cut sagittally through the lateral into the central part of the TMJ utilizing a scroll saw and then cut in 5  $\mu\text{m}$  sections for histological and biomolecular analyzes (III-IV).

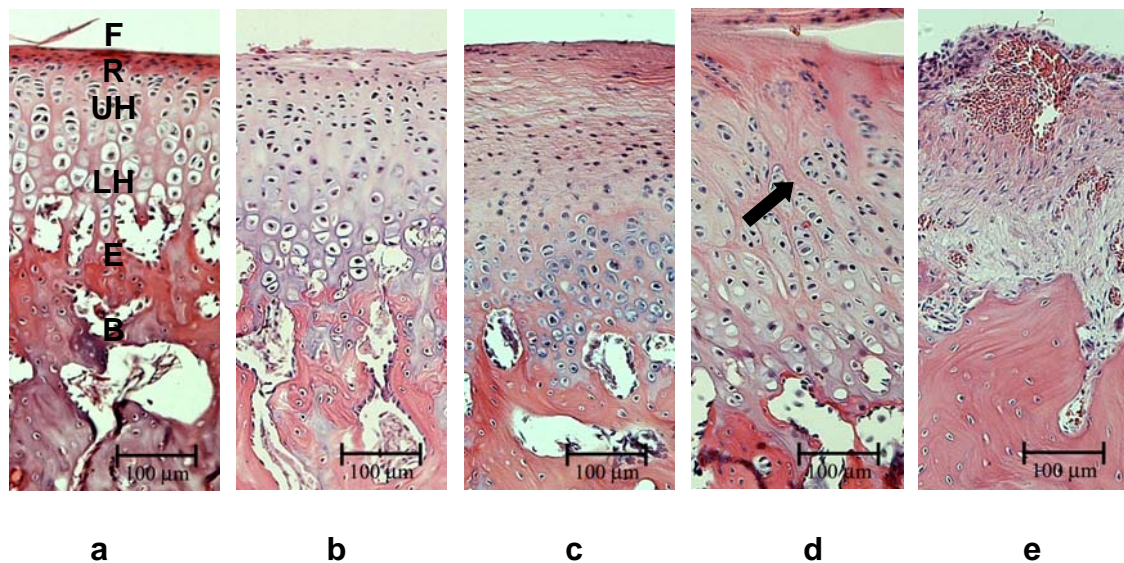
## **Condylar cartilage features**

One set of histological sections was stained with hematoxylin and eosin for general morphology. The histological sections were evaluated under a light microscope and classified, in consensus, by two observers (III).

The anterosuperior part of the condyle, opposing the articular tubercle, was chosen as the region of interest, as this part of the condyle is most susceptible to altered articular biomechanics due to displacement of the disk (Ali and Sharawy, 1995; Legrell *et al.*, 1999; Long and Li, 2000; Berteretche *et al.*, 2001)

The histological condition of the condylar cartilage of each joint was categorized in five different classes of severity with increased divergence from normal cartilage configuration; the graded severity classes starting with "no changes", *i.e.* normal cartilage and ending with "destructive changes", *i.e.* osteoarthrotic degeneration. The three intermediate classes: "minor changes", "moderate changes", and "severe changes" illustrating increased histological adaptation to biophysical stress (Fig. 8).





**Figure 8**

Cartilage classification: **a)** *No changes* - normal condylar cartilage with five well organized basic layers; fibrous layer (F), reserve cell layer (R), upper hypertrophic layer (UH), lower hypertrophic layer (LH), and the erosive zone (E) with endochondral ossification toward the subchondral bone (B). **b)** *Minor changes* - intact layers but with slightly altered thickness of separate layers, indication of cellular atrophy. **c)** *Moderate changes* - absence of specific layers, moderate cartilage hypo- or hyperplasia, modest cellular atrophy, indication of vertical fibrous bundles between the fibrous layer and the subchondral bone. **d)** *Severe changes* - loss of layer organization, pronounced cartilage hypo- or hyperplasia, severe cellular atrophy, apparent vertical fibrous bundles between the fibrous layer and the subchondral bone (arrow). **e)** *Destructive changes* - splitting or absence of condylar cartilage – osteoarthrotic degeneration.

## Assessment of subchondral bone

One set of histological sections was stained with periodic acid and Schiff's reagent (PAS) for the identification of endochondral new bone formation. Stained with PAS, the newly formed bone takes on a distinctive magenta color (IV).

Three regions of interest for subchondral bone analyses were defined and marked by equal sized frames of about 1 mm<sup>2</sup> (954708 µm<sup>2</sup>) placed; (i) as far anteriorly as possible inferior to the articulating cartilage, (ii) opposing the articulating tubercle of the temporal bone and, (iii) in the posterior region inferior to the articulating cartilage. The two anteriorly placed frames were therefore situated in the articulating part of the condyle, and the posteriorly placed frame situated in the non articulating part of the condyle.

## Expression of VEGF and Ihh

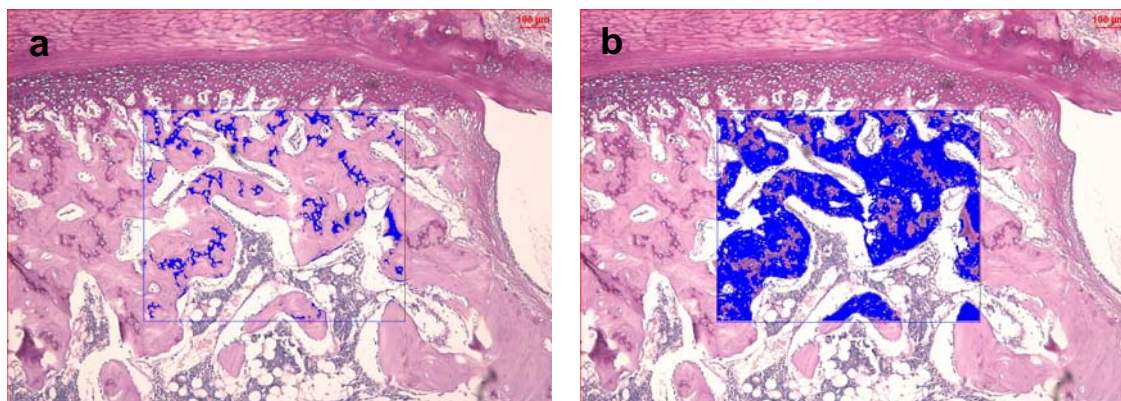
One set of sections intended for *in situ* hybridization (ISH) was placed on Super Frost Plus adhesive slides. ISH for evaluation of the expression of VEGF and Ihh, respectively, was performed following routine procedures previously described in detail (Wong and Rabie, 2005) (IV).

Three additional regions of interest for evaluation of expression of VEGF and Ihh were defined and marked by equal sized frames of about  $0.2 \text{ mm}^2$  ( $219771 \text{ } \mu\text{m}^2$ ) placed in the cartilage adjacent to the positions of the subchondral frames.

## Quantitative evaluation

The expression of VEGF and Ihh as well as the amount of mature bone and newly formed bone was quantified within the three regions of interest in the condyle utilizing a true-color RGB computer-assisted image analyzing system. This system obtains high-definition digital images from the histological sections. Different staining intensity can be automatically selected and recognized by identification of color, shade, shape, or texture. The system was used to recognize the distinction in staining density between newly formed bone and mature bone in the condylar subchondral bone in the PAS stained sections, as well as the expression of VEGF and Ihh in the adjacent cartilage in the ISH stained sections (Fig. 9) (IV).

The quantifications of bone, VEGF and Ihh followed methods previously described in detail (Rabie *et al.*, 2001; Tang *et al.*, 2004; Leung *et al.*, 2004).



**Figure 9**

Example from quantification of newly formed and mature bone on PAS stained sections using the image analyzing system. **a)** The amount of newly formed bone digitally identified within the measure frame in the anterior part of the condyle (blue color). **b)** Mature bone digitally identified within the same measure frame.

## **Statistics**

The formula used for calculating precision (s) was  $s = \sqrt{\sum d^2 / 2n}$ , where d is the difference between dual measurements and n is the number of measurements performed (I, IV).

Mann-Whitney's non-parametric test was used to test for (i) differences between the experimental group and the control group regarding weight gain and regarding mean mandibular and maxillary growth during each month and at the end of the study period (II), (ii) differences in manifestation of histological cartilage changes between experimental condyles and control condyles (III), and (iii) differences in the amount of subchondral bone as well as in the expression of VEGF and Ihh between experimental and control condyles (IV).

T-test for equality of means was used to test whether cartilage changes in the mandibular condyles were associated with deviant mandibular growth (III).

Spearman's non-parametric correlation test was used for intra-individual correlation between the histologically classified features of the condylar cartilage and the assessed ipsilateral mandibular growth (III).

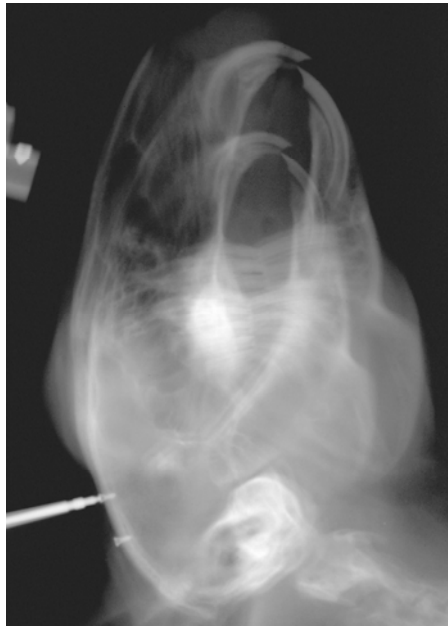
Pearson correlation test was used for intra-individual correlation between the amount of subchondral bone and the expression of VEGF and Ihh in adjacent cartilage (IV).

P-values less than 0.05 were considered as statistically significant in all papers.

## RESULTS

### *The experimental method*

A match between the steel pin and the anterior screw was achieved in each animal at every examination. The positioning of the skull in the cephalostat was thereby constant relative to the film-focus assembly, giving periodically congruent depiction of the titanium alloy screws in the calvarium intended as reference structures at superimposition (I).



**Figure 10**

Superimposition of two cephalograms.

Example showing a cephalogram from the initial examination and a cephalogram from the examination after three months superimposed.

Note match between the titanium screws in the calvarium in spite of major craniofacial growth.

An average growth of approximately 13 mm took place in the part of the maxilla where the tantalum implant was situated. Although the histological analysis indicated that the screws in the calvarium were not osseointegrated, no changes in the overall distance between the paired screws were registered (Fig. 10).

The 10 computer-aided superimpositions of the masked pairs of cephalograms revealed a precision of superimposition only with the aid of the geometric structure of a screw, with an s-value less than 0.06 mm at the site of the maxillary implant. The 30 computer-aided superimpositions of serial cephalograms using the posterior screw as a single reference structure, and using both screws as paired structures, revealed precision in reproducibility of  $s = 0.39$  mm and  $s = 0.41$  mm respectively, at the site of the maxillary implant (I).

The method errors were of no significance to the results of the growth measurements in the project (II-III).

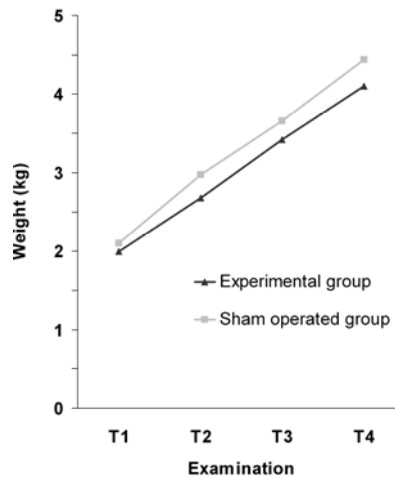
## General Growth

In the experimental series a difference of 0.2 kg in mean weight gain between groups occurred during the first postoperative month ( $p=0.011$ ). There was no significant difference in mean weight gain between groups during the second or third month (II). The temporary difference during the first month was of no significance to the results (Putz *et al.*, 2002) (Fig. 11).

**Figure 11**

In the experimental group the mean body weight increased from 2.0 kg to 4.1 kg during the study period.

The corresponding figures for the control group were 2.1 kg to 4.4 kg



## Craniofacial Growth

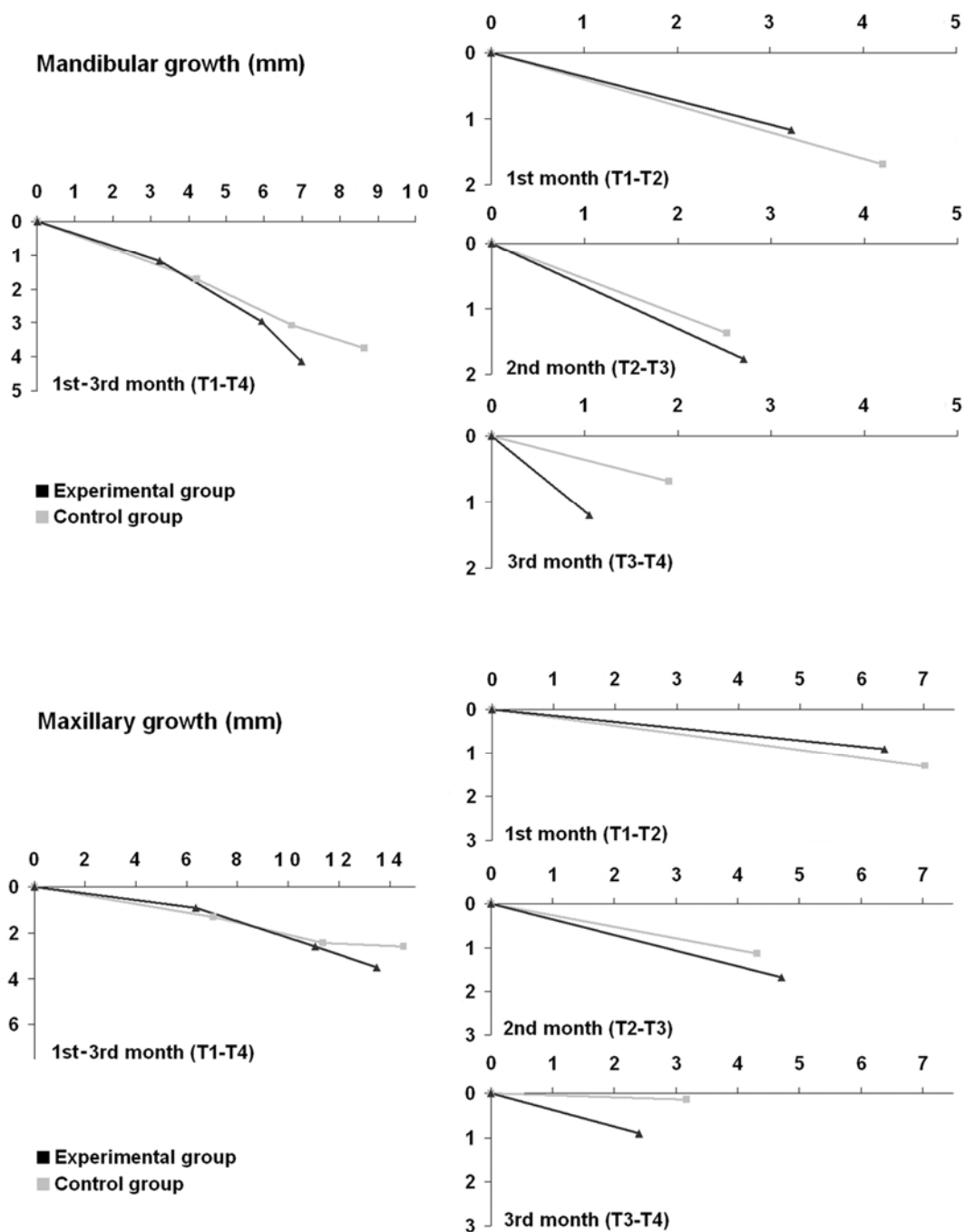
### Horizontal growth

#### Mandible

At T4 mandibular horizontal growth was reduced by 19 % in the experimental group compared with the control group ( $p=0.011$ ). The experimental animals grew 24 % less than the control animals during the first month ( $p=0.001$ ), and 42 % less than the control group during the third month ( $p=0.003$ ) with no significant difference between groups during the intermediate month ( $p=0.631$ ) (II) (Fig. 12).

#### Maxilla

At T4 maxillary horizontal growth was reduced by 7 % in the experimental group compared with the control group ( $p=0.023$ ). The experimental animals grew 9 % less than the control animals during the first month ( $p=0.004$ ) and 25 % less during the third month ( $p=0.001$ ) with no significant difference between groups during the intermediate month ( $p=0.247$ ) (II) (Fig. 12). The growth impairment was consistently bilateral in both jaws.



**Figure 12**

Growth of the mandible and the maxilla. Throughout the study period (T1-T4) the experimental animals displayed a 19 % reduction in mean mandibular horizontal growth and a 7 % reduction in mean maxillary horizontal growth in comparison with the control group. Horizontal growth was significantly impaired both throughout the study period as well as during the first and third month. There was no significant difference in horizontal growth during the second month, nor were there any significant differences in mandibular or maxillary vertical growth.

## Vertical growth

### *Mandible and maxilla*

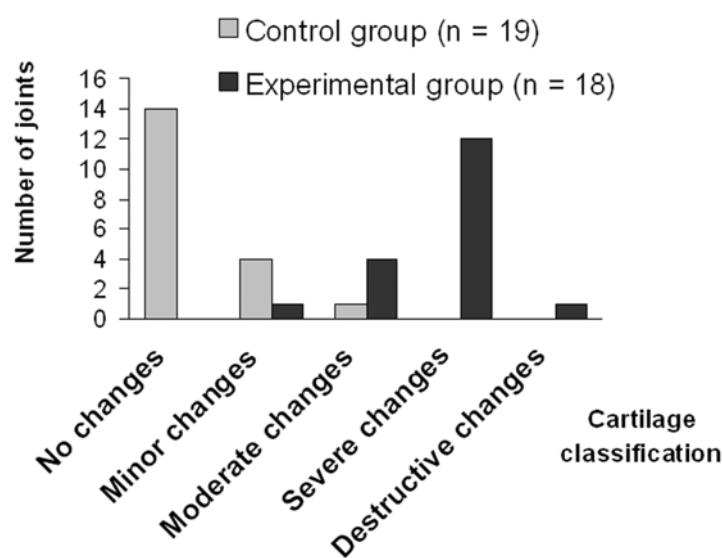
There was no statistically significant difference in mean vertical mandibular or maxillary growth between the two groups either at the termination of the study or during any specific period. The mean amount of vertical growth among the experimental animals was, however, larger compared to the control animals, with a more downward backward rotational growth pattern during the third month (II) (Fig. 11).

### **Condylar cartilage features**

The articulating fibrous layer was intact in all control condyles and in all but one experimental condyle (III).

Each experimental condyle showed some degree of cartilage change. Fourteen of the sham operated control condyles showed normal cartilage. The remaining five control condyles displayed minor or moderate cartilage change. These tissue reactions opposed iatrogenic irregularities in the temporal bone after sham surgery.

Cartilage changes were consistently found in areas predisposed to articular loading, and were significantly more frequent and more severe in the experimental condyles than in the control condyles ( $p < 0.001$ ) (Fig. 13).



**Figure 13**

Distribution of normal condylar cartilage and different grades of cartilage changes in the two groups of animals.

Cartilage changes were of adaptive nature in all condyles but one experimental condyle where gross maladaptive changes were found.

## ***Correlation between cartilage features and ipsilateral mandibular growth***

When cartilage changes were present in mandibular condyles, mandibular growth on the same side was significantly reduced and the horizontal growth vector was significantly shorter, compared to mandibular growth when condylar cartilage was unaffected (III) (Table 1).

Cartilage classification	Mandibular growth		Horizontal growth vector		Vertical growth vector	
	Mean	SD	Mean	SD	Mean	SD
No changes (n=14)	9.5	0.96	8.7	0.80	3.7	1.25
Minor changes (n=5)	9.4	0.36	8.4	0.24	4.1	0.75
Moderate changes (n=5)	9.2	0.72	7.8	1.44	4.7	1.09
Severe changes (n=12)	8.4	1.16	7.0	1.16	4.0	2.47
Destructive changes ( n=1)	6.6	-	5.2	-	4.1	-
No cartilage changes (n=14)	9.5	0.96	8.7	0.80	3.7	1.25
Cartilage changes (n=23)	8.7	1.10	7.4	1.27	4.2	1.86
T-test Sig. (2-tailed)	0.025	*	0.001	***	0.345	ns

**Table 1**

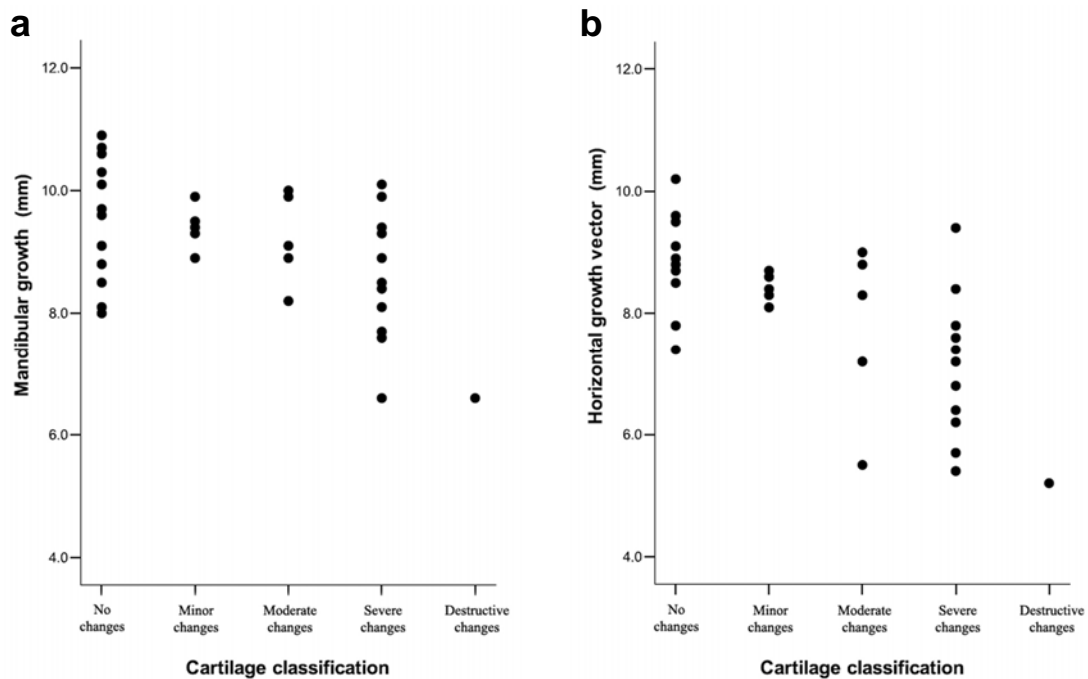
Table shows mean and standard deviation (SD) of mandibular growth in mm and its horizontal and vertical growth vectors. T-test for equality of means was used to test whether cartilage changes in the mandibular condyles were associated with altered magnitude and direction of mandibular growth.

The test for intra-individual correlation between cartilage features and mandibular growth revealed a negative correlation between the five grades of histologically classified features of the condylar cartilage and the magnitude of ipsilateral mandibular growth ( $p=0.006$ ). A strong negative correlation was also seen between the five grades of cartilage features and the length of the horizontal growth vector ( $p=0.000$ ).

Inverse correlations were present also between the four different degrees of cartilage changes and the magnitude of ipsilateral mandibular growth ( $p=0.020$ ) and between cartilage changes and the length of the horizontal growth vector ( $p=0.006$ ).

No statistically significant correlation was found between cartilage features and the vertical aspect of mandibular growth (III) (Fig. 14).



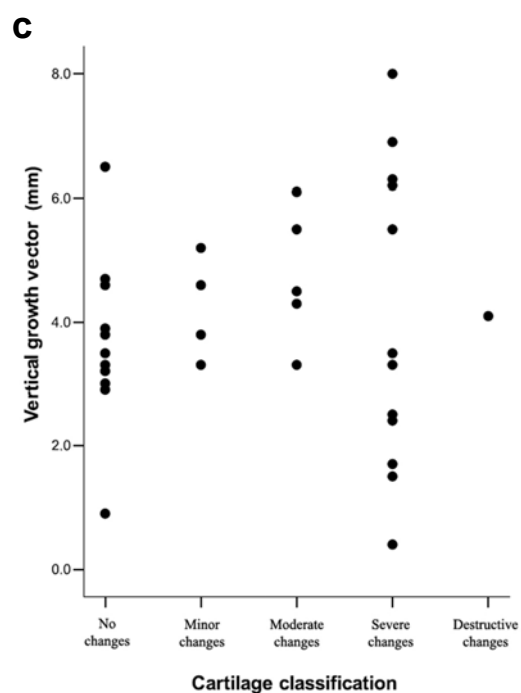


**Figure 14**

Scatter plots for visual presentation of the material showing growth in mm for each mandibular side in relation to the five grades of histologically classified cartilage features of the ipsilateral condyle.

Graphs show each individual measurement of mandibular growth **a)** as well as its horizontal **b)** and vertical **c)** growth vectors.

Strong negative correlation was seen between the classified cartilage features and the amount and horizontal aspect of ipsilateral mandibular growth.



## ***Amount of condylar subchondral bone***

Fifteen of the 20 experimental condyles displayed modeling with loss of convexity and a flattened enlargement of the articulating surface (IV). One experimental condyle lacked cartilage due to osteoarthrotic destruction and the bone was instead covered by synovial tissue.

Because of lack of cartilage the effect of disk displacement on subchondral bone, as well as on expression of local growth factors in the cartilage, could not be evaluated. The condyle was discarded from further analysis.

The proportion of newly formed and mature bone differed significantly between the experimental group and the control group (IV). The mean of the assessed amount of subchondral bone from the three regions of interest in each condyle, were chosen to represent the entire condyle. There was a general increase of the new-to-mature bone quotient in the experimental condyles. This was assigned to a significantly lower amount of mature bone, as well as to a significantly higher amount of newly formed bone in the experimental condyles. Despite the increased amount of new bone formation in the experimental condyles, there was a significant decrease of the total amount of subchondral bone in the experimental condyles compared to the control condyles.

As a consequence of the adaptive modeling observed in experimental condyles the articulating surface extended to include the anterior part of the condyle. In the anterior region of the condyle, and in the region opposing the tubercle, the new-to-old bone quotient was higher in the experimental group compared with the controls. The total amount of subchondral bone was lower in both regions in the experimental condyles, assigned to a significantly lower amount of mature bone. In these articulating parts of the condyle the amount of mature bone was reduced by 14 % and 20 % as a mean respectively in the experimental condyles compared with the control condyles. In the posterior region of the condyle the total amount of bone was similar in both groups. The new-to-old bone quotient was higher in the experimental group in the non-articulating part of the condyle, but in this region the quotient increase was assigned to a higher amount of newly formed bone in the experimental condyles compared to the control condyles (IV) (Table 2).

### ***Expression of VEGF and Ihh***

There was no statistically significant difference between the experimental and control group in the expression of VEGF and Ihh in the articular cartilage adjacent to the areas of bone measurement (Table 2). The expression of VEGF in the experimental group showed a negative correlation between its level and that of the amount of newly formed bone ( $p=0.036$ ). There was also a negative correlation between VEGF and the quotient of new-to-mature bone ( $p=0.031$ ). No such correlation was seen in the control condyles (IV).

	<b>Control group</b> <i>N = 20</i>		<b>Experimental group</b> <i>N = 19</i>			
<b>CONDYLE</b>	<b>mean</b>	<b>SD</b>	<b>mean</b>	<b>SD</b>	<b>p-value</b>	<b>Sig.</b>
Total bone area ( $\mu\text{m}^2$ )	571 729	60 757	518 498	81 968	0.038	*
Mature bone area ( $\mu\text{m}^2$ )	497 434	56 573	422 193	84 765	0.003	**
New bone area ( $\mu\text{m}^2$ )	74 295	25 057	96 305	32 467	0.018	*
Quotient new-to-mature bone	0.155	0.053	0.250	0.104	0.002	**
VEGF (%)	1.6	0.9	1.9	0.9	0.365	ns
Ihh (%)	2.0	1.1	2.3	1.5	0.708	ns
<b>Anterior region</b>	<b>mean</b>	<b>SD</b>	<b>mean</b>	<b>SD</b>	<b>p-value</b>	<b>Sig.</b>
Total bone area ( $\mu\text{m}^2$ )	605 868	64 207	541 627	86 276	0.022	*
Mature bone area ( $\mu\text{m}^2$ )	541 561	57 737	463 475	88 044	0.003	**
New bone area ( $\mu\text{m}^2$ )	64 307	22 881	78 152	32 562	0.194	ns
Quotient new-to-mature bone	0.120	0.042	0.178	0.086	0.022	*
VEGF (%)	1.5	1.0	1.8	1.0	0.478	ns
Ihh (%)	1.4	0.9	2.1	1.7	0.158	ns
<b>Region opposing tubercle</b>	<b>mean</b>	<b>SD</b>	<b>mean</b>	<b>SD</b>	<b>p-value</b>	<b>Sig.</b>
Total bone area ( $\mu\text{m}^2$ )	622 479	68 972	530 584	75 650	0.001	***
Mature bone area ( $\mu\text{m}^2$ )	540 133	65 062	434 780	90 375	0.000	***
New bone area ( $\mu\text{m}^2$ )	82 346	32 883	95 804	34 850	0.224	ns
Quotient new-to-mature bone	0.155	0.067	0.241	0.127	0.021	*
VEGF (%)	1.9	1.2	2.2	1.4	0.607	ns
Ihh (%)	2.1	1.5	2.7	2.1	0.550	ns
<b>Posterior region</b>	<b>mean</b>	<b>SD</b>	<b>mean</b>	<b>SD</b>	<b>p-value</b>	<b>Sig.</b>
Total bone area ( $\mu\text{m}^2$ )	486 839	117 424	483 282	144 871	0.708	ns
Mature bone area ( $\mu\text{m}^2$ )	410 607	102 197	368 324	124 521	0.194	ns
New bone area ( $\mu\text{m}^2$ )	76 232	36 266	114 958	55 901	0.014	*
Quotient new-to-mature bone	0.189	0.083	0.332	0.162	0.001	***
VEGF (%)	1.5	0.7	1.6	0.7	0.569	ns
Ihh (%)	2.5	2.1	2.1	1.4	0.461	ns

**Mann-Whitney non-parametric test, Sig. not corrected for ties**

**Table 2**

Table shows mean and standard deviation (SD) of the amount of subchondral bone in  $\mu\text{m}^2$ , and the expression of VEGF and Ihh in percent of adjacent cartilage, in the three regions of interest in the mandibular condyles. Intra-individual mean values from the three regions of interest in the condyle presented as "CONDYLE".

The amount of subchondral bone and of mature bone was significantly decreased in the experimental condyles compared with control condyles, in spite of a general increase of new bone formation. In the anterior region of the condyle, the amount of subchondral bone was decreased, assigned to a 14 % reduction of mature bone. In the region opposing the articulating tubercle, the decrease of subchondral bone was assigned to a 20 % reduction of mature bone. In the posterior region of the condyle the total amount of bone was similar in both groups in spite of a significantly higher amount of newly formed bone in the experimental condyles.

## DISCUSSION

The findings of this prospective project documented that bilateral non-reducing TMJ disk displacement caused bilateral impairment of mandibular growth, resulting in a retrognathic growth pattern (II). The assessment of cartilage changes and quantitative analysis of subchondral bone in the experimental and control condyles, revealed that growth impairment following non-reducing disk displacement was associated with adaptive cartilage reactions (III), and that the cause of growth impairment was to be found in an intra-condylar reduction of subchondral bone (IV).

### ***TMJ disk displacement and condylar cartilage***

As a result of the altered functional biomechanics following non-reducing disk displacement, adaptive cartilage changes with an intact articulating surface were observed in all but one of the experimental condyles (III). The vast majority of the experimental condyles therefore managed to withstand maladaptive development in spite of the three month duration of non-reducing disk displacement. Experimentally it has been shown that the progression of cartilage reaction as a sequel to displacement of the TMJ disk is directly correlated to the severity of the displacement (Berteretche *et al.*, 2001). It has also been shown that the longer a non-reducing TMJ disk remains displaced, the more pronounced the cartilage changes in the condyle will be (Ali and Sharawy, 1995; Long and Li, 2000).

Besides these established progressive factors, age appears to play a significant role in the severity and progression of cartilage changes. This was illustrated by two experimental studies utilizing the same surgical technique for the creation of non-reducing TMJ disk displacement, with the age of the animals being the only difference between the experimental models (Ali and Sharawy, 1995; Legrell *et al.*, 1999). Both studies demonstrated gross morphological modeling of the mandibular condyles as a sequel to disk displacement. While the fully-grown experimental animals developed degenerative changes with erosion and loss of articulating cartilage after only two weeks, the articulating surfaces remained intact in the growing animals throughout a three month study period. This is the same finding as in this project (III). Given the same excessive functional demands on the joint, the higher plasticity and regenerative capacity of the condylar cartilage in growing individuals compared to adults, can explain the dissimilarities of adaptive versus maladaptive cartilage response between age groups.

Adaptive modeling is characterized by slow morphologic changes under physiologic circumstances that permit alteration of the joint components in order to maintain adequate joint function. The process takes place as long as the functional demands do not exceed the adaptive capacity of the joint tissues. If the adaptive capacity of the joint is exceeded, maladaptive tissue reactions and joint degeneration develop (Berteretche *et al.*, 2001; Stegenga, 2001). Maladaptation was found in only one of the experimental condyles in this project. The degenerative changes in this case included destruction of the condylar cartilage and synovial tissue covering the bone (III).

It has been declared that the mandibular condyle is morphologically tailored to resist excessive changes in biomechanical function (Miekle, 2007). One unique character of the condylar cartilage is the capability to drop collagen anchors from the articulating fibrous layer into the subchondral bone during overloading conditions, in order to prevent bone-cartilage interface splitting (Berteretche *et al.*, 2001). This protective fibrous anchorage was evident in those experimental animals in this project with the largest mandibular growth impairment as a sequel to non-reducing TMJ disk displacement (III).

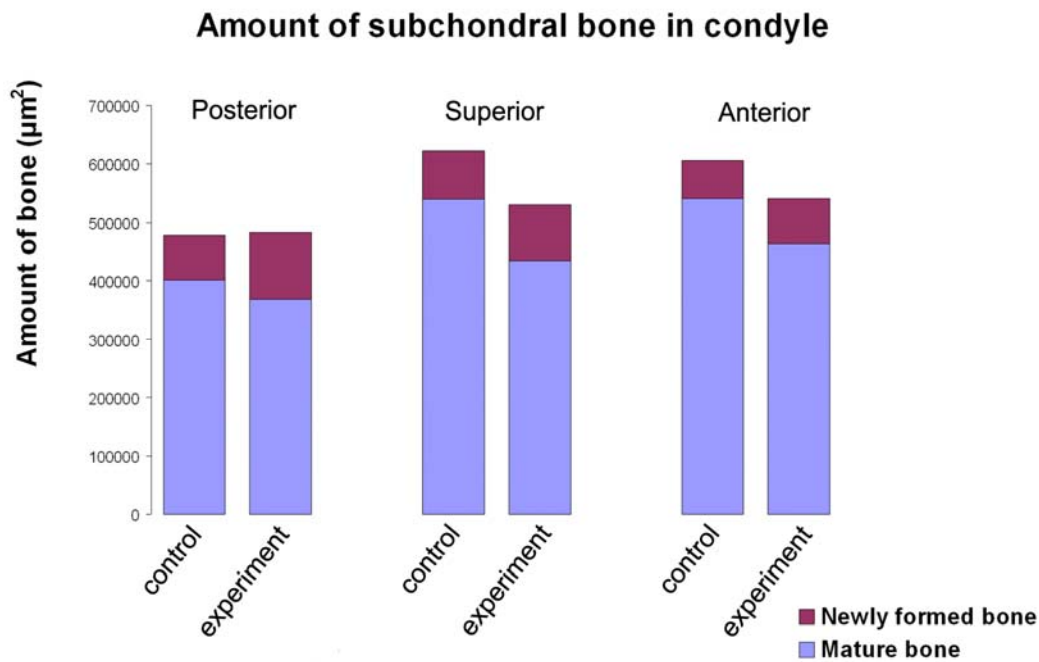
These results strongly imply that the adaptive capacity of the growing condyle is usually sufficient that the excessive functional demands following non-reducing displacement of the TMJ disk will not lead to destruction of the articular tissues. Then again, the findings implied that the cost for upholding the functional integrity of the articulating surfaces was an impaired adaptive growth capacity of the condyle (III).

### ***TMJ disk displacement and condylar subchondral bone***

A striking observation was the significant decrease in the amount of condylar subchondral bone in the experimental condyles (IV), with the articulating cartilage remaining intact (III). The extensive loss of condylar bone was not referable to surface erosion or to diminished endochondral bone formation. On the contrary, the results from the quantification of subchondral bone in the condyle as a whole implied that the articular cartilage initiated increased formation of new bone, but that an even larger subchondral bone resorption had outpaced bone formation.

However, the bone reactions were different in loaded and unloaded parts of the condyle. In the posterior non-articulating part of the experimental condyles, the substantial increase of endochondral new bone production compensated for the resorption, but also contributed to the maintenance of the total amount of subchondral bone (IV). In the articulating anterior and superior parts, the total amount of subchondral bone was considerably

reduced in the experimental condyles. Even though the endochondral production of new bone was no less than that in the control condyles, production was limited to compensation for the excessive resorption of bone. This insufficiency was plausibly related to the severity of the adaptive cartilage reaction in this area caused by non-reducing displacement of the TMJ disk (III) (Fig. 15).



**Figure 15**

Figure shows the mean amount of mature and newly formed subchondral bone. Bars illustrating control condyles and experimental condyles are paired for the three regions of interest, to exemplify the differences in subchondral bone proportions between groups in different parts of the condyle.

### ***TMJ disk displacement and mandibular growth***

An established explanation for impaired mandibular growth, as a sequel to intra articular TMJ afflictions, is degeneration of the condylar cartilage and subsequent erosive destruction of the condylar bone (Stabrun *et al.*, 1988; Schellhas *et al.*, 1993; Kjellberg *et al.*, 1995; Yamada *et al.*, 1999). Destruction of the condylar growth layers might incapacitate further growth, as well as reduce already established condylar dimensions. Because of this well accepted causality, degeneration of the mandibular condyle is regularly assumed in children and adolescents, when a retrognathic mandible is found

contemporaneous to TMJ affliction. The results of this project challenge such generalized assumptions; instead the cause of growth impairment is to be found in the intra-condylar reduction of bone (IV).

In bone metabolism the functional balance between the simultaneous production and resorption of bone is essential (for review see Lerner, 2006). In the mandibular condyle the endochondral production of new subchondral bone is appositional and therefore directly linked to condylar growth (Rabie *et al.*, 2003; Meikle, 2007). As illustrated by Figure 15, the posterior region was the only part of the experimental condyles where increased endochondral formation of new bone compensated for the resorption of subchondral bone and also contributed to the maintenance of the normative total amount of bone. As a result, the capacity of appositional bone formation was sustained in this part of the condyle (IV) (Fig. 15). In the anterior and superior parts of the experimental condyles, the formation of new bone was merely enough to compensate for the resorption of bone (IV); therefore, the formation of new bone in these parts did not contribute to normative appositional bone formation, *i.e.* growth. These results indicate that condylar growth, in effect, was incapacitated in the superior and anterior articulating parts of the experimental condyles (Fig. 15).

The findings of incapacitated appositional condylar growth in the anterior and superior parts of the condyle (IV) leave posteriorly directed growth as the only remaining potential direction for condylar development. Posteriorly directed condylar growth is most unfavorable regarding the development of mandibular retrognathia, since it strongly relates to a downward and backward directed mandibular growth rotation (Björk, 1969).

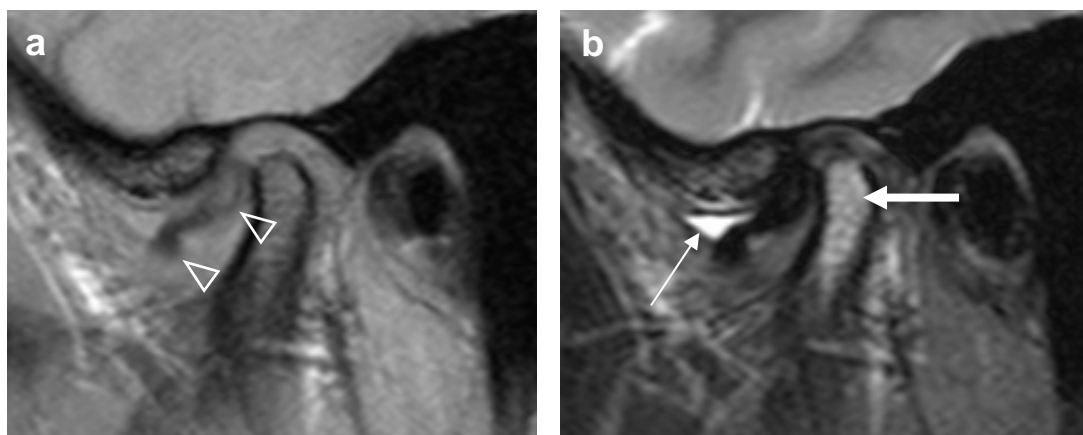
The amount of growth impairment was found to fluctuate distinctly over time (II). This inconsistency, induced by TMJ disk displacement, had not previously been revealed and the result was of major clinical significance. In the rabbit, the blood level of skeletal regulating growth hormones peaks between 10 and 14 weeks of age, with a general acceleration of the craniofacial growth rate. A smaller peak appears between 20 and 22 weeks (Masoud *et al.*, 1986). The general acceleration of facial growth rate in the rabbit model therefore corresponded to the first and third study month of this project, *i.e.* when growth retardation was observed (II). This unexpected result was interpreted as a locally induced growth reduction that counteracted growth acceleration induced by the general hormonal regulation. A simultaneous subchondral bone resorption that equals or even surpasses the maximum capacity of endochondral production of subchondral bone (IV) inevitably hampers the ability of the joint tissues to respond to growth acceleration. This will consequently "cut the peak" of

the expected increase of condylar growth during a period of elevated hormonal regulation (II).

The combined findings of this project disclosed the essential mechanisms of adverse mandibular development following non-reducing TMJ disk displacement in growing individuals (II-IV). Impaired mandibular growth not only related to a generally hampered and posteriorly directed condylar growth, but just as important, to a failure of the condylar growth layers to respond to increased growth velocity during the growth spurt.

### ***TMJ disk displacement and inflammatory reactions***

It is well established that non-reducing disk displacement induces inflammatory reactions in the TMJ. MR-imaging studies have determined that increased volume of synovial fluid, *i.e.* joint effusion, is correlated to synovial inflammatory activity (Østergaard *et al.*, 1997; Segami *et al.*, 2003). Joint effusion, as a radiographic sign of inflammation, is significantly associated with non-reducing TMJ disk displacement (Sano and Westesson, 1995; Westesson and Brooks, 1992; Larheim *et al.*, 2001a; Tomas *et al.*, 2007) (Fig. 16).



**Figure 16**

MR-images of non reducing TMJ disk displacement in an adolescent patient. **a)** Anteriorly displaced non-reducing TMJ disk (arrowheads) seen inferior to the articular tubercle. **b)** T2-weighted MR-image showing intense signal in the upper joint compartment indicating joint effusion (thin arrow), and a slightly increased signal in the bone marrow of the condyle (bold arrow).

In the non-deranged TMJ the biconcave disk fills the gap between the two incongruent bony joint components during all jaw movements. Because of the viscoelastic properties of the disk, the articular load is dispersed over a larger surface, the size of the disk (Koolstra and van Eijden, 2007).



A functional disk position is therefore crucial for prevention of damage to the articulating surfaces in areas susceptible to load concentrations.

When the disk becomes displaced, the loose connective tissue of the posterior disk attachment is pulled in between the two convex articular bony joint components, which now articulate with a pointed load uptake and without the disk's protective dispersal of the articular load. Hence, a traumatic load stress is concentrated in the smaller area, where the condyle opposes the articulating tubercle. Furthermore, the anterior part of the condyle becomes exposed to intermittent load stress, when condylar translation is obstructed by the displaced disk. Accordingly, these two parts of the condyle were where adaptive condylar cartilage changes were the most severe (III) and the reduction of subchondral bone the most extensive (IV).

Instead of the disk, the tissue between the articulating components of the joint will now be the loose connective tissue of the posterior disk attachment. In contrast to the disk, the posterior attachment is richly innervated and vascularized and the surface is covered by a layer of synovial tissue (Isberg, 2001; Segami *et al.*, 2003). Traumatic loading of the richly innervated posterior attachment is proposed to release pro-inflammatory neuropeptides, resulting in a local neurogenic inflammatory response of the articular tissues (Milam, 2005). At inflammation, active macrophages induce neovascularization (Rabie, 1997), and along with new invading blood vessels, mesenchymal cells are brought to the inflamed area. The mesenchymal cells differentiate to osteoblasts, which further enhance the osteoclastic activity as a preparation for new bone formation (Rabie and Hägg, 2002).

Osteoclasts have been observed in the subchondral bone in patients with inflammatory TMJ disease, and there is firm evidence that loss of bone in the vicinity of inflammatory processes can be attributed to increased bone resorption (for review see Lerner, 2006).

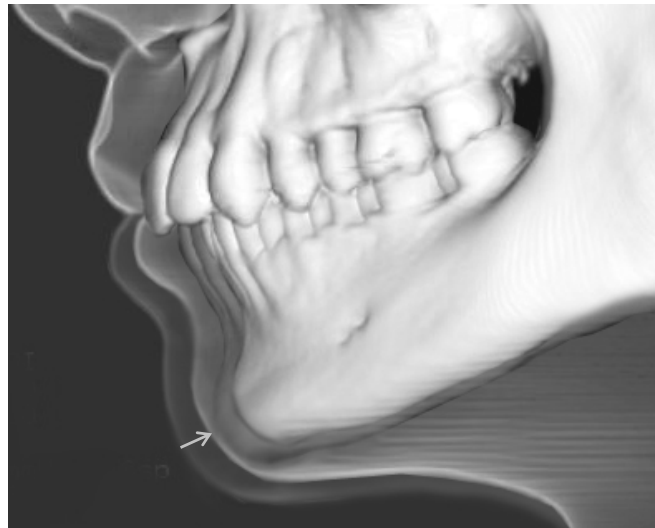
In a recent study, synovial tissue was experimentally compressed to mimic the traumatic loading of the synovial membrane that occurs when the disk becomes displaced without reduction (Ichimiya *et al.*, 2007). Compressive force was observed to enhance osteoclast formation through inflammatory cascade reactions and concluded to induce subsequent osteoclastic bone resorption in the TMJ, a sequel that was verified by the results of this project (IV).

## ***Clinical implications of the results***

Translation of results from animal to man in skeletal growth studies is feasible during equivalent developmental periods. The experimental period in this project was chosen to correspond to the ages of 6 to 18 years in man (Losken *et al.*, 1992). According to the *Bolton Standards of Dentofacial Development*, the length of the human mandible increases by approximately 25 mm (Ar-Gn) during this interval of growth (Broadbent *et al.*, 1975). When the 19 % reduction of mandibular horizontal growth observed in the rabbit is translated to the growth of the human mandible, it corresponds to a skeletal shortening of approximately 5 mm at the end of the growth period (Fig. 17).

**Figure 17**

Figure illustrates the resulting change of the chin profile (arrow) when the 19 % reduction of mandibular growth is conveyed to the corresponding growth period in man.



During the same developmental period, the human maxilla increases only by approximately 9 mm in length (PNS-ANS) (Broadbent *et al.*, 1975). The 7 % reduction of maxillary horizontal growth in this project therefore corresponds to a 0.6 mm shortening of the maxilla in man. The implication of the results in this project was that bilateral non-reducing TMJ disk displacement in man would result in retrognathia, mainly assigned to the mandible (II).

As discussed earlier, age seems to play a significant role in the progression of TMJ tissue reactions following TMJ disk displacement. The experimental finding that the articulating cartilage usually remains intact in growing individuals, in spite of gross morphological changes with flattening of the condyle and enlargement of the articulating surfaces, implicates an obvious clinical risk of false positive radiographic diagnosis of degenerative TMJ changes in children and adolescents.

The findings of a hampered superior and anterior condylar growth direction (IV) are in accordance with the clinically and experimentally well-known retrognathic skeletal appearance associated with acquired mandibular growth impairment (Schellhas *et al.*, 1993; Kjellberg *et al.*, 1995; Legrell *et al.*, 1999; Tavakkoli-Jou *et al.*, 1999; Alkhamrah *et al.*, 2002; Stoustrup *et al.*, 2008). A shortened mandibular corpus and ramus, increased antegonial notching, flattening of the superior and anterior part of the condyle, and a short and posteriorly inclined condylar process, are morphological similarities frequently associated with different forms of TMJ affliction during growth. These similarities suggest a more common cause for mandibular growth impairment following intra articular TMJ affliction than previously assumed. The findings in this project might plausibly be useful in the appreciation of impaired mandibular growth as a sequel, not only to TMJ disk displacement, but also to inflammatory joint disease and trauma involving the growing TMJ.

The one animal in this project showing maladaptive degeneration in a joint also demonstrated particularly severe impairment of mandibular growth on the ipsilateral side (III) (Fig. 18).

### Figure 18

One experimental animal displayed degeneration of the left TMJ at the end of the study period. During growth this individual showed unbalanced tooth attrition because of the large growth impairment of the left mandibular side.

With time this animal developed a forced midline shift towards the opposite side.



This rabbit was the only animal demonstrating severe dentoalveolar effects of the TMJ affliction. It developed a forced midline shift of the mandible to the right side, with loss of contact between the incisors at the end of the study period. Rabbit incisors grow constantly and without the reduction of tooth mass due to masticatory grinding, the teeth grow too long. The animal displayed osteoarthrotic degeneration of the left TMJ. Because of the subsequent major reduction of mandibular growth on the ipsilateral side (see Fig. 14) the attrition of the incisors became

asymmetrical. The asymmetrical incisors successively forced the chin to the right, *i.e.* to the side opposite the osteoarthrotic joint. At termination a midline deviation of about 6 mm could be registered in this animal, and the upper and lower incisors lacked contact and had become elongated.

This animal constitutes a good example of the distinction between the growth reduction revealed in this project, as primarily associated with adaptive TMJ tissue reactions (III), and growth impairment following maladaptive joint degeneration. As previously discussed there is reason to assume that the growing temporomandibular joint is better able than the adult to withstand maladaptive development of joint affliction such as disk displacement. However, there is also reason to consider that the articular tissues in these cases are pushed to their absolute limit of adaptive capacity. Signs of inflammatory reaction in subchondral bone inferior to an intact cartilage layer have been described in clinical MR-imaging studies. These studies showed high T2-signal in the condylar bone marrow as a frequent finding in the pediatric population with TMJ disk displacement (Schellhas *et al.*, 1990; 1993) (Fig. 16). It was discussed that this stage of TMJ pathology might rapidly develop into maladaptive degeneration of the articular surface of the condyle, if the functional demands on the joint were further increased.

Orthodontic functional therapy is shown to enhance condylar growth through adaptive cartilage reactions (Rabie *et al.*, 2003) with subsequent positive effect on the growth of the mandible (Konik *et al.*, 1997; Mills and McCulloch, 2000). In this project displacement of the TMJ disk caused adaptive cartilage reactions (III), but resulted in impaired mandibular growth (II). There are close points of similarity between the two situations in the primarily adaptive cartilage response following changes in functional demands of the joint. Both situations have a significant effect on the adaptive growth of the condyle. Hence, the diametrical difference in the resulting mandibular growth implies clinical consequences that need to be discussed.

TMJ disk displacement is common in the non-symptomatic population (Ribeiro *et al.*, 1997; Larheim *et al.*, 2001b). Clinicians have therefore been advised to consider that the condition may be overlooked because signs and symptoms of joint affliction might not occur until growth has ceased (Nebbe *et al.*, 1999; Gidarakou *et al.*, 2003). Because bilateral non-reducing TMJ disk displacement induces mandibular retrognathia (II) the prevalence of non-reducing disk displacement ought to be higher in children and adolescents with mandibular retrognathia, than in the population at large. In line with this presumption, MR-imaging studies of children and adolescents with Class II

malocclusion revealed a pre-treatment disk displacement frequency of approximately 25 % in 11-year old asymptomatic children (Chintakanon *et al.*, 2000) and 20 % in 14-year old adolescents (Ruf and Pancherz, 2000). In the latter study 40 % of the displaced disks were non-reducing and one out of seven patients had TMJ symptoms, the majority of them in association with non-reducing disk displacement.

It has been pointed out that orthodontic functional treatment with anterior positioning of the mandibular condyle could cause loss of condylar vertical height in patients with non-reducing TMJ disk displacement (Nebbe *et al.*, 1999). In these patients, subsequent degenerative condylar erosions are proposed to cause lack of treatment response, *i.e.* failure of growth stimulation, or even to aggravate mandibular retrognathia. The recommendation in these cases has been to postpone treatment until growth is completed. The results of the present project (II-IV) suggest that instead early treatment implying normalization of disk position should be considered, and in doing this the need for future corrective therapy might be reduced.

Clinical studies rarely conclude any general negative effects on the TMJ following bite jumping therapy with concomitant TMJ disk displacement. On the contrary, positive effects on small pre-treatment structural hard tissue changes of the mandibular condyles have been proposed (Ruf and Pancherz, 2000). This is to be expected in the majority of patients with reducing TMJ disk displacement, as the forward positioning of the condyle is likely to recapture the disk, and the limited retrusive translation to hinder the disk from re-displacing (Pancherz *et al.*, 1999). In these cases the bite jumping situation mimics the recommended treatment of symptomatic reducing disk displacement (Summer and Westesson, 1997).

In the case of non-reducing displacement of the TMJ disk, however, the forward positioning of the condyle would predictably provoke an increased biomechanical stress on the condyle. Such a biomechanical change might be enough to push the functional demands on already severely affected articular tissues (III) to a limit that exceeds the joint's adaptive capacity, leading to maladaptive tissue reactions and joint degeneration. Results supporting this concern are found in a longitudinal clinical study, revealing post-treatment osteoarthrotic degeneration exclusively in joints with pre-treatment non-reducing disk displacement (Ruf and Pancherz, 2000). This finding indicates that forward positioning of the condyle at functional treatment might lead to degenerative aggravation and joint destruction. On the other hand, orthodontic functional therapy is plausibly a most feasible choice for treatment for a large number of orthodontic patients with orthopedic TMJ

affliction, *i.e.* children and adolescents with reducing disk displacement. This reasoning provided that the functional position of the TMJ disk is known prior to treatment and controlled throughout the course of treatment.

The results of this project illustrate that full appraisal of TMJ disk position in the orthodontic adolescent population should not be neglected, and shows the value of consideration of TMJ disk function prior to, and just as important, during orthodontic functional therapy. A paradigm shift should be considered regarding indications for radiographic evaluation of the TMJ disk status prior to growth-stimulating orthodontic treatment.

## CONCLUSIONS

- The longitudinal experimental method presented allowed congruent positioning of the animal skull relative to the film-focus assembly at repeated radiographic examinations allowing high precision superimposition of serial radiographs.
- Bilateral non-reducing TMJ disk displacement in growing rabbits caused significant bilateral reduction of mandibular and maxillary horizontal growth. The amount of craniofacial growth reduction was estimated to result in mandibular retrognathia in man.
- Non-reducing TMJ disk displacement during the growth period induced condylar cartilage adaptive reactions which correlated to both the amount and direction of mandibular growth, manifesting in a retrognathic growth pattern.
- Non reducing TMJ disk displacement during the growth period significantly decreased the overall amount of subchondral bone in the mandibular condyle, offering an explanation for the subsequent adverse mandibular growth.
- The locally induced growth reduction caused by a displaced non-reducing TMJ disk counteracted general growth acceleration induced by the general hormonal regulation, and can be expected to significantly diminish the spurt of mandibular growth during puberty.
- A displaced non-reducing TMJ disk will plausibly neutralize growth stimulation intended to result from mandibular advancement with the aid of jaw-protrusion devices, and functional treatment might aggravate the primarily adaptive reaction of the joint to a state of maladaptation and joint degeneration.
- The findings of intact articular layers in spite of gross histological and morphological soft and hard tissue changes as a sequel to TMJ disk displacement in growing individuals, implicate a clinical risk of false positive radiographic findings of degenerative changes of the TMJ in children and adolescents.

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