Journal of Oral Rehabilitation

*Journal of Oral Rehabilitation* 2013 **40**; 279–295

**Review Article**

# The difficult relationship between occlusal interferences and temporomandibular disorder – insights from animal and human experimental studies

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SUMMARY **The aetiology of temporomandibular disorder (TMD) is multifactorial, and numerous studies have addressed that occlusion may be of great importance. However, whether occlusion plays a crucial role in the pathogenesis of TMD remains controversial. Study designs utilising animal models have been used to study the effects of artificial occlusal alterations. Experimental traumatic occlusion affects blood flow in the temporomandibular joint and results in changes in the condylar cartilage, and artificial occlusal interference induces masticatory muscle nociceptive responses that are associated with peripheral sensitisation and lead to central sensitisation, which maintains masticatory muscle hyperalgesia. The possibility that occlusal interference results in TMD has been investigated in humans using a double-blind randomised design. Subjects without a history of TMD show fairly good adaptation to interferences. In contrast, subjects with a history of TMD develop a significant increase in clinical signs and self-report stronger symptoms (occlusal discomfort and chewing difficulties) in response to interferences. Meanwhile, psychological factors appear meaningful for symptomatic responses to artificial interferences in subjects with a history of TMD. Thus, individual differences in vulnerability to occlusal interferences do exist. Although there are advantages and disadvantages to using human and animal occlusal interference models, these approaches are indispensable for discovering the role of occlusion in TMD pathogenesis.**

KEYWORDS: **occlusion, temporomandibular disorder, animal, human**

**Accepted for publication 3 January 2013**

## Introduction

Occlusal alteration is commonly seen among patients from prosthodontic, orthodontic, periodontic and surgical department clinics (1–4). Dentists need to consider that many common treatments, such as the use of dentures for missing teeth, correction of malocclusion or extraction of teeth, can significantly alter occlusion in patients. However, what will happen following occlusal alteration? Clinical experience suggests that procedures which alter occlusion can result in acute patient discomfort, which may then subside over a few days or progress to result in patient complaints of chronic pain in the stomatognathic system and include the development of a temporomandibular disorder (TMD).

Temporomandibular disorder is comprised of a group of chronic pain conditions that can involve the temporomandibular joints (TMJs), masticatory muscles and associated structures and tissues (5). Temporomandibular disorder is most commonly observed in individuals between the ages of 20 and 40 years (1, 6, 7). Approximately 33% of the population has at least one TMD symptom, and 3·6% to 7% of the



doi: 10.1111/joor.12034

population has TMD with sufficient severity to cause them to seek treatment (1, 8). The aetiology of TMD is multifactorial, as biomechanical, neuromuscular, biopsychosocial and neurobiological factors may contribute to the disorder (9). These factors are classified as predisposing (structural, metabolic and/or psychological conditions), initiating (trauma or repetitive adverse loading of the masticatory system) and aggravating (parafunctional, hormonal or psychosocial factors) to emphasise their roles in the progression of TMD (10).

Studies have evaluated the important role of occlusal alteration in the aetiology of TMJ disorders. Nevertheless, there is no consensus on the importance of this factor for the development of TMJ dysfunctions. Occlusion is frequently cited as a major aetiological factor of TMD (11–14). Numerous aetiological and therapeutic theories are based on this presumed association and have been applied to justify the use of several therapeutic approaches, such as occlusal appliance (15) and anterior repositioning appliance (16) therapies, occlusal adjustment (17), restorative procedures (17) and orthodontic (18) and orthognathic (19) treatments. Conversely, many TMD experts hold opposing views (20–22), and various types of dental interventions, including routine orthodontic treatment, have been reported as causes of TMD (23).

The large number of clinical variables associated with TMD has made explaining the aetiological role of occlusal maladjustment difficult, hindering study standardisation and subsequent discussion. In the light of the elevated controversy and extreme importance of this theme for clinical practice, many clinical studies have focused their efforts here. A number of studies have demonstrated that experimental occlusal interferences (EOIs) might cause changes in the myoelectric contraction patterns of the human jaw muscles (24–29). It is apparent that EOIs are associated with short-term clinical symptoms and signs, such as pain and fatigue of the jaw muscles, headache and pain and clicking in the TMJs. However, due to ethical concerns regarding the potential for irreversible damage to human subjects in long-term occlusal interference studies, it has not yet been unequivocally established that the observed changes result in specific long-term detrimental effects. Animal models have thus become a valuable means to investigate this issue.

## Animal studies

### *Creating occlusal alterations*

Various study designs utilise animal models with artificial occlusal alterations. In general, the animal models can be grouped into three types: (i) occlusal elevation (bite-raising), that is, a device is placed on the local position to form the interference or raise the occlusal plane; (ii) occlusal elimination (occlusal loss), that is, exodontias or grinding is performed until occlusal contact is lost; and (iii) occlusal extrusion, that is, an orthodontic approach is conducted to disturb the occlusion.

Occlusal elevation, which can be achieved by direct filling, restoration or application of an adhering band, either unilaterally or bilaterally, is the most commonly used of the three model types. Direct filling for occlusal elevation means preparing a hole on the occlusal surface of the tooth and filling it with silver amalgam (30). This direct filling method cannot be quantified easily due to operating inside the mouth and usually destroys the pulpal tissues of rats, strongly stimulating pulpal nerve fibres. Pre-made onlays, pre-made crowns and orthodontic bands were cemented to one or more teeth to raise the bite (31–36). These methods are more easily and precisely quantified because the aforementioned appliances can be measured at multiple points out of the mouth before being cemented. Occlusal interference can also be achieved easily by cementing a square wire to the tooth. However, maintaining the bond of the wire to the tooth for a long-term study can be problematic. An appliance can be bonded to the mandibular incisors to open the bite (37).

In addition to exodontias and grinding, occlusal loss can be induced by amputation of the cusps of the molars either unilaterally or bilaterally (38). However, this method is difficult to quantify because it requires direct amputation from inside the animal's mouth. To achieve unilateral mastication, extraction of all the molars on one side can result in a large amount of trauma (39). Another approach is daily trimming of the upper and lower incisors on one side to maintain an incisor disclusion (40).

### *Occlusal alterations and TMJ*

Animal studies have been used to address the putative role of occlusal interference on TMJ remodelling (41,

42). In a monkey study, the vertical dimension of occlusion (VDO) was increased by 4 mm through the use of bilateral metal splints at the first molar area over the course of 15 and 55 days (41). Histological analysis of the TMJ showed evidence of destructive bony tissue changes in the condyles, glenoid fossa and neck of the condyle in both the 15- and 55-day specimens. In the 55-day specimens, the condyle was displaced mesio-inferiorly, and the articular eminence and condylar head were flattened. One likely explanation for these changes is the overloading of the TMJ stemming from occlusal change. Both flat and pivoted splints caused traumatic changes in the TMJs (41). Similarly, a miniature pig study showed that 2 months of wearing the open-bite splint and protrusive-bite splint induced remodelling and injury of TMJ tissues (42). Despite the clinical effectiveness of occlusal splints in relieving symptoms of TMD (43), their mechanisms of action are unknown.

The effect of occlusal interference on the TMJ was assessed using a rat model with 1-mm-high EOI unilaterally on the right maxillary first molar (44). Fluorescent microspheres were injected into the rat to allow the observation of changes in blood flow in TMJs. After 15–20 days, blood flow was increased on the ipsilateral side relative to the contralateral side. There was also an increase in blood flow in both the ipsilateral and contralateral TMJs in the experimental animals compared with the controls (44). These results were interpreted as a demonstration of altered joint loading due to the interference and a change in blood flow possibly related to tissue damage and inflammation.

The articular surface of the TMJ is considered a load-bearing region that accommodates the compressive, shearing and tensile stresses generated by various jaw functions (45). Temporomandibular joint cartilage is comprised mostly of type I collagen. Although type II collagen is present to a lesser extent, it contributes to the biomechanical properties of TMJ cartilage and is functionally important in tissues subjected to compressive forces as well as tensile forces (46–48). The histological changes seen in the immediate response to artificial abnormal occlusion in animal models have most commonly included the thickening of the condylar cartilage (49), hyperplasia and alterations in the morphology of chondrocytes (49, 50), changes in the proliferation and maturation of chondrocytes (40) and an elevated level of sulphated glycosaminoglycans (40, 49).

Findings from previous studies show that proteoglycan expression and glycosaminoglycan synthesis increase in response to increasing compressive forces applied to TMJ articular tissues (50–54) and decrease as a result of a reduction in TMJ compressive forces (47, 55, 56). Thus, the enhanced expression of proteoglycans resembling aggrecan and versican may suggest an increase in the magnitude of compressive forces projected onto the mandibular condyle in response to unilateral bite raise in the rat. The apparent increase in the loading of the ipsilateral (treated) TMJ, as opposed to the contralateral TMJ, is likely due to the fact that the two halves of the rat mandible are linked by a fibrous joint at the mandibular symphysis (50).

Alteration of loading at the rat TMJ brings about changes in type II collagen. Immunohistochemical analysis showed that increased VDO decreased the amount of type II collagen in the condylar cartilage of a rabbit (57). In a rat model with removal or shortening of incisors and a change in diet (such as providing a soft diet), the deposition of type II collagen is decreased, as shown by immunohistochemistry and image analysis (48, 58), and the type II collagen is more wavy, rough and irregular than normal (59). The condylar cartilage and disc adapt to the imbalance induced by unilateral extraction of teeth through chondrocyte repair responses involving type II collagen expression that appears to differ between the functional and non-functional sides of TMJs (49, 60). Huang *et al.* (60) showed an increase in antitype II collagen antibody binding to the disc and condylar cartilage after unilateral extraction of teeth, in accordance with some previous studies on altered loading of the TMJ (48, 57, 58, 61, 62), but contrary to others (59). It seems likely that these contrasting results are due to the different animal models used, a difference in the magnitude and nature of the external joint loading forces and differences in the timing of observation. Huang's findings may thus provide insight into a basic mechanism of molecular changes in an adaptive response of the TMJ to unilateral mastication. Further studies are necessary to explore whether differences in the expression of type II collagen in the TMJ cartilage persist in the long term and whether the expression of this molecule in the extracellular matrix of the

articular cartilage changes with progression to osteoarthrosis.

Controversy remains as to the effect of an altered occlusion on the mandibular condylar cartilage. Functional changes in some animal models produce an increase in the growth of the mandibular cartilage (63), while other studies have exhibited the opposite response (64). A histomorphological study has demonstrated that experimentally created disordered occlusion leads to an obvious degradation in the mandibular condylar cartilage of rats, characterised by the reduced proliferation and increased death of chondrocytes, predominantly in females (65). Further studies, including biomechanical analysis of the rat TMJ under the same experimental conditions, are needed. The rehabilitative ability of the degraded TMJ, as well as the role of oestrogen in the process, is also worthy of further study (65).

*Occlusal interference and masticatory muscles*

In the stomatognathic system, masticatory muscles, such as the masseter, temporalis and lateral pterygoid muscle, play a central role in the masticatory mechanism. Spatial orientation and physiological cross section of masticatory muscle fibres are not the only determining factors for adjusting masticatory strength; fibre composition is also crucial. In general, the fibres of skeletal muscles can be classified into two categories: rapidly contracting, phasic, fast-twitch type II fibres and slowly contracting, tonic, slow-twitch type I fibres (39).

To study the effects of occlusal interference on masticatory muscle activity, electromyography (EMG) has been employed to record muscle potentials during mandibular movements using surface electrodes or needle electrodes. An electromyographic study demonstrated the preference of pigs to chew food on the bite splint side and the resulting increase of integrated activities of the jaw-closing muscles, especially on the contralateral side (66). However, in rat experiments using inserted bilateral bite-raising splints, reduced EMG activity was observed in the deep masseter of some rats, while others showed increased activity in the anterior temporalis and superficial masseter muscles during the late phase of opening (67). After approximately 7 days, all of the rats exhibited decreased EMG activity (67). This result indicates that splint treatment in rats is effective at reducing EMG activity and that some animals may have the ability, through certain biological mechanisms, to adapt to physical disturbance.

Bilateral muscle changes following insertion of a unilateral occlusal splint may occur in human jaw muscles. Physiopathological and morphological changes were observed bilaterally in the deep masseter muscle of rats having a unilateral occlusal splint fixed to their mandibular molars (68). The muscles on the splint side progressively recovered to their natural state, but those on the contralateral side displayed a persistent increase in type IIb fibres. It has been indicated that type IIb myosin is not found in human muscle (69, 70). Thus, these observations in rats might not be applicable to the human masseter.

The experimental rat model was set up by amputating the cusps of the superior and inferior molars of the left side and excising the ipsilateral and contralateral masseter muscles 26 days later. The occlusal dysfunction resulted in microvessel constriction, morphological damage of muscular fibres and capillary endothelium and elevation of tissue calcium content in the ipsilateral masseter muscle (38). These changes are probably related to muscle fatigue and ischaemia, and because tissue areas rich in type I muscle fibres are characterised by a predominantly aerobic metabolism, early signs of injury are readily exhibited. The ipsilateral muscle damage became more extended and severe with time, while the contralateral muscles showed only slight alterations that were reversible with time, possibly due to an adaptive response (38). These muscle changes were nearly abrogated by dantrolene, thus supporting it as a possible new therapeutic tool for the treatment of malocclusion-induced muscle diseases (71). Amputation of the cusps, however, cannot be quantified and may lead to pulp damage, potentially resulting in erroneous data and confusion of the results.

Muscle spindles are mechanoreceptors that are sensitive to changes in muscle length, and they are important proprioceptors responsible for controlling jaw position and movement. They are present in large numbers in jaw-closing muscles but not in jaw-opening muscles, so that they are considered to be involved in stabilising jaw position (72–74). The spindle afferents in jaw-closing muscles are thought to play a significant functional role in the regulation of the activity of these muscles. The rat experiment of occlusal loss demonstrates this point with immunohistochemical

evidence for the re-expression of growth-associated protein-43 (GAP-43) in some parts of fully differentiated masseter and temporal muscle spindle nerve endings, which are associated with ultrastructural changes (75). Using an electrophysiological method, Brunetti *et al.* (76) demonstrated that muscle fatigue affected jaw-closing muscle spindle activity, causing a decrease in the discharge frequency and potential amplitude of masseter muscle spindle afferents. In the rat study of unilateral molar cusp amputation, occlusal alteration resulted in severe damage to the extrafusal muscle and affected predominantly capsular cells, intra-fusal muscle fibres and sensory nerve endings (77). These findings indicate that occlusal alteration and related muscle fatigue might be involved in the pathogenesis of human TMD.

*Occlusal interference and the nervous system*

Over the last few decades, many studies have reported the effects of occlusal trauma on oro-facial tissue, and more and more attention has been paid to the possible effects of occlusal trauma on the nervous system.

*Mechanical hyperalgesia.* To detect the mechanism of hyperalgesia induced by occlusal trauma, Chen *et al.* (36) used nociceptive behaviour assessment to examine a rat model having an orthodontic square wire bonded onto the occlusal surface of the first upper molar to raise it approximately 1 mm unilaterally. The rats were assessed for nociceptive behaviour at 1, 2, 4, 8, 24 and 72 h after bite-raising, and a bilateral mechanical hyperalgesia response to mechanical stimulation was noted at 8, 24 and 72 h (36). Another rat study revealed mechanical hyperalgesia of the skin in the temporalis and masseter muscles after 1-mm bite-raising (78).

Cao *et al.* (32) created an occlusal interference animal model by directly bonding a crown to a maxillary molar to raise the masticating surface to three different heights (0·2, 0·4 and 0·6 mm) for 1 month. They quantitatively measured mechanical nociceptive thresholds of the temporal and masseter muscles on both sides using the electronic von Frey anaesthesiometer with a round cap (diameter 3 mm). Mechanical hyperalgesia was significantly associated with the height of the occlusal alteration; animals with 0·4- and 0·6-mm crowns showed more decreased nociceptive thresholds than those with 0·2-mm crowns, demonstrating a cause and effect relationship between occlusal interferences and muscle hyperalgesia (32). Removal of the crown 6 days after occlusal interference did not terminate the 1-month duration of mechanical hyperalgesia in the masticatory muscles. These findings suggest that occlusal interference can directly induce nociceptive responses of masticatory muscles and that central sensitisation is involved in maintaining this mechanical hyperalgesia. The research group developed a modified tip for the von Frey filament capable of transmitting pressure onto the masticatory muscles, avoiding skin stimulation that would evoke cutaneous nociceptive responses typically elicited by sharp von Frey filaments. In their experiments, the NMDA antagonist MK801 attenuated the occlusal interference–induced hyperalgesia in a dose-dependent manner, and no evidence of ongoing muscle trauma was found (33), which suggests that central sensitisation mechanisms are involved in the maintenance of the occlusal interference–induced mechanical hyperalgesia. Their data strongly indicate that occlusal interference can directly cause long-term masticatory muscle response in a laboratory animal model. Whether this mechanism may account for cases of TMD in humans requires further investigation.

Cao *et al.* further studied the histological changes of masseter muscles from rats having 0·4-mm-thick crowns bonded to their first maxillary molar using H&E stains and substance P (SP) and protein gene product 9·5 (PGP9·5) immunohistochemical stains. Intense staining of PGP9·5 was observed at day 1 in occlusal interference groups, and this level of staining was maintained for the duration of the experiment. Substance P expression in masseter muscles of both sides peaked at day 5 and then gradually decreases to the level of the control. There were no inflammatory cells present in either side (33). Their study suggests that although no evidence of muscle damage and inflammation was found, peripheral sensitisation appears to be involved in the mechanism of EOI–induced masticatory muscle response. However, peripheral sensitisation of nociceptive neurones cannot fully account for the long-standing nociceptive responses of masticatory muscles; a central sensitisation mechanism may also be involved.

*Spinal trigeminal nucleus complex.* Sensory information from the cranial oro-facial region is first relayed in the spinal trigeminal nucleus complex (SpV), which

consists of the subnucleus oralis (Vo), subnucleus interpolaris (Vi) and subnucleus caudalis (Vc) (79, 80). Oro-facial nociceptive input is initially processed in the Vc (81), which due to morphological similarities to the spinal dorsal horn and having been accepted as the first-order relay nucleus to the higher centres for trigeminal nociceptive information, is also called the medullary dorsal horn (MDH) (82, 83). However, a recent study has identified that trigeminal pain processing in the MDH is distinctly different from that of the spinal dorsal horn (84). The superficial laminae of the Vc are known to contain nociceptive-specific neurones (85, 86), and the transganglionic tracing method shows that the dorsomedial region of Vo (Vodm) receives intra-oral primary afferent innervation.

In a rat experimental model, Zhu *et al.* (87) inserted and bonded a retention nail in the pulp chamber to raise the bite by 0·3–0·8 mm. They then observed the up-regulated expression of SP and NMDAR1 in Vc and a significant change in grey scale staining between the experimental and control sides at day 7, implying that sensitisation of the primary centre is a mechanism of chronic oro-facial pain following occlusal trauma. Because the insertion of a retention nail into the pulp chamber caused a nociceptive stimulus, this result cannot be interpreted solely as occlusal trauma.

The role of astrocytes in Vc during occlusal trauma has been investigated in rats having a section of square orthodontic steel (0·5 mm high) bonded to the occlusal surface of the left first maxillary molar (88). The presence of glial fibrillary acidic protein (GFAP) was used as an indicator of astrocyte activation. The number of GFAP-containing astrocytes was increased in Vc 3 days after occlusal trauma, and the density of GFAP immunoreactivity in the experimental side peaked at day 7 and decreased at day 14. The increased presence of GFAP-containing astrocytes implied the possible involvement of astrocytes in traumatic occlusal pain.

*Parabrachial nucleus.* The parabrachial nucleus (PBN) is a bilateral cluster of neurones surrounding the superior cerebellar peduncle at the border between the pons and mesencephalon (87, 89–91). Anatomical and physiological studies have shown that this nucleus plays an important role as a relay nucleus in somatosensory functions, particularly in nociception originating from neurones located in the superficial layers of the spinal and MDH (92–96). The trigeminal nociceptive endings stimulated by oro-facial pain, including trauma, terminate mostly in the caudal portion of the parabrachial external medial, external lateral and Kölliker–Fuse subnuclei (95, 97, 98). Although traditionally accepted as a complement to the classical trigeminal–thalamic tract, the trigeminal–parabrachial tract appears to play a major role in mediating oro-facial pain (36). Some neurones in the SpV project certain areas of the PBN (99–102), and various stimuli to the trigeminal nerve induce the expression of Fos protein in the PBN (103).

The effects of bite-raising on astrocytes in the PBN were detected with the expression of GFAP in PBN of ipsilateral and contralateral sides with and without administration of fluorocitrate (FCA), an inhibitor of glia metabolism (104). The expression of GFAP in PBN of both sides was up-regulated 4 h after molar occlusal alteration, reaching peak levels at 24 h and then gradually becoming down-regulated (36). Pain response in the PBN of the FCA-treated bite-raising group following occlusal trauma was significantly inhibited at both the 8- and 24-h time points relative to the untreated group (36). However, because FCA is a small molecule, it can also easily diffuse further into the Vc, hypothalamus, thalamus, amygdala or the rodent anterior cingulated equivalent and affect pain processing there. Although astrocytes in the PBN are most likely involved in causing post-occlusal hyperalgesia, this could not be confirmed as the researchers were unable to present evidence that astrocytes in these other locations were not affected by FCA and/or not up-regulated following occlusal trauma. This issue will undoubtedly require attention for future studies.

Bidirectional communication between neurones and astrocytes has been well described, but the extent to which neurones and astrocytes interact under the various conditions and at the different levels of the nervous system remains controversial. Neurones and astrocytes act as a multifunctional unit in the brain (105). By releasing neuroactive molecules, including neurotransmitters, astrocytes may directly participate in information processing in the neurone–astrocyte network. Astrocytes are known to respond to the synaptic release of various neurotransmitters, such as glutamate (106, 107), gamma-aminobutyric acid (108), noradrenaline (109, 110) and acetylcholine (111). Chen *et al.* (36) showed that the time to peak Fos expression (2 h) was shorter than that of GFAP

(24 h) in rat PBN. The difference observed could simply reflect the different lag time in the expression of these two proteins, but a functional interaction between astrocytes and neurones cannot be excluded. The mechanism of possible interaction between neurones and astrocytes in PBN is, however, difficult to explain. Further research is needed to assess whether the neurones that are excited more quickly are those that initiate the activation of the astrocytes in PBN after bite-raising.

*Other brain structures.* In addition to trigeminal sensory subnuclei and PBN, other brain structures have been studied for their roles in modulation of nociceptive information (112). Adjustment of the non-activated appliance on incisors increases Fos expression in many areas, including the dorsal raphe (DR), periaqueductal grey matter (PAG) and locus coeruleus (LC). The descending pathways modulating pain are composed of several neural connections that form a circuitry (112). The DR is an important nucleus in pain modulation that participates in a central antinociception circuitry together with the PAG and LC (113–115), and LC is a source of noradrenergic input to the Vc (112, 116). Because the ventrolateral part of the DR also projects to the Vc and LC, its neurones could affect pain transmission in the Vc both directly and indirectly (via the LC pathway) (113, 117, 118). The PAG can also send descending inhibitory fibres directly to the trigeminal sensory complex (119).

The effect of bite-raising on LC in the pons was also investigated (35). The expression of Fos, GFAP and OX42 of LC peaked in rats at 2, 24 and 24 h, respectively, indicating that neurones, astrocytes and microglia in LC were involved in the bite-raising response (35).

### *Discussion*

Temporomandibular disorder is a multifactorial pathology that is inherently difficult to study because a direct correlation between cause and effect, such as the relationship between occlusal interference and TMD-related pain, cannot effectively be demonstrated. The factors are so mixed that we do not have adequate diagnostic instruments to establish a clear correlation or to know if, how or when occlusal alteration can unbalance the stomatognathic system.

Based on the information gathered from the animal experiments, it seems evident that occlusal interferences can lead to the development of TMD and to an increase in their severity. However, we should be aware that in the animal studies, the occlusal alteration is created acutely, which does not appear in humans except for inadequate prosthesis and incorrect occlusal adjustment. Many scholars have also questioned the extent to which we have altered the occlusion in the animal model. Assuming proportional scaling, most occlusion alterations are quite large for a small animal, such as a rat. Because experimental conditions in animals are inconsistent with the clinical situation in humans and there are intrinsic anatomical and behavioural differences between the masticatory apparatus of humans and rats, results from animal studies cannot be directly extrapolated to humans.

Experimental occlusal interferences in human subjects are associated with short-term clinical symptoms and signs, such as pain and fatigue of the jaw muscle as well as pain and clicking in the TMJs (120, 121). However, due to ethical concerns regarding the potential for irreversible damage to human subjects from long-term occlusal interference studies, it has not yet been unequivocally established that the observed changes result in specific long-term detrimental effects. Despite the noted problems associated with using animal models, they remain a valuable means to study this issue (Table 1). Animal models that mimic, as much as possible, clinical occlusal alterations are needed to further explore the role of this factor in TMD pathogenesis.

## Human studies

Since 1999, human studies dealing with experimental occlusal alteration have primarily focused on changes in jaw movement, muscle activity, clinical symptoms, etc., resulting from different artificial occlusal interferences.

### *Occlusal alteration and jaw movement*

In a study of 20 healthy human subjects, metallic occlusal overlays were made for the lower canine, canine to the second molar on the working side and second molar on the balancing side to simulate a canine protected occlusion (CO), group function

**Table 1.** Sample characteristics, outcome assessment methods and main results of selected animal studies

| Reference article | Size of EOI | Type of EOI | Duration of study | No. of animals | Outcome assessment method | Results |
|---|---|---|---|---|---|---|
| Ruben and Mafla (41) | 4 mm | Bite-raising: metal flat and pivoted splints, bilaterally | 15–55 d | 6 monkeys | TMJ by histological analysis | Destructive bony tissue changes in the condyles, glenoid fossa and neck of the condyle. In 55-day specimens, the condyle was displaced mesio-inferiorly and there was flattening of the articular eminence and the condylar head |
| Kvinnsland *et al.* (44) | 1 mm | Bite-raising: composite, unilaterally | 20 d | 30 rats | Blood flow in the TMJ by injecting fluorescent microspheres | Increase in blood flow at 15–20 d in ipsilateral TMJ and increase in blood flow in both TMJs compared with the controls |
| Rashed *et al.* (57) | 1·5 mm | Bite-raising: composite, bilaterally | 6 w | 10 rabbits | TMJ by histomorphometrical and immunohistochemical techniques | Increase in volume of experimental condylar cartilage. No evidence of production of type I collagen |
| Zhang *et al.* (66) | 2–3·8 mm | Bite-raising: splint, unilaterally | Same day | 3 mini pigs | EMG and jaw movement | Increase in integrated activities of jaw-closing muscles, especially on contralateral side, mainly due to prolongation of burst durations. Jaw-opening activity increasing. Preferred to chew food on bite splint side |
| Bani *et al.* (38) | N/A | Occlusal loss: unilateral amputation of molar cusps | 14–26 d | 20 rats | Muscles by microscopy, morphometry and calcium content of tissue | Occlusal dysfunction leading to microvessel constriction, morphologic damage of muscular fibres and blood capillary endothelium and elevation of tissue calcium content in the ipsilateral masseter muscle. Early signs of injury mainly restricted to areas rich in type I (slow) muscle fibres |
| Miehe *et al.* (39) | N/A | Occlusal loss: bilateral extraction of lateral teeth | 14–98 d | 168 rats | Mass proportions and muscle fibre composition in the masticatory muscles | Reduction in muscle dry weight and shift in muscle fibre composition in favour of type IIb fibres, indicating less masticatory strength required. Adaptation occurred on macroscopic and microscopic levels |

(continued)

**Table 1.** *(continued)*

| Reference article | Size of EOI | Type of EOI | Duration of study | No. of animals | Outcome assessment method | Results |
|---|---|---|---|---|---|---|
| Muller *et al.* (68) | 1·5 mm | Bite-raising: occlusal splint, unilaterally | 4–30 d | 38 rats | Proportion of MHC isoforms in deep masseter muscle | Increasing proportion of MHC type I isoforms found in deep masseters until 7 d after splint insertion. Type I fibres distributed on either side of muscles. At 15 d, a decrease in the percentage of the type IIb MHC isoform was observed on the splint side, and at 30 d, the proportion returned to baseline. On the contralateral side, this proportion was increased |
| Bani and Bergamini (71) | N/A | Occlusal loss: amputation of molar cusps, unilaterally | 26 d | 22 rats | Masseter muscles by microscopy, morphometry and calcium content of tissue | In the absence of dantrolene, occlusal alteration leading to microvessel constriction, morphologic damage of masseter muscle fibres and blood capillaries and elevation of tissue Ca2 + content. These changes nearly abrogated by dantrolene. |
| Zhu *et al.* (87) | 0·3–0·8 mm | Bite-raising: unilaterally | 7,15, 30 d | 16 rats | Immunohistochemistry: expression of SP and NMDAR1 in trigeminal subnucleus caudalis (Vc) | At 7 d, expression of SP and NMDAR1 was up-regulated and grey scale of staining was significantly different from the control side; at 15 d, higher expression with no statistical significance; at 30 d, density of SP- and NMDAR1-immunoreactive structures in experimental side higher or lower than the control side |
| Santiwong *et al.* (75) | N/A | Occlusal loss: the upper molars extracted and the upper and lower incisors cut-off every other day | 1–4 w | 64 rats | PGP 9·5 or GAP-43 in muscle spindles by immunohistochemistry | GAP-43 immunoreactivity reappeared in nerve endings of muscle spindles by 3 d and persisted for at least 28 d. Afferent terminals exhibited various fine structural abnormalities. Some sensory-terminal profiles completely engulfed by intra-fusal fibres. GAP-43 expression and ultrastructural alterations became undetectable within a week of the end of incisal cutting and the recovery of incisal contact |

(continued)

**Table 1.** *(continued)*

| Reference article | Size of EOI | Type of EOI | Duration of study | No. of animals | Outcome assessment method | Results |
|---|---|---|---|---|---|---|
| Sindelar *et al.* (42) | 5 mm | Bite-raising: intra-oral open-bite splint, protrusive-bite splint | 2 m | 9 miniature pigs | TMJ morphology in general and collagen orientation of the intra-articular disc | The discs of the protrusively-splinted group showed an increased thickness of the posterior band and minor changes in collagen orientation of the anterior band. Presence of a degenerative osseous defect on the medial side of the mandibular condyle in half of the splinted animals |
| Huang *et al.* (49) | N/A | Occlusal loss: lower right teeth extracted | 3–6 w | 15 rabbits | TMJ by morphological analysis and glycosaminoglycans (GAGs) in the TMJ condyle and disc | Thickening of the condylar cartilage, alterations in the morphology of chondrocyte nuclei in the condylar cartilage and disc and increases in levels of negatively charged ions in the hypertrophic layer of condylar cartilage. Small differences observed between functional and non-functional sides of TMJ |
| Chen *et al.* (36) | 1·0 mm | Bite-raising: bonding an orthodontic square wire, unilaterally | 1, 2, 4, 8, 24, 72 h | 84 rats | Nociceptive behaviour assessment and immunofluorescence double labelling for c-Fos and GFAP in PBN of bite-raising and FCA-pre-treated rats | Expression of GFAP in PBN of ipsilateral and contralateral sides up-regulated 4 h after occlusal changes in molars, peaking at 24 h and gradually down-regulated. Inhibitor of glia metabolism, FCA, reduced astrocyte activation and attenuated development of pain hypersensitivity |
| Cao *et al.* (32) | | Bite-raising: metal crown, unilaterally | 28 d | 60 rats | Nociceptive behaviour measurement in masseter and temporalis | A stimulus–response relationship disclosed between height of occlusal interference and muscle hyperalgesia. Removal of the crown 6 d after occlusal interference could not terminate 1-month duration of mechanical hyperalgesia. MK801 dose dependently attenuated this occlusal interference–induced hyperalgesia |
| Cao *et al.* (33) | 0·4 mm | Bite-raising: metal crowns, vvunilaterally | 1, 5, 10, 21 d | 15 rats | Nociceptive behaviour assessment and H&E, SP and PGP 9·5 immunohistochemical staining of masseter muscles | Decreased mechanical threshold in masseter muscles on both sides and no inflammatory cells found. Intensely stained PGP 9·5 observed from 1 to 21 d. SP expression peaking at 5 d and then decreasing to control level |

(continued)

**Table 1.** *(continued)*

| Reference article | Size of EOI | Type of EOI | Duration of study | No. of animals | Outcome assessment method | Results |
|---|---|---|---|---|---|---|
| Jia *et al.* (88) | 0·5 mm | Bite-raising: bonding square wire, unilaterally | 1, 3, 7 d<br>2, 4, 5 w | 35 rats | Vc by immunofluorescence and histochemical technique | Number of c-Fos-containing neurones increased significantly in experimental side of Vc at 7 d after occlusal trauma; maximum number of c-Fos-containing neurones found at 2 w and returned to control level at 4 w |

EOI, experimental occlusal interference; EMG, electromyography; MHC, myosin heavy chain; SP, substance P; NMDAR, *N*-methyl-D-aspartate receptor; Vc, subnuclei caudalis; PGP, protein gene product; GAP, growth-associated protein; GFAP, glial fibrillary acidic protein; FCA, fluorocitrate; PBN, parabrachial nucleus; d, day(s); w, week(s); m, month(s).

occlusion (GO) and bilateral balanced occlusion (BO), respectively (122). Three-dimensional displacements of the bilateral condyles were recorded during maximal clenching. Researchers noticed that the simulated CO and BO caused a statistically significant decrease in the superior displacements of the balancing-side condyle as compared to the simulated GO, implying that TMJ loading of CO and BO may be reduced. Three years after these initial tests, they investigated clenching-induced condylar displacements with controlled submaximal clenching levels (123, 124). Subjects were asked to perform clenching tasks at a 50% level of maximal voluntary contraction with simulated GO via visual feedback of EMG signals from an oscilloscope screen. Compared with the simulated CO, the simulated GO caused smaller working-side condylar displacement, and the BO caused smaller balancing-side and working-side condylar displacements. The findings may suggest that under the control of the clenching level, the increased working-side tooth contacts have the potential to reduce working-side joint loadings, and a balancing-side contact has the potential to reduce balancing-side joint loadings. To support this point, further study exploring bilateral condyle displacements, EMG levels of masticatory muscles under conditions of maximal voluntary contraction at the lateral occlusion and comparison of the recording data among groups CO, GO and BO is needed.

In 2010, Yashiro *et al.* (125) applied an occlusal interference to 10 healthy adults with good occlusion. A golden onlay was fixed to the upper first molar contralateral to the side of preferred chewing in each subject, covering the antagonistic lingual cusp of the lower first molar by 2·5 mm at full intercuspation. Mandibular incisor-point movement for chewing gum was recorded with a 3D tracking device before and after insertion of the balancing interference. An obvious increase was observed in the normalised jerk-cost (NJC), prolonged duration of the decelerative phase and lowered peak velocity of the jaw-closing movement during chewing induced by the occlusal disturbance. However, the NJC and velocity profile recovered significantly after about 90 repetitive chewing cycles. The research group inferred that significant recovery contributes to rapid adaptation of the skilfulness of chewing jaw movements to the occlusal interference, although the adaptation is limited to the degree that impedes normal movement.

*Occlusal alteration and muscle response*

The theory that occlusal interference increases activity in the jaw muscles and results in the development of TMD was tested in a double-blind randomised crossover experiment on 11 young, healthy females (126). One-quarter millimetre golden strips were cemented either to an occlusal contact area (active interference) of the lower first molar of the preferred chewing side or to the vestibular surface of the same tooth (dummy interference) for 8 days each. Electromyography recording of masseter showed that active interference induced a significant decrease in the number of activity periods per hour and their mean amplitude. Dummy interference did not change EMG activity significantly. None of the subjects developed signs and/or symptoms of TMD throughout the entire study, and most adapted to the occlusal disturbance. Pressure pain thresholds of the masseter and anterior temporalis muscles were assessed using pressure algometry under the same conditions (127). The results indicated that the application of active interference did not significantly affect the pressure pain thresholds of these muscles in healthy individuals.

The inferior head of lateral pterygoid (IHLP) is thought to play a critical role in the generation and control of lateral jaw movements. Huang *et al.* (124) investigated the effect of a working-side occlusal alteration on the activity of the IHLP. A cast metal overlay was cemented onto the upper right first molar in 14 subjects to disclude all other teeth during right laterotrusion, which did not interfere with intercuspal contact. Inferior head of lateral pterygoid activity was significantly increased with the occlusal alteration during the outgoing and return phases of laterotrusion, while bilateral anterior and posterior temporalis, masseter and submandibular muscles presented no change or a significant decrease in activity, which meant that a change to the occlusion on the working side in the form of a steeper guidance necessitates an increase in IHLP activity to move the mandible down the steeper guidance.

In 2008, Li *et al.* (121) attempted to seek the relationship between an occlusal high spot and oro-facial pain symptoms. A 0·5-mm cast onlay was placed on the lower right first molar of six volunteers to act as an intercuspal occlusal interference for 6 days. The induced oro-facial symptoms were collected, and the oro-facial pain was scored on a visual analogue scale (VAS) by the subjects during the experiment. Meanwhile, the surface EMG of the bilateral masseter and anterior temporalis was recorded before and during the intervention and again after its removal. The study demonstrated that the unilateral occlusal high spot did induce pain and various symptoms in the oro-facial and temporomandibular area and lead to subjective complaints of headache in the right temporal region (VAS 3·7). The EMG of the bilateral anterior temporalis became more unsymmetrical during clenching. The authors speculated that the changes in muscular activity may have had some relationship with the onset of a tension-type headache in the temporal region. However, the sample size was relatively small, and a longer-term study of the effects of occlusal high spots is needed.

*Occlusal alteration and individual characteristics*

The majority of human studies have mainly recruited normal, healthy people as subjects, usually resulting in adaptation to the artificial interference within a short period of time and therefore creating doubt as to the true role of occlusal factors in the aetiology of TMD.

In 2002, Le Bell *et al.* (120) carried out a randomised double-blind clinical study including 26 healthy women and 21 women with a history of TMD, both randomly divided into true and placebo interference groups. Centric relation and balancing-side interferences were introduced bilaterally for 2 weeks by adding composite resin to the palatal cusps of the upper second molars to disclude the incisors by 0·3 mm at the centric relation position. Results indicated that the subjects without a TMD history showed fairly good adaptation to the interferences, but the subjects with a TMD history and true interferences developed a significant increase in clinical signs relative to the other groups. Furthermore, the subjective reactions of these individuals were measured according to several symptom scales (128). Each day during the 2-week follow-up period, the subjects rated the intensity of their symptoms on nine VAS scales (occlusal discomfort, chewing difficulties, tender teeth, fatigue in the jaws, headache, facial pain, opening difficulty, bruxism and ear symptoms). Subjects with a history of TMD and true interferences reported stronger symptoms than subjects with no TMD history and placebo interferences. The most noteworthy symptoms were occlusal discomfort and chewing difficulties. Thus, individual

differences in vulnerability to occlusal interferences do indeed exist.

Le Bell's (129) group continued to evaluate the associations of psychological factors with symptom responses and adaptation to interferences. Before the intervention, the subjects filled in questionnaires dealing with personality traits, level of psychological and somatic stress symptoms, coping strategies and health beliefs. Analysis showed that health hardiness, positive socialisation history and inhibition of aggression were associated with weaker symptom responses and better adaptation to true artificial interferences. Some personality characteristics in subjects with a history of TMD tended to be associated with higher symptom responses despite the type of intervention. Psychological factors appear meaningful in determining symptom responses to artificial interferences, and they seem to play a different role in responses in subjects with a TMD history compared to those without.

*Discussion*

Changes in jaw movement and muscle function are among the symptoms induced by experimental occlusal alteration that have been verified in these human studies and seem to be reversible or adaptable in the subjects. Discoveries must still be made before we can finally answer the question put forth at the beginning of this review: what will happen following occlusal alteration? Although the aforementioned conclusions are reasonable enough to be applied into practice, quite a few limits for human studies remain constrained by ethics and morality: small sample size, short observation time, low intensity of interference and few developed experimental approaches, which always lead to uncertain results and/or superficial conclusions. In addition to animal studies aimed at mechanistic investigation, large sample size human studies that are rigorously designed and standardised are desperately needed.

## Summary

Upon thorough review of pertinent animal and human studies, we can conclude that experimental occlusal alteration has been a hot topic for occlusion-focused scientific research. In animal models, different artificial occlusal alterations can result in disorders or damage of TMJs, masticatory muscles and the nervous system. Long-term mechanistic nociception is related not only to peripheral sensitisation of nociceptive neurones but also to central sensitisation. However, results from animal studies cannot be directly extrapolated to humans due to many inherent differences between experimental conditions and clinical states. In human experimental studies, subjects without a history of TMD may adapt well to experimental interferences, but subjects with a history of TMD seem to adapt less well to this introduced interference. Furthermore, individual differences in vulnerability to occlusal interferences do exist. Although there are advantages and disadvantages to utilising human and animal experimental studies, each approach is indispensable for making new discoveries in this area of research.

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