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The effects of increasing occlusal vertical dimension on the deep masseter of rat at different ages

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ABSTRACT

Objective: To investigate the influence of increasing the occlusal vertical dimension (iOVD) on the fibre-type distribution and ultrastructure of deep masseter of rat at different ages.
Design: A total of forty-eight male Wistar rats were divided into two groups according to age: 'teenage' group (n = 24, 1.5 months) and 'young adult' group (n = 24, 8 months). Both the teenage and the young adult rats were then randomly divided into the control group (n = 12) and the experimental group (n = 12). The occlusal vertical dimensions of the rats in the experimental groups were increased by placing composite resin on all maxillary molars. The fibre-type distribution and ultrastructure of the deep masseter were subsequently observed on day 7 and day 14 after iOVD.
Results: In the teenage experimental group, the proportion of type IIa fibres increased, while the proportion of type IIb and type IIx fibres decreased by day 7 after iOVD ($P < 0.05$). However, no significant fibre phenotype transformation was observed in the young adult experimental group until day 14 after iOVD. In addition, the proportion of type IIa in the teenage experimental group was higher than that of the young adult experimental group on day 7 and 14 ($P < 0.05$). Under the transmission electron microscope, muscle fibre reconstruction and the compensatory increase in the number and volume of mitochondria appeared earlier in the teenage experimental group. The cellular traumatic reaction was less than that in the young adult experimental group.
Conclusion: The teenage rat alters masseter muscle structure to a slower phenotype earlier and to a greater degree than that of the young adult rat when increasing the occlusal vertical dimension.

1. Introduction

A "deep overbite" refers to the extent of vertical overlap of the maxillary central incisors over the mandibular central incisors measured in centric occlusion (Fattahi, Pakshir, Afzali, & Shahian, 2014). Deep overbite can result in severe tooth wear and periodontal damage on the maxillary incisors, and even temporomandibular joint disease. Despite widespread focus on the dangers of a deep overbite, relapse of the deep overbite remains a challenging issue in orthodontics. Literature reviews have implicated several factors related to overbite relapse, including the mandibular incisors angle, proper occlusal relationship, craniofacial growth patterns and so on (Lee & Graber, 2011, Chapter 27). Recently, increasing numbers of researchers have emphasised that establishing the appropriate masticatory muscle balance is very important for the stability of orthodontics (Kondo, 2004; Sciote et al., 2013). The period of retention may partly depend on the extent of structural and functional reconstruction. Therefore, unadapted change in the structure and function of the masticatory muscle could be a primary reason for relapse.

Skeletal muscles consist of different types of muscle fibres and show a high plasticity; accordingly, they can change their fibre type according to the relevant muscle load (Pette & Staron, 1997). As the main jaw-elevators in masticatory muscles, the structure of the masseter is closely related to the occlusal vertical dimension. Increasing the occlusal vertical dimension (iOVD) stretches the fibres of the deep masseter muscle. Some reports have focused on whether the masseter muscle changes the way other skeletal muscles do after iOVD (Ohnuki, Saeki, Yamane, Kawasaki, &

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Yanagisawa, 1999; Widmer, Nguyen, & Chiang, 2013; Ohnuki et al., 2009). Such studies have primarily examined adolescent animals, which is most likely because orthodontic treatment is typically concentrated during adolescence. In today's society, however, more and more young adult patients are seeking orthodontic treatment as malocclusion can have adverse effects on appearance and pronunciation clarity, and can even impact one's chance to obtain employment (Kerosuo, Kerosuo, Niemi, & Simola, 2000).

Although the need for treatment is increasing among young adults, we have observed that deep overbite corrections obtained during growth periods are less likely to relapse than those obtained during young adulthood, a finding which may be related to the pattern of craniofacial growth as described in previous literature (Nemeth & Isaacson, 1974; Simons & Joondeph, 1973). However, except for the growth factor, little is known about the extent to which the masseter plays an important role in maintaining stability and whether different muscle changes exist in different age groups after iOVD. Due to ethical limitations, masseter changes are rarely studied on clinical samples before and after iOVD. For the aforementioned reasons, the aim of present study was to incorporate an animal model to investigate the differential influence that iOVD has on the deep masseter at different ages. We can then hopefully imitate the iOVD in clinical practice and provide a theoretical basis for the long-term stability of iOVD at different ages from a musculature view.

2. Materials and methods

2.1. Animals and experimental protocol

A total of forty-eight male Wistar rats were divided into two groups according to their age: a 'teenage' group (n = 24, 1.5 months) and a 'young adult' group (n = 24, 8 months). The average weight of the animals was 144.33 g ± 4.35 g in the group of teenage and 487.71 g ± 8.68 g in the group of young adults. Both of teenage and young adult rats were then randomized divided into control group (n = 12) and experimental group (n = 12). So there were four subgroups according to the age and experimental intervention, namely teenage control group, teenage experimental group, young adult control group and young adult experimental group respectively. The animals were individually raised in plastic cages, and standard laboratory food and water was available ad libitum.

The local Animal Care & Use Committee approved the entire experimental procedure. The occlusal vertical dimension was increased for the teenage and young adult experimental groups by placing the composite resin on all maxillary molars as indicated by previous methods (Yabushita et al., 2006). Rats in the experimental group were anesthetized with 10% chloral hydrate by intraperitoneal injection (0.3 ml/100 g of the body weight). The mandibular was carefully opened, and both left and right upper molars were etched using 35% phosphate gel (GLUMA™ Etch, Heraeus Kulzer, Germany) for 20 s. After rinsing completely, the adhesive (Adper™ Single Bond 2, 3M, USA) was coated on the surface of the molars and light-cured for 10 s. The composite resin (Filtek™ Z250, 3M, USA) was applied on the first molar 1.5 mm thick and the third molar 1 mm thick, then light-cured for 20 s. The teenage and young adult control groups performed the same procedures as the experimental groups with the exception of resin placement.

To evaluate the iOVD animal model, splint maintenance, behaviour and body weight were recorded twice during the whole experimental period. Meanwhile, radiographs were obtained on the day prior to iOVD, as well as 0 days, 7 days and 14 days after iOVD to evaluate the degree and stability of iOVD during the experimental period. The X-ray machine (GENDEX Dental Systems, USA) was used with digital radiography film under the electric condition of 65 kVp, 7 mA with an exposure time of 0.125 s. The distance from the tube centre to the film and the animal mid-sagittal plane was standardized at 32 cm and 25 cm, respectively. The cephalometric landmarks were determined according to Yagi's method (Yagi et al., 2003). Cephalometric landmarks were defined as follows: E, the intersection between the frontal bone and the most superior-anterior point of the ethmoid bone; A, the most anterior point on the nasal bone; Gn, the most inferior-posterior point of the angular process of the mandible; Pg, a point on the most inferior contour of the lower border of the mandible, adjacent to the incisor; U1, the most anterior point of the upper first molar; V, the intersection between the E-A line and the line perpendicular to the E-A line through U1; T, the intersection between the Pg-Gn line and the U1-V line; and V-T, vertical dimension. Each landmark was measured using FUJI software. The VT distance and the difference in the VT value between the experiment group and control group were recorded (Fig. 1).

2.2. Preparation of the muscle sample

After bite-rising for 7 days, six rats were randomly chosen from the four subgroups and sacrificed by anaesthetic overdose. And the rest of the animals were sacrificed on the 14 days of the experiment. The mid-portions of the deep masseters were excised perpendicularly to the direction of the muscle fibres. Sites for specimen collection were kept consistent, stretching or squeezing of the muscles was avoided. Each muscle was then divided longitudinally into two parts: one part was used for histochemistry analysis (8.0mm × 8.0mm × 3 mm), while the other was used for

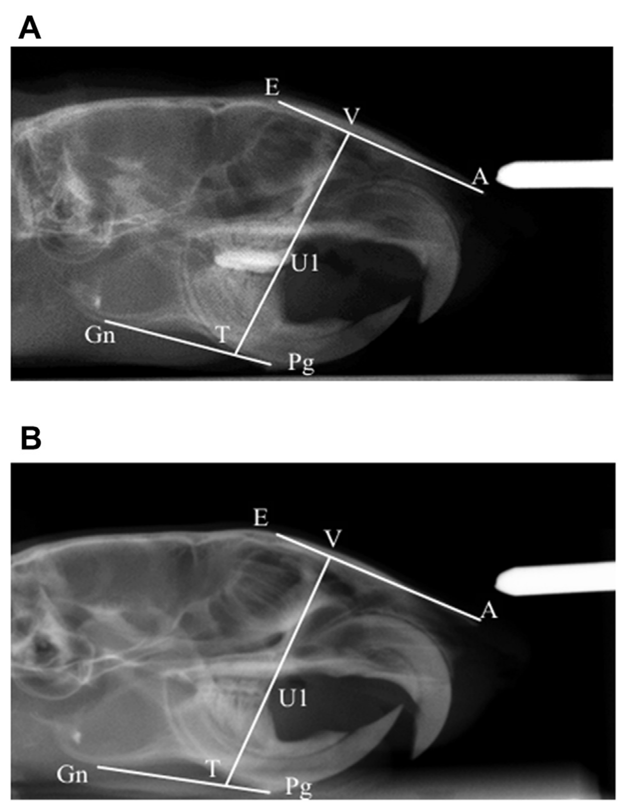

Fig. 1. The landmarks of the lateral cephalograms. (A) after increasing the vertical dimension (B) before increasing the vertical dimension. A: The most anterior point on the nasal bone. Po: The most posterior point on the cranial vault. Pg: A point on the most inferior contour of the lower border of the mandible, adjacent to the incisor. Gn: A point on the most inferior contour of the angular process of the mandible. U1: A point on the most anterior edge of the upper first molar. V-T: Vertical dimension.