European Journal of Orthodontics 35 (2013) 659–663
doi:10.1093/ejo/cjs083
Advance Access publication 11 November 2012

Molecular changes in detrained & retrained adult jaw muscle

Anna M. Ödman*, Nigel P. Hunt**, Hadwah A. Matloub Moawad,**,***, Andrea C. M. Sinanan**, Stavros Kiliaridis**** and Mark P. Lewis**,*****

*Department of Orthodontics, Institution of Odontology, The Sahlgrenska Academy at the University of Gothenburg, Sweden, **UCL Eastman Dental Institute, London, UK, ***Department of Orthodontics, Riyadh Military Hospital, Riyadh, Saudi Arabia, ****Department of Orthodontics, University of Geneva, Switzerland and *****The School of Sport, Exercise and Health Sciences, Loughborough University, Leicestershire, UK

Correspondence to: Anna M. Ödman, Department of Orthodontics, Institution of Odontology, the Sahlgrenska Academy at the University of Gothenburg, Box 450, 405 30 Göteborg, Sweden.
E-mail: anna.odman@odontologi.gu.se

SUMMARY A hypofunctional masticatory system was developed in 21-day-old male rats by feeding them a soft diet for 27 weeks. Retraining of a parallel group for 6 weeks was achieved by switching back to a hard diet after 21 weeks. A control group was fed a hard diet for 27 weeks. At the end of the experimental period, the expression levels of the myosin heavy chain isoform genes *MYH 1* and *2* (fast), *3* (embryonic) and *7* (slow) in the deep masseter were compared using qRT-PCR analysis.

The gene expressions of *MYH 3* and *MYH 7* were significantly higher in the rehabilitation group compared with the normal and hypofunctional group, but no significant differences were found in regards to the gene expression of *MYH 1* and *2*.

Retraining made it possible for the slow (*MYH 7*) isoform levels to adapt to the increased mechanical load. The increased level of embryonic (*MYH 3*) isoform could be due to the need of creation of new MYH isoforms.

Introduction

Adults with an improved masticatory muscle function following orthognathic surgery or after receiving implant-retained bridges may have different functional conditions that could induce significant changes in the myosin heavy chain (MyHC) composition of their jaw closing muscles. This is of particular interest for future therapies and procedures whether functional changes can be reversed or rehabilitated. For example, in finding ways to reduce the risk or to prevent relapse after orthodontic surgery.

Skeletal jaw muscles have an inherent adaptability (Goldspink, 2003), and when muscles adapt to new functional demands, fibre-type changes occur (Adams *et al*., 1993; Oishi *et al*., 1998). These changes allow the muscle to optimize its contractile function (Korfage *et al*., 2005). Changes in muscle structure and properties can be brought about by switching on one subset and repressing another subset of genes. (Goldspink *et al*.,1992).

Fibre-type changes normally follow a strict order: from slow fibre-type towards fast and then towards faster (I → IIA → IIX → IIB) and *vice versa* (Schiaffino and Reggiani, 1994). It has been shown that the switch from fast to slow is activity dependant (Barton-Davis *et al*., 1996). In humans, for example, exercise against load increases slow fibres in favour of fast fibres (Hather *et al*., 1991; Staron *et al*., 1994).

Histologically, muscle fibres are classified in relation to which MyHCs they contain: this scheme is no longer particularly simple due to the wide number of MyHCs that are expressed in the same muscle fibre. A MyHC 'profile' (protein and/or gene expression) of muscle fibres/groups is more usually reported.

Although regimes of detraining followed by rehabilitation/retraining have been reported for non-masticatory muscle (e.g., immobilization of limbs), similar studies of adult jaw muscle have not been conducted.

Studies in human subjects with varying growth patterns have shown that different craniofacial morphologies are associated with variations in masticatory muscle physiology; individuals with a long face have a weaker bite force than individuals with normal vertical dimensions. (Proffit *et al*., 1983; Hunt and Cunningham, 1997). Individuals with a short face have a thicker masseter muscle than long-face individuals (Kiliaridis and Kälebo, 1991; Bakke *et al*., 1992; Benington *et al*., 1999). The masticatory muscles of an individual with short-face characteristics contain more type II fibres than an individual with normal facial dimensions, while a long-face individual has a reduction in both the size and number of type II fibres (Hunt *et al*., 2006).

However, these observations have been made in a non-experimental setting where the degree of 'detraining' and 'retraining' has not been controlled. Animal studies lend themselves to such controlled experiments where adaptive changes have been induced experimentally, e.g., by changing diets. Changing masticatory muscle function, for example, by feeding rats with a soft diet leads to a reduced

masticatory muscle strength (Kiliaridis and Shyu, 1988) and a change of the fibre composition in their deep masseter muscles (Kiliaridis *et al*., 1988). This in turn causes changes in bone modelling in growing rats. (Mavropoulos *et al*., 2004; Abed *et al*., 2007) and alterations in craniofacial growth. It has been shown that rehabilitation of masticatory function in adult rats led to a significant improvement of alveolar bone architecture and change the alveolar process (Mavropoulos *et al*., 2010).

In order to investigate the effect of masticatory functional changes on deep masseter muscle contractile protein gene expression, an experimental study in non-growing, adult rats was performed.

Material and methods

Material

Forty male rats of the Sprague–Dawley strain (average age 21 days) from Charles River, Uppsala, Sweden were used. The experimental protocol obtained approval by the Ethics Committee of University of Gothenburg.

Experimental design (Fig. 1)

The 40 rats were randomly divided into two groups. Fourteen received ordinary (hard) food during the whole experimental period (normal group). The remaining 26 received a soft diet to develop a hypofunctional masticatory system (Kiliaridis *et al*., 1988). The soft diet was made of ordinary (hard) food (R34, Lactamin, Södertälje, Sweden) mixed with water in standardized proportions (2:5, R34:water). After a period of 21 weeks on a soft diet these animals, now adults, were divided into two equal groups (matched for weight): one continued on the soft diet (hypofunctional group), and the other changed to an ordinary (hard) diet with the aim of functionally rehabilitating

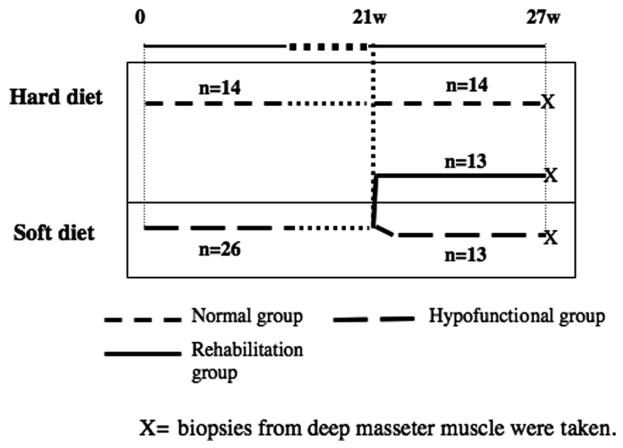

Figure 1 Experimental design.

Table 1 Amount of rats in the different groups included in the results.

Variables	Amount of rats		
	Normal	Rehabilitation	Hypofunctional
MYH 1 and *2*	12	9	12
MYH 3	13	12	12
MYH 7	12	9	12

their masticatory system (rehabilitation group). Due to losses during the experiment (e.g., loss during qRT-PCR and during preparation for qRT-PCR), the total number of samples reported is lower than the total number of animals that completed the experimental protocol (Table 1). The total experimental period was 27 weeks. Throughout the experimental period, the rats were weighed every second week and were fed and watered *ad libitum*. At the end of the experimental period, the animals were sacrificed in a CO_2 chamber. The deep masseter muscle was excised. The muscle biopsies were immediately frozen in thawing isopentane (−150°C) and stored at −80°C (Kiliaridis *et al*., 1988; Suchak *et al*., 2009).

Quantitative reverse transcriptase polymerase chain reaction (qRT-PCR). In order to successfully extract ribonucleicacid (RNA) from the rat deep masseter muscle samples, the tissue was initially disrupted using the FastPrep FP120A bench-top reciprocating device (MP Biomedicals Europe, Illkirch, France). Muscle tissue (weighing more than 30 mg) was 'pulverised' in 800 µl buffer RLT mixed with 10 µl b-mercaptoethanol per 1 ml buffer (RNeasy Mini Kit, Qiagen Ltd, W Sussex, UK) against silica ceramic beads (Lysing Matrix D, MP Biomedicals Europe) using the FastPrep device. The resulting solution was processed with the RNeasy Mini Kit (Qiagen). The RNA's quality (A260/A280) and quantity (concentration) was controlled/measured with a Spectrophotometer (Ultrospec 2000 machine, Amersham Pharmacia Biotech, GE Healthcare Ltd, Buckinghamshire, UK). RNA was then converted into complementary deoxyribonucleic acid (cDNA) using a high capacity cDNA archive kit (Applied Biosystems, Cheshire, UK). Quantitative PCR was performed with these cDNA templates using the TaqMan® System (Applied Biosystems). This system uses specific gene probes conjugated to a FAM (6-Carboxyfluorescein) reporter dye that bind to the sequence of interest (e.g., MyHC specific transcripts). During the PCR reaction, the probe is cleaved by the action of DNA polymerase that results in increased fluorescence. Accumulation of PCR products is detected directly by monitoring the increase in fluorescence of the reporter dye using the ABI PRISM® 7300 Sequence Detection System (All from applied Biosystems). Relative quantification studies were conducted using an endogenous control, GAPDH (Genebank NM_017008.3) (Suchak *et al*.,

Table 2 Myosin heavy chain isoforms investigated in this study.

Gene of interest	Myosin heavy chain protein	Muscle contraction	Genebank messenger RNA
MYH 1	Myosin heavy chain II x	Fast	AF111785.1 BC114545.1
MYH 2	Myosin heavy chain II a	Faster	AF111784.1 BX510904.2 BC126409.1
MYH 3	Embryonic myosin heavy chain	Fairly fast	X13988.1 BP232245.1 BQ956249.1
MYH 7	Beta-cardiac myosin heavy chain, Myosin heavy chain I	Slow	M58018.1 X51591.1 AY518538.1 AB209708.1 DQ248310.1 BC112173.1 BC112171.1 EF560725.1

Table 3 Mean values and standard deviation (in parentheses) of the variables under study for each of the reference and experimental groups.

Variables	ANOVA			Significance
	Normal	Rehabilitation	Hypofunctional	
Gene expression (2^-DDCt)				
MYH 1 and *2*	0.80 (0.71)	3.65 (5.05)	2.81 (4.48)	$P = 0.214$
MYH 3	2.18* (0.80)	8.95*,** (7.14)	4.47 ** (3.92)	$P = 0.003$
MYH 7	1.29* (1.24)	142.05*,** (137.43)	27.6** (34.92)	$P = 0.000$

Analysis of variance (ANOVA) was applied in order to investigate the difference between the three experimental groups (Normal group; Rehabilitation group; Hypofunctional group).
*, **, superscript characters denote statistical significant pairwise differences (*post hoc* comparisons).

2009). Masseter muscle derived cDNA was targeted for sequences of MyHC genes (Table 2).

Statistical analysis

All data are represented as means and standard deviations (mean ± SD). To investigate the morphological differences between the three experimental groups, analysis of variance (ANOVA) was performed. All statistical analyses were performed using the SPSS statistical package (SPSS 16; SPSS, Chicago, IL, USA). A result was considered statistically significant at $P < 0.05$.

Results

Body weight

No statistically significant differences in body weight were found at week 27 between the three groups at the end of the experimental period (week 27): the mean body weight (±SD) for the hypofunctional group was 595 g (±48 g), for the rehabilitation group was 595 g ± (43 g), and for normal group was 631 g (±81 g).

Contractile protein gene expression in adult rats

The results showed that the gene expression of *MYH 3* was significantly higher in the rehabilitation group compared with the normal group ($P = 0.001$) and with the hypofunctional group ($P = 0.025$). When comparing between the normal and the hypofunctional group, there were no significant differences.

There were no significant differences in the comparison of the three groups regarding the gene expression of MYH 1 and 2.

The analysis showed that there were distinct differences in gene expression of *MYH 7* between the rehabilitation and normal group ($P = 0.000$) and between the rehabilitation and hypofunctional groups ($P = 0.001$), where the levels were highest in the rehabilitation group and lowest in the normal group. However, again there were no significant differences between the normal and the hypofunctional group. The mean values and standard deviation of the variables under study for each of the groups are found in Table 3.

Discussion

In the present study, we found that a functional rehabilitation of the masticatory system for 6 weeks during adolescence and early adulthood after a prolonged period with low masticatory demands (the rehabilitation group) had a significant effect on the gene expression of *MYH 3* and *MYH 7*, but not on *MYH 1* and *2*.

The significant effect on the *MYH 7* (slow) is in line with earlier muscle studies (Caiozzo *et al*., 1996), in that activation of the masseter muscle increased the amount of slow MyHC. The increase of slow MyHc is due to the muscle fibres' capacity to adapt to new functional demands (Korfage *et al*., 2005, Part I).

The level of the *MYH 7* was lowest in the normal group and highest in the rehabilitation group. The low level of *MYH 7* in the normal group is in line with Sano and coworkers' description of the fibre-type composition in a normal rat deep masseter muscle (Sano *et al*., 2007)

The change of diet from soft to hard in order to rehabilitate their masticatory system had a significant effect on *MYH 3* (coding for embryonic MyHC) gene expression in the rehabilitation group compared with both the normal group and the hypofunctional group. That *MYH 3* was detected in all three groups, with the lowest amount in the normal group and the highest in the rehabilitation group, is not in line with, for example, Jung *et al*. (1998). Using competitive PCR, they could not detect any *MYH 3* at all in the masseter muscle of adult rats. However, it is known that in the limb muscles,

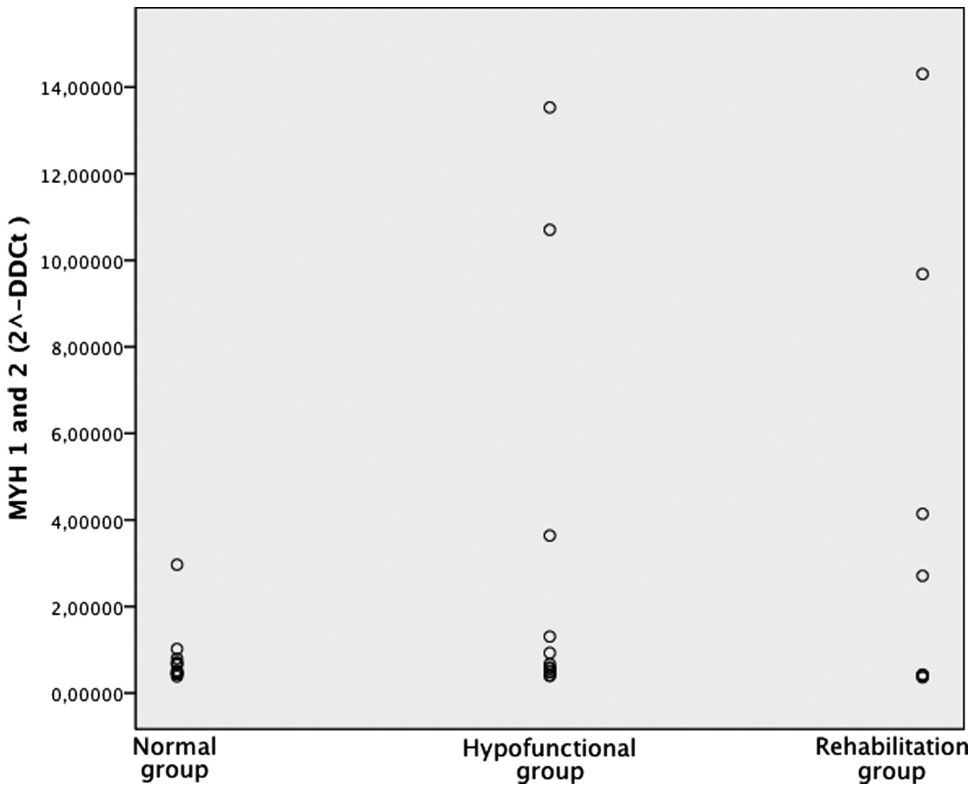

Figure 2 Scatterplot of the gene expression of MYH 1 and 2 in the different groups.

developmental isoforms can be re-expressed in the case of regeneration and injury (Jung *et al*., 1998). It seems that this is the case even with the deep masseter muscle of adult rats.

The analysis showed no significant differences in the comparison of the three groups regarding the gene expression of *MYH 1* and *2*, but when studying the rehabilitation group in the *MYH 1* and *2* scatterplot (Fig. 2), it shows that five out of nine are clustered together. In four of the animals, *MYH 1* and *2* were not affected, whereas five of them showed a reduced expression of *MYH 1* and *2*. These data indicate that there is a large degree of individual variation in how jaw muscle reacts to rehabilitation. The decrease in gene expression of *MYH 1* and *2* that can be seen in the scatterplot with the five clustered rats in the rehabilitation group is in line with earlier studies where it has been shown that the fast fibres decrease in favour of the slow fibres (Hather *et al*., 1991, Staron *et al*., 1994).

It is known from a description of the fibre-type composition in a normal rat deep masseter muscle (Sano *et al*., 2007) that two of the fast fibre types exist in all parts of the muscle and that is type IIa and IIx. (*MYH 2* and *MYH1*).

The findings from our rat model experiment cannot be directly extrapolated to humans, but it can give an insight into a better understanding of the mechanisms that influence the interaction between muscle function and craniofacial morphology. For example, it may help in finding ways to reduce the risk or to prevent relapse after orthodontic surgery.

Conclusion

A 6-week period of masticatory functional rehabilitation in deep masseter muscle of adult rats made it possible for the slow contractile gene expression isoform (*MYH 7*) levels to increase, an adaptation to the increased mechanical load. The increased level of embryonic gene expression isoform (*MYH 3*), which is novel, can be due to the need for the creation of new MyHC isoforms.

Funding

The European Orthodontic Society; the Swedish Dental Society.

Acknowledgments

We are grateful to Mrs Stina Olsson and Dr Andrea Bresin for their technical assistance.

References

Abed G S, Buschang P H, Taylor R, Hinton R J 2007 Maturational and functional related differences in rat craniofacial growth. Archives of Oral Biology 52: 1018–1025

Adams G R, Hather B M, Baldwin K M, Dudley G A 1993 Skeletal muscle myosin heavy chain composition and resistance training. Journal of Applied Physiology 74: 911–915

Bakke M, Tuxen A, Vilmann P, Jensen B R, Vilmann A, Toft M 1992 Ultrasound image of human masseter muscle related to bite force,

electromyography, facial morphology, and occlusal factors. Scandinavian Journal of Dental Research 100: 164–171

Barton-Davis E R, LaFramboise W A, Kushmerick M J 1996 Activity-dependent induction of slow myosin gene expression in isolated fast-twitch mouse muscle. The American Journal of Physiology 271: C1409–C1414

Benington P C, Gardener J E, Hunt N P 1999 Masseter muscle volume measured using ultrasonography and its relationship with facial morphology. European Journal of Orthodontics 21: 659–670

Caiozzo V J, Haddad F, Baker M J, Baldwin K M 1996 Influence of mechanical loading on myosin heavy-chain protein and mRNA isoform expression. Journal of applied physiology 80: 1503–1512

Goldspink G, Scutt A, Loughna P T, Wells D J, Jaenicke T, Gerlach G F 1992 Gene expression in skeletal muscle in response to stretch and force generation. The American Journal of Physiology 262: R356–R363

Goldspink G 2003 Gene expression in muscle in response to exercise. Journal of Muscle Research and Cell Motility 24: 121–126

Hather B M, Tesch P A, Buchanan P, Dudley G A 1991 Influence of eccentric actions on skeletal muscle adaptations to resistance training. Acta Physiologica Scandinavica 143: 177–185

Hunt N P, Cunningham S J 1997 The influence of orthognathic surgery on occlusal force in patients with vertical facial deformities. International Journal of Oral and Maxillofacial Surgery 26: 87–91

Hunt N, Shah R, Sinanan A, Lewis M 2006 Northcroft Memorial Lecture 2005: muscling in on malocclusions: current concepts on the role of muscles in the aetiology and treatment of malocclusion. Journal of Orthodontics 33: 187–197

Jung H H, Lieber R L, Ryan A F 1998 Quantification of myosin heavy chain mRNA in somatic and branchial arch muscles using competitive PCR. American Journal of Physiology 275: C68–C74

Kiliaridis S, Engström C, Thilander B. 1988 Histochemical analysis of masticatory muscle in the growing rat after prolonged alteration in the consistency of the diet. Archives of Oral Biology 33: 187–193

Kiliaridis S, Shyu B C 1988 Isometric muscle tension generated by masseter stimulation after prolonged alteration of the consistency of the diet fed to growing rats. Archives of Oral Biology 33: 467–472

Kiliaridis S, Kälebo P 1991 Masseter muscle thickness measured by ultrasonography and its relation to facial morphology. Journal of Dental Research 70: 1262–1265

Korfage J A, Koolstra J H, Langenbach G E, van Eijden T M 2005 Fiber-type composition of the human jaw muscles–(part 1) origin and functional significance of fiber-type diversity. Journal of Dental Research 84: 774–783

Mavropoulos A, Bresin A, Kiliaridis S 2004 Morphometric analysis of the mandible in growing rats with different masticatory functional demands: adaptation to an upper posterior bite block. European Journal of Oral Sciences 112: 259–266

Mavropoulos A, Ödman A, Ammann P, Kiliaridis S 2010 Rehabilitation of masticatory function improves the alveolar bone architecture of the mandible in adult rats. Bone 47: 687–692

Oishi Y, Ishihara A, Yamamoto H, Miyamoto E 1998 Hindlimb suspension induces the expression of multiple myosin heavy chain isoforms in single fibres of the rat soleus muscle. Acta Physiologica Scandinavica 162: 127–134

Proffit W R, Fields H W, Nixon W L 1983 Occlusal forces in normal- and long-face adults. Journal of Dental Research 62: 566–570

Sano R *et al.* 2007 Heterogeneity of fiber characteristics in the rat masseter and digastric muscles. Journal of Anatomy 211: 464–470

Schiaffino S, Reggiani C 1994 Myosin isoforms in mammalian skeletal muscle. Journal of Applied Physiology 77: 493–501

Staron R S *et al.* 1994 Skeletal muscle adaptations during early phase of heavy-resistance training in men and women. Journal of Applied Physiology 76: 1247–1255

Suchak A, Hunt N P, Shah R, Sinanan A C, Lloyd T, Lewis M P. 2009 Myosin proteins identified from masseter muscle using quantitative reverse transcriptase-polymerase chain reaction–a pilot study of the relevance to orthodontics. European Journal of Orthodontics 31: 196–201