The Journal of Experimental Biology 213, 775-781 © 2010. Published by The Company of Biologists Ltd doi:10.1242/jeb.037762

Changes in rabbit jaw-muscle activity parameters in response to reduced masticatory load

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Accepted 29 November 2009

SUMMARY

Mechanical food properties influence the neuromuscular activity of jaw-closing muscles during mastication. It is, however, unknown how the activity profiles of the jaw muscles are influenced by long-term alterations in masticatory load. In order to elucidate the effect of reduced masticatory load on the daily habitual activity profiles of three functionally different jaw muscles, the electromyograms of the masseter, temporalis and digastric muscles were recorded telemetrically in 16 male rabbits between seven and 20 weeks of age. Starting at eight weeks of age the experimental animals were fed significantly softer pellets than the control animals. Daily muscle activity was quantified by the relative duration of muscle use (duty time), burst number and burst length in relation to multiple activity levels. The daily duty time and burst number of the masseter muscle were significantly lower in the experimental group than in the control group at 5% and 10% of the maximum activity during the two weeks following the change in food hardness. By contrast, altered food hardness did not significantly influence the activity characteristics of the temporalis and digastric muscles. The findings suggest that a reduction in masticatory load decreases the neuromuscular activity of the jaw-closing muscles that are primarily responsible for force generation during mastication. This decrease is most pronounced in the weeks immediately following the change in food hardness and is limited to the activity levels that reflect muscle contractions during chewing. These findings support the conclusion that the masticatory system manifests few diet-specific long-term changes in the activity profiles of jaw muscles.

Key words: activity burst, adaptation, duty time, electromyography, functional load.

INTRODUCTION

The masticatory system is a complex musculoskeletal system, in which the activity of jaw muscles, the mechanical loading of muscles and bones, and the phenotypic properties of these tissues are closely interrelated (Burr, 1997; Ferretti et al., 2003). If one of these factors changes as a result of intrinsic or extrinsic causes, it will affect all of the other factors. This evidently concerns the activity patterns of the jaw muscles adapting to a new environment, which may occur as a consequence of, for instance, craniofacial growth and development (van Wessel et al., 2006) or as a result of an alteration of the functional load (He and Kiliaridis, 2003).

At the experimental level, it has been shown that the functional load of the jaw muscles can be reproducibly altered by changing the consistency of the available food during growth and development (Kiliaridis and Shyu, 1988; Liu et al., 1998). Employing this experimental design, a number of studies have observed changes in the phenotypic properties of the jaw muscles related to changes in masticatory function brought about by changing the ordinary hard diet to a soft one (Kitagawa et al., 2004; Taylor et al., 2006). It has been suggested that the changes in the jaw-muscle properties are signs of a structural adaptation caused by a reduction in the neuromuscular activity of the muscles involved (Miyata et al., 1993). However, the effect of altered masticatory functional loading, induced by feeding on a diet of reduced hardness, on the neuromuscular activity of the jaw muscles has received very little attention.

Neuromuscular activity is most commonly studied by electromyography, which is a recording of the amplified action potentials of the muscle. The evaluation of long-term electromyograms (EMG) frequently involves the determination of the duty time as the relative duration of muscle activity during the day (Hensbergen and Kernell, 1997) and the quantification of the number and duration of activity bursts (van Wessel et al., 2005c). Although these parameters are valuable measures of neuromuscular activity, they can only provide information on the intensity of muscle activation when related to the level of muscle activity.

Several studies have demonstrated that altering the food hardness changes the neuromuscular activity of jaw-closing muscles during mastication (Agrawal et al., 1998; Peyron et al., 2002; Piancino et al., 2008). Up to now, however, it is unknown if these changes are also reflected in the daily activity profiles of the jaw muscles, if they are temporary or permanent, if they differ among the jaw muscles or which of the jaw-muscle activity parameters, such as duty time, burst number and burst length, change under conditions of altered masticatory functional load.

The aim of this study was, therefore, to assess the daily habitual activity profiles of the jaw muscles under conditions of different masticatory functional loading. The effect of a reduction in the hardness of the diet on the daily activity of the masseter, temporalis and digastric muscles was examined in growing rabbits over the time course from weaning to puberty. Because the jaw muscles are functionally distinct, it was hypothesized that they would be differently affected by altered masticatory loading.

Potential changes in the daily activity profiles in response to altered function were expected to be limited to activity levels that reflect mastication. Because changes in neuromuscular activity can be regarded as first signs of muscle adaptation, the changes were assumed to be most pronounced shortly after the introduction of the functional change.

MATERIALS AND METHODS Animals

Sixteen male juvenile New Zealand White rabbits (*Oryctolagus cuniculus*, Linnaeus; Harlan, Horst, The Netherlands) were used. When obtained, they were six weeks old and weighed $1402\pm189\,\mathrm{g}$. The animals were individually housed in metal cages ($87\,\mathrm{cm} \times 75\,\mathrm{cm} \times 38\,\mathrm{cm}$) with perforated plastic floors, kept in a climate controlled room ($22.0\pm0.9^{\circ}\mathrm{C}$, $57.8\pm7.2\%$ relative humidity) with a $12\,\mathrm{h}:12\,\mathrm{h}$ light:dark cycle with lights on at $07:00\,\mathrm{h}$, and fed a commercially manufactured pelleted diet (Arie Blok, Woerden, The Netherlands) and water *ad libitum*.

Experimental design

After an acclimatization period of one week, the animals were randomly assigned to either the experimental or control groups, and were each implanted with a three-channel transmitter device (F50-EEE, Data Sciences International, St Paul, MN, USA) for telemetrical recording of intramuscular EMGs of the masseter, temporalis and digastric muscles as detailed below. Between eight and 20 weeks of age, the experimental animals (N=8) were fed a diet of soft pellets requiring significantly reduced peak loadings (10 N cm⁻²), and thus level of jaw-muscle contractions, to break the pellet in comparison with the standard pellets (120 N cm⁻²) fed to controls (*N*=8). The pellets did not differ in size or nutritional value. No environmental enrichment was provided in order to prevent the animals from gnawing. Except for daily care and a weekly check of weight, health and occlusal condition, the animals were left undisturbed to minimize external influence. The experiment had been approved by the Animal Ethics Committee of the Medical School of the University of Amsterdam, and was performed in accordance with the animal care and welfare guidelines of the National Institute of Health.

Transmitter implantation and muscle activity recording

The transmitters were implanted subcutaneously in the shoulder area. Bipolar fine wire electrodes, each consisting of two silicone-insulated stainless steel wires (diameter 0.45 mm), were used to pick up muscle potentials. Two electrodes were led subcutaneously to an incision in the submandibular region and from there were inserted into the centers of the right superficial masseter and the digastric muscles. The third electrode was led subcutaneously to a mediofrontal incision and from there was inserted into the center of the right superficial temporalis muscle. The electrodes were placed parallel to the fiber direction by means of a longitudinally ground hypodermic needle (Nuijens et al., 1997) and sutured at the muscle surfaces to prevent them from dislodging. The effective electrode length was 7 mm with a distance of 2 mm between the tips of each bipolar electrode.

All surgical procedures were performed under aseptic conditions and with the animals under general anesthesia induced by subcutaneous injection of 0.8 ml kg⁻¹ body mass of a 1:3 mixture of xylazine (Sedazine, AST Farma, Oudewater, The Netherlands) and ketamine (Ketamine, Alfasan, Woerden, The Netherlands), followed by inhalation of an individually adjusted mixture of isoflurane (PCH Pharmachemie, Haarlem, The Netherlands) and

oxygen. Local anaesthesia in the areas of incision was achieved by subcutaneous injection of lidocaine (Lidocain 2%, Braun, Melsungen, Germany).

Muscle activities were recorded during a 13-week period, starting two days after surgery when the animals had regained normal feeding and locomotor activity. Muscle potentials were simultaneously sampled at 250 Hz on the input of each channel (21,600,000 samples per channel per day), filtered in the device (first-order low-pass filter, 158 Hz), transmitted to a group of four receivers (RMC-1, Data Sciences International) placed directly beneath the cage and stored on a computer hard disk using a data acquisition system (DataQuest ART 2.3, Data Sciences International).

At the end of the experiment, the animals were sedated by a subcutaneous injection of $0.6\,\mathrm{ml\,kg^{-1}}$ body mass of a 1:3 mixture of xylazine and ketamine, and killed by an intravenous overdose of sodium pentobarbital (Euthesate, Ceva Sante Animale, Naaldwijk, The Netherlands). The signals were then sampled for another five minutes to determine the level of recorded noise for each muscle as previously described (Grünheid et al., 2005). Only signals with amplitudes clearly exceeding the maximum noise amplitudes (5.6–24.3 μV) were processed further. Inaccurate electrode location at the time of dissection led to the exclusion of one superficial masseter muscle, one superficial temporalis muscle and two digastric muscles in each experimental and control groups.

Data processing and analysis

For each animal, the continuous EMG recordings of a predefined day per week were analyzed, as described in detail elsewhere (Van Wessel et al., 2005a; Van Wessel et al., 2005b). In brief, the recordings were filtered (5 Hz high pass), freed from artifacts, fullwave rectified and averaged over 20 ms (Spike2 version 5.19, Cambridge Electronic Design, Cambridge, UK). Signal loss because of transmission problems and artifact removal accounted for 0.62±1.94% of the total time. All EMG samples were indexed for their amplitudes (resolution 1 µV), and the 0.001% samples with the largest EMG amplitudes (i.e. 43 samples) were excluded to eliminate any potentially remaining artifacts (Van Wessel et al., 2005c). The largest amplitude of the remaining 99.999% of the samples was identified as peak-EMG. This peak-EMG indicated the maximum activity of this muscle for that day and was used for EMG normalization (Knutson et al., 1994). Activity levels were defined as fixed percentages of the peak-EMG.

The daily muscle activity was quantified by the duty time, total burst number and average burst length. These parameters were determined using custom-made codes within the Spike2 software at activity levels of 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80% and 90% of the peak-EMG (Fig. 1). A burst was defined as a series of consecutive samples exceeding a specified activity level. Duty time was defined as the relative time per day during which a muscle was active and was calculated as the cumulative length of the EMG samples having amplitudes larger than the predefined activity levels (Grünheid et al., 2006). For example, the duty time at the 5% activity level is the percentaged duration of all EMG samples with amplitudes larger than 5% of the peak-EMG of that day.

Statistical analysis

For each group of animals, mean values and standard deviations of duty times, burst numbers and burst lengths were calculated separately for each muscle, experimental day and activity level. Differences between the experimental and control groups were tested for statistical significance, for each muscle and activity level separately, using

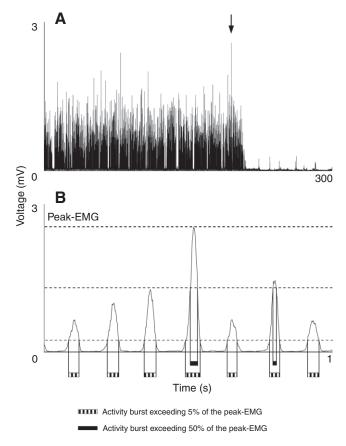


Fig. 1. Example of an electromyogram (EMG) recording and details for quantification of individual bursts. (A) Segment of a processed and rectified EMG recorded from the superficial masseter muscle. (B) Expanded view at the location of the arrow shown in A. The broken lines indicate activity levels, expressed as percentages of the maximum activity (peak-EMG) of this muscle for that day. EMG activity was based on the number and lengths of bursts with amplitudes exceeding 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80% and 90% of the peak-EMG. Bursts were grouped to quantify the duty times at these activity levels. Note that the depicted period is not representative of the EMG recording of the entire day.

Student's t-test for normally distributed data (Kolmogorov–Smirnov test), and Mann–Whitney rank sum test for non-normally distributed data. Differences among experimental days were tested for statistical

significance, for each group and activity level separately, using one-way analysis of variance (ANOVA) with Holm–Sidak's method as *post hoc* pairwise comparison procedure for normally distributed data, and Kruskal–Wallis one way ANOVA on ranks with Dunn's method as *post hoc* pairwise comparison procedure for non-normally distributed data. Statistical analyses were performed using SigmaStat 3.5 (Systat Software Inc., Point Richmond, CA, USA) with *P*-values of less than 0.05 considered statistically significant. For data presentation, mean values and standard deviations of the parameters assessed were calculated for the period during which the animals were fed diets of different hardness.

RESULTS

All animals grew continuously throughout the experimental period. Their body mass did not differ significantly between the groups at any time. The peak-EMG values were highest in the masseter muscles and lowest in the temporalis muscles in both groups of animals. They did neither differ significantly between the groups nor over the time course of the experiment.

The mean daily duty times, burst numbers and burst lengths of the masseter, temporalis and digastric muscles at the various activity levels studied are summarized in Tables 1–3. Please note that these values were calculated for the entire period during which the animals were fed diets of different hardness. Statistical analysis of differences between groups was performed using data of each experimental day.

Feeding the animals pellets of lower physical consistency statistically significantly decreased the activity of the masseter muscle; in the two weeks following the change in food hardness both the duty times (Fig. 2) and the burst numbers (Fig. 3) were significantly lower in the experimental group than in the control group at the 5% and 10% activity levels. By contrast, these parameters did not differ significantly between groups in the temporalis and digastric muscles. The mean burst lengths did not differ significantly between the groups in any muscle or at any activity level examined.

The muscles also showed some changes in their activity parameters with age. The burst numbers of the masseter muscles of the control animals at the 10%, 20% and 30% activity levels (Fig. 3) and those of the digastric muscles of both the experimental and control animals at the 5% activity level (Fig. 4) decreased significantly over the time course of the experiment. By contrast, the duty times and the mean burst lengths did not change significantly over time in any muscle studied.

Table 1. Daily duty time of rabbit jaw muscles at various activity levels

Activity level (%)*	Duty time (%)							
	Masseter		Temporalis		Digastric			
	Experimental	Control	Experimental	Control	Experimental	Control		
5	7.5667±0.5915	9.2707±1.4248	10.0771±3.7942	21.3237±2.9645	11.1746±1.4996	11.2094±0.8773		
10	4.0039±0.2836	4.6598±0.9159	3.2153±1.1683	5.7242±0.7207	6.0685±0.8037	6.6405±0.6026		
20	1.6394±0.1610	1.9081±0.4103	1.0420±0.2728	1.2340±0.2250	2.2920±0.4399	2.8123±0.4747		
30	0.7226±0.1088	0.8517±0.1731	0.4561±0.1176	0.4497±0.1214	0.8658±0.2205	1.1607±0.2708		
40	0.3171±0.0566	0.3747±0.0689	0.2031±0.0554	0.1867±0.0565	0.3235±0.0902	0.4604±0.1281		
50	0.1348±0.0253	0.1578±0.0260	0.0884±0.0239	0.0800±0.0239	0.1222±0.0326	0.1796±0.0545		
60	0.0549±0.0093	0.0640±0.0090	0.0375±0.0093	0.0333±0.0083	0.0467±0.0098	0.0697±0.0201		
70	0.0217±0.0032	0.0255±0.0034	0.0157±0.0037	0.0144±0.0040	0.0187±0.0028	0.0278±0.0079		
80	0.0097±0.0020	0.0131±0.0034	0.0086±0.0014	0.0056±0.0014	0.0092±0.0011	0.0127±0.0075		
90	0.0007±0.0020	0.0051±0.0040	0.0007±0.0009	0.0001±0.0001	0.0018±0.0018	0.0042±0.0082		

Results are mean values ± standard deviations of the mean duty times calculated for the period from 8 to 20 weeks of age, during which groups were fed diets of different hardness.

^{*}Expressed as a percentage of the peak-EMG

90

Burst number (counts) Masseter Temporalis Digastric Activity level (%)* Experimental Control Experimental Control Experimental Control 77,149±8080 93,372±13,208 113.632±31.059 206,054±30,625 99.842±12.393 103,176±9746 10 50,520±4529 57,727±9690 44,149±16,077 79,264±9394 73,290±9082 80,595±8191 20 28.208±2715 31,459±6387 17,225±4474 21.842±3453 39,585±7214 49,333±7905 30 15,423±2060 17,353±3623 9068±2229 9298±2373 18,317±4701 25,397±6118 40 7931±1315 8915±1827 4728±1272 4412±1341 7803±2306 11,601±3546 2097±666 50 3790±677 4221±818 2324±654 3236±937 5035±1670 60 1688±297 1867±325 1088±289 970±289 1343±324 2124±741 70 710±117 790±92 487±113 435±111 563±97 895±299 80 360±112 281+41 316 + 29214 + 39189 + 41238+27

Table 2. Daily burst number of rabbit jaw muscles at various activity levels

Results are mean values ± standard deviations of the mean burst numbers calculated for the period from 8 to 20 weeks of age, during which groups were fed diets of different hardness.

81±13

89±12

104±11

DISCUSSION

118±11

The present study provides a comprehensive examination of the habitual activity patterns of the rabbit masseter, temporalis and digastric muscles and is, to our knowledge, the first to investigate the effect of a reduction in masticatory load on the daily activity characteristics of these muscles over the time course from weaning to puberty. The results show that the reduction in dietary hardness caused a decrease in the duty times and burst numbers of the masseter muscle at low activity levels.

A relationship has been demonstrated between the mechanical food properties and the neuromuscular activity of jaw-closing muscles during mastication (Agrawal et al., 1998). Typically, the electrical activities are higher during chewing of hard food (Peyron et al., 2002; Piancino et al., 2008). The present investigation shows that the hardness of the ingested food also has an effect on the daily activity profiles of jaw muscles. Statistically significant differences between the groups of animals that were fed pellets of different hardness were limited to the 5% and 10% activity levels. Muscle activity at these levels comprises a broad range of oral behavior (van Wessel et al., 2005a). However, it has been demonstrated that in rabbits jaw-muscle contractions during chewing rarely exceed 20% of the peak-EMG (Langenbach et al., 2004). The significant differences in the daily activity patterns at the 5% and 10% activity levels are thus most likely to be attributable to differences in the neuromuscular activity during

chewing. This assumption is corroborated by the finding that higher activity levels, which include only the most powerful tasks such as grinding, gnawing or clenching, were completely unaffected by the change in dietary hardness.

100±8

134±46

The reduction in masticatory load had a differential effect on the jaw muscles studied. While the intake of soft pellets altered the daily activity profile of the masseter muscle, those of the temporalis and digastric muscles remained unchanged. This disparity is most likely to be based on the function of these muscles during mastication. The masseter muscle elevates the mandible and generates occlusal force during the power stroke (Widmer et al., 2003), while the superficial temporalis and digastric muscles stabilize and open the jaw, respectively (Weijs et al., 1989). During chewing, less muscle activation is most probably required to break soft food than to break hard food whilst the activation necessary to stabilize or open the jaw is, in all likelihood, not influenced by the food hardness.

Not all activity parameters, i.e. duty time, burst number and burst length, of the masseter muscle were affected by the alteration in masticatory functional load. The decrease in duty time and burst number in response to the change in food hardness contrasted with the constancy of the mean burst length. The mean burst lengths computed in the present study were very similar to those reported for both juvenile (van Wessel et al., 2005b) and adult rabbits (Grünheid et al., 2006), and did not differ significantly between

Table 5. Daily butst length of tabbit jaw muscles at various activity levels									
Activity level (%)*	Burst length (ms)								
	Masseter		Temporalis		Digastric				
	Experimental	Control	Experimental	Control	Experimental	Control			
5	83.46±1.20	86.63±2.43	74.29±5.12	84.43±5.44	95.82±3.29	94.37±4.95			
10	67.21±0.79	69.81±2.30	64.97±1.98	62.78±1.86	70.97±2.12	71.40±4.17			
20	48.88±1.15	51.25±1.18	52.77±1.56	48.18±1.35	49.41±1.06	48.84±2.43			
30	39.24±0.68	40.75±0.75	42.58±1.05	41.84±1.54	39.89±0.61	38.57±1.15			
40	33.35±0.29	34.27±0.49	36.08±0.70	37.97±1.42	34.68±0.75	33.35±0.56			
50	29.38±0.32	29.94±0.64	32.00±0.53	34.78±1.51	31.24±1.12	30.01±0.74			
60	26.83±0.85	26.96±0.92	29.02±0.84	31.96±2.04	28.43±1.10	27.37±0.79			
70	24.47±0.69	24.69±1.08	26.86±1.18	29.27±1.35	26.20±1.15	25.14±0.90			
80	22.68±0.80	23.08±1.56	24.83±1.25	27.31±1.66	24.43±1.06	22.89±0.91			

Table 3. Daily burst length of rabbit jaw muscles at various activity levels

Results are mean values ± standard deviations of the mean burst lengths calculated for the period from 8 to 20 weeks of age, during which groups were fed diets of different hardness. Burst lengths at the 90% activity level are not shown because they were partially below the measuring limit (20 ms) of the system used.

^{*}Expressed as a percentage of the peak-EMG.

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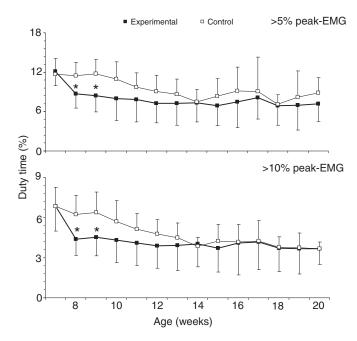


Fig. 2. Mean values and standard deviations of the daily duty times of the masseter muscle at the 5% and 10% activity levels as a function of rabbit age. Significant differences (*P*<0.05) between the experimental and control groups are indicated by asterisks. Only activity levels with statistically significant differences are shown.

groups or over the time course of the experiment at any activity level. This finding is in accord with a previous study, which has shown that the burst lengths of rabbit jaw muscles are very stable during postnatal development (van Wessel et al., 2005b). Furthermore, in a study on nine genera of mammals, Ross et al. showed that bite force modulation during mastication is mainly achieved by modulating the rate at which force is generated within a chewing cycle, and less so by varying temporal parameters (Ross et al., 2007). These authors suggest that significant variation in chewing cycle time would be difficult to accommodate in the context of a central pattern generator producing rhythmic bursts of activity at a relatively constant frequency. It is therefore reasonable to conclude that, under physiological conditions, the mean burst length of a muscle is largely unaffected by environmental changes and can be considered a relatively constant biological parameter.

The alteration in the activity profile of the masseter muscle was most pronounced shortly after the reduction in masticatory load, with statistically significant differences between the experimental and control groups limited to the two weeks after the change in food hardness. It appears that the softer pellets were easier to process for the experimental animals. Most likely, this resulted in a decrease in time necessary for food uptake, which is reflected in the decrease in masseter activity observed in this group of animals. The limitation of significant differences between the groups to the two weeks after the dietary change appears to imply that the effect of the dietary change was transient. However, this apparent transience might be the result of the slowly decreasing duty time and burst number in the control group, which over the time course of the experiment approached the values in the experimental group. Most likely, the masseter muscles of the experimental animals also adapted to the functional change. This adaptation probably involved feedback from the muscle spindle systems, resulting in a sensitivity modification of the muscle with higher probability of action potential firing. It

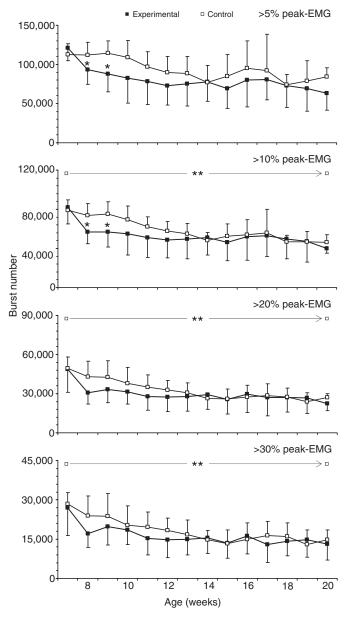


Fig. 3. Mean values and standard deviations of the daily burst number of the masseter muscle at the 5%, 10%, 20% and 30% activity levels as a function of rabbit age. Significant differences (*P*<0.05) between the experimental and control groups are indicated by single asterisks; significant differences over the time course are indicated by double asterisks. Only activity levels with statistically significant differences are shown.

has been suggested that loading changes, even chronic ones, may lead to compensatory sensory inputs, without apparent changes in central circuitry (Carvalho and Gerstner, 2004). It is conceivable that the reduction in neuromuscular activity was paralleled by a structural adaptation of the masseter muscle. Skeletal muscles can adapt to altered functional demands by changing from one fiber type into another and altering their cross-sectional areas (Adams et al., 1993; Pette and Staron, 1997). The changes in the phenotypic properties of muscle fibers might, in turn, have influenced the daily muscular activity.

Rabbit jaw muscles undergo substantial functional and anatomical changes during maturation (Weijs et al., 1987; Bredman et al., 1992).

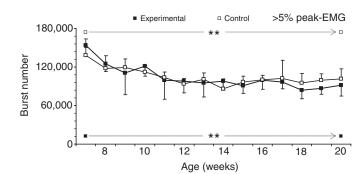


Fig. 4. Mean values and standard deviations of the daily burst number of the digastric muscle at the 5% activity level as a function of rabbit age. Significant differences (*P*<0.05) over the time course are indicated by double asterisks. Only activity levels with statistically significant differences are shown.

The results of the present study suggest that these changes are accompanied by alterations in the daily activity profiles of the jaw muscles. Under conditions of unaltered masticatory load, the total burst numbers of the masseter and the digastric muscles decreased significantly over the time course. These changes might represent developmental changes in oral behavior or an increased efficiency with food handling. As the animals matured and their jaws, oral cavities and jaw muscles got bigger, it may have become progressively easier for them to process pellets of a given size, resulting in fewer chews per bite. Neuromuscular activation patterns are typically established early in development (Bekoff and Lau, 1980; Cazalets et al., 1990), long before the anatomical changes in the muscles are completed. However, it is known that the execution of functional motor behavior is modified throughout development depending on factors such as muscular strength, peripheral feedback mechanisms and effective synaptic input to the muscles (Navarette and Vrbová, 1993). These latter modifications might also have contributed to the significant decrease in burst numbers over time observed in the present study.

A few remarks need to be made about the methods used in the present study. Implantable radio-telemetry enabled continuous wireless EMG recording and ensured that the behavior of the animals was as natural as possible. The telemetric system, recording technique and subsequent analysis are well characterized (Langenbach et al., 2002) and have been previously used in longterm studies of daily jaw-muscle activity (Langenbach et al., 2004; van Wessel et al., 2005a; van Wessel et al., 2005b; Kawai et al., 2007). The absence of significant changes in the peak-EMG values over the time course of the experiment indicates that the findings of the present study are not confounded by slow changes in equipment. However, it is recognized that the technique has limitations. The range of transmitted frequencies and the sampling frequency of the system render the signal not suitable for further characterization, such as frequency analysis (Langenbach et al., 2002). In the present study, the low sampling frequency of the system affected the accuracy of the burst length calculation, which decreased with increasing percentage of short bursts approaching the duration of time resolution.

In conclusion, the results of the present study show that a reduction in masticatory load may cause a decrease in the daily duty times and burst numbers of the jaw-closing muscles that are mainly responsible for the generation of occlusal force during mastication. The results suggest that this decrease is most pronounced in the

weeks immediately following the change in food hardness and that it is limited to activity levels that reflect muscle contractions during chewing. It appears that the masticatory system is relatively rigid, manifesting few diet-specific long-term changes in the activity profiles of jaw muscles.

ACKNOWLEDGEMENTS

This work was supported by a European Orthodontic Society research grant. The authors express their gratitude to Hans Korfage for his expert assistance with many aspects of this study. The surgical assistance and animal care of Klaas-Walter Meyer, Carla Prins and Erwin van Gelderop are gratefully acknowledged.

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