

The buccinator during mastication: A functional and anatomical evaluation in minipigs

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ABSTRACT

Objective: The buccinator muscle forms the lateral wall of the oral cavity. It is presumed to aid mastication by maintaining bolus position. Such a function would involve thickening the cheek, possibly compressing the alveolar bone and contributing to malocclusions. However, neither buccinator deformation nor its effect on pressure has been demonstrated. Our objective was to evaluate buccinator EMG during feeding, its changes in length and thickness, and the pressure exerted on its alveolar attachment, using miniature pigs as an animal model. Methods: EMG of the buccinator and other oral muscles was recorded with fine-wire electrodes. Anteroposterior length and mediolateral thickness of the buccinator were evaluated with implanted sonomicrometry crystals, and pressure was measured by flat transducers placed beneath the mandibular origin of the buccinator. Recordings were made during feeding and muscle stimulation. Tissues were collected postmortem for histology. Results: During mastication, buccinator EMG showed regular peaks that preceded those of the jaw closers. Pattern differences clearly distinguished working and balancing sides. The buccinator shortened and thickened when it contracted. Positive pressures were observed at the mandibular attachment of the buccinator, increasing when the muscle was active. Histological evaluation showed a complex interweaving of fibres closely associated with salivary tissue.

Conclusions: Buccinator contraction does thicken the cheek, and during mastication this activity takes place just as the closing stroke begins. In addition to controlling the bolus, there may be an effect on salivation. Despite the fact that the muscle pulls on its attachment, the local mechanical environment at the alveolar bone is one of positive pressure.

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1. Introduction

The buccinator, a muscle innervated by the facial nerve, forms the lateral wall of the oral cavity in mammals. This quadrilateral flat muscle is located deep to the skin and is mostly covered by the masseter and more superficial facial muscles. It is usually described as having predominantly horizontal fibres arising from the pterygomandibular raphe and from the alveolar bone of the maxilla and mandible and running anteriorly to interdigitate with the fibres of the orbicularis oris in the corner of the mouth.

Most textbooks briefly and superficially describe the role of the buccinator as controlling the bolus during mastication, keeping food between tooth surfaces by "compressing the

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cheeks," and preventing injury of the buccal mucosa.¹ Because the buccinator contributes fibres to the parotid duct, it has also been speculated that the muscle could assist salivary secretion.² Early electromyographic (EMG) evaluation of the buccinator in humans indicated it was strongly but irregularly active during most oral functions including sucking, blowing, swallowing, smiling, and speech, generally in association with the orbicularis oris muscle.³⁻⁵ More recent and complete work in humans⁶ and in rabbits⁷ has concentrated instead on the activity of the buccinator in mastication. These studies describe strong activity, differing in both timing and amplitude between working and balancing sides, and changing in response to different quality or quantity of food. Oddly, humans and rabbits differ somewhat in the specific pattern of masticatory activity of the buccinator. In humans, the buccinator and orbicularis oris EMG bursts occur simultaneously at the end of the opening phase, just prior to strong contraction of the masseter muscle. The working-side buccinator has higher amplitude and longer duration than the balancing side, but the basic timing is similar on the two sides.⁶ In rabbits, on the other hand, only the balancing side has its major burst just prior to the jaw-closing muscles; the working side burst begins after closing muscle activity has begun and ends during the opening phase. Orbicularis oris activity in rabbits is bilaterally symmetrical and resembles the balancing buccinator pattern.7

Regardless of the detailed pattern of activity, these studies do support a role in mastication as a principal function of the buccinator. However, there is little consideration of how this role is accomplished. Although buccinator fibres attach to both upper and lower jaws, their predominantly horizontal direction indicates that the muscle is not a jaw closer. Rather, the muscle's anatomy suggests it would retract the oral angle. However, several other facial muscles, such as zygomaticus major, are better suited for this action. Most likely, the muscle functions by thickening the entire cheek, basically acting as a muscular hydrostat.⁸ This thickening could be the mechanism by which the cheek seems to be able to push a food bolus towards the tongue.⁹

Because of the proximity of the buccinator to the alveolar bone and dental arches, its functional effects on these structures have intrigued dental investigators for decades.¹⁰ It has been suggested that hyperactivity of the buccinator (presumably thickening it and putting pressure on the underlying hard tissues) causes narrow arches and malocclusion.^{10,11} Pressures measured in the oral cavity of humans are, in fact, usually positive on buccal surfaces, typically rising with mastication and rising even more for swallowing.10,16-18 Appliances with buccal shields have been created to remove the "restricting" effects of the facial muscles surrounding the dental arches, and clinicians have demonstrated the utility of these appliances for expanding the dental arches transversely.^{12–15} However, debate remains on whether contraction of the thin buccinator muscle is the actual cause of these pressures, especially because the muscle is quiescent at rest, and in the only previous study combining EMG and pressure measurements, the buccinator EMG recording was overwhelmed by crosstalk from the much larger masseter.¹⁷ Another reason sometimes given for the success of buccal shields is that the passive tension produced by stretching the buccinator provides an osteogenic stimulus to the alveolar attachment areas.¹¹ A

tensile load on the alveolar attachment areas might also arise from the active contraction of the buccinator. However, noninvasive studies on humans cannot access the actual alveolar attachment areas to test this suggestion.

Therefore, in order to better understand the function of the buccinator during mastication and the effects on the alveolar bone, we undertook a study using minipigs (Sus scrofa), the nonprimate animal model most appropriate for the study of human mastication.¹⁹ EMG activity of the buccinator was compared with that of the masticatory muscles, including two jaw closers, masseter and temporalis, and one jaw opener, digastric. Because of the close relationship of the buccinator with the orbicularis oris, EMG of this muscle was examined as well. The recordings were performed repeatedly over a period of weeks during ingestion and mastication of foods of different consistency and size. In a final experiment, EMG activity was supplemented by simultaneous measurements of buccinator length and thickness and of pressure at the buccinator attachment to the mandibular alveolar bone. In addition, in order to understand the muscle anatomy better, dissection was followed by histological study of the cheek so that the fibre direction could be discerned.

The purposes of this study were: (1) to evaluate the EMG activity of the buccinator during feeding in pigs and to compare it with that of the surrounding masticatory and facial musculature; (2) to establish whether buccinator contraction thickens the cheek, and if so whether this is accompanied by other dimensional changes in the muscle; (3) to determine whether the buccinator attachment loads the alveolar bone either in compression or in tension; and (4) to provide a description of the pig buccinator muscle for future studies.

2. Materials and methods

2.1. Subjects

Five Hanford (3 males and 2 females) and one Sinclair (male) miniature pigs (Sinclair BioResources, Columbia, MO) were used. The animals were 3–4 months old at the beginning of the study.

2.2. Electromyography (EMG)

After a period of acclimation (3-5 days), EMG activity of the facial muscles (buccinator and orbicularis oris), jaw closers (masseter and temporalis) and (in two of the pigs) jaw depressor (digastric) was recorded bilaterally during normal mastication. Recordings were made five times per week for about 4 weeks for each animal. Fine-wire electrodes (0.05 mm insulated nickel-chromium wire with 1 mm of insulation scraped from the ends, interelectrode distance 1-2 mm, 25 G hypodermic needles) were used for the buccinator, orbicularis oris and digastric (Fig. 1). Surface electrodes were used for the masseter and temporalis (interelectrode distance 2-3 cm). A ground electrode was affixed to the forehead. The pigs were anaesthetized with isoflurane and nitrous oxide during electrode placement. After recovery, foods of varying consistency were offered: pig chow pellets, pieces of apple with skin, shelled almonds, dried apricot, pistachios with shells and pieces of orange. The pigs ate



Fig. 1 – Diagram of a pig head showing positioning of instrumentation. Only the part of the buccinator not covered by the masseter is shown. Surface electrodes were positioned over the masseter (M) and temporalis (T). Finewire electrodes (stars) were placed in buccinator (B), orbicularis oris (OO), and digastric (D) muscles. Sonomicrometry crystals in the buccinator (small ovals within dotted circles) were placed anterior (1), posterior (2), superficial (3) and deep (4). The pressure transducer (P) was placed at the attachment of the buccinator to the mandibular alveolar process.

normally whilst EMG signals were recorded to computer (500 Hz sampling rate) using Acqknowledge III (version 3.9, Biopac Systems, Santa Barbara, CA). Lateral videorecording synchronized with the EMG activity was performed for a small number of recordings (Motus, Peak Performance Tech., Englewood, CO).

2.3. Surgical procedures and terminal experiment

In a terminal experiment, buccinator dimensions and alveolar loading were recorded along with EMG. The surgical procedures were identical for the six pigs and were performed bilaterally. Pigs were anaesthetized as usual and positioned in lateral recumbency. First, an incision of about 10 mm was made close to the lower border of the mandible. A tunnel from this incision to the molar region accessed the mandibular alveolar origin of the buccinator. A flat titanium-bodied pressure transducer (Model P19F, 5 mm diameter, 1.8 mm thick; Konigsberg Co., Pasadena, CA) was inserted and sutured in place (Fig. 1). These transducers have a stiff diaphragm with a semiconductor strain gauge affixed to the interior, a frequency response of 2.6 kHz, an operational pressure range of -13.3 to +53.3 kPa, and a capacity up to 400 kPa. They were calibrated prior to each use by immersion in a sealed pressurized bottle filled with water at 37 °C. Pressure (0-40 kPa), produced using a blood pressure bulb, was plotted against voltage output. Linear relationships ($r^2 = 0.98-0.99$) were consistently obtained, and the resulting regression equations were used to calculate experimental values.

After the pressure transducer was placed, four ultrasonic crystal transducers (2 mm diameter with barbs or suture loops for tissue retention, Sonometrics Co., London, ON, Canada) were implanted in the buccinator to evaluate anteroposterior length and mediolateral thickness. These crystals continuously send and receive ultrasound from other crystals in the array, thus measuring distance in real time. The four crystals were placed through small skin incisions. As illustrated in Fig. 1, the anterior crystal was positioned close to the commissure of the lips and the posterior close to the masseter. However, this was not the muscle's total anteroposterior length; the most posterior part of the buccinator was inaccessible because of the overlying masseter. The crystals used in the anterior and posterior locations were equipped with "A-barbs" which prevented backing out of the tissue. To insert the anterior and posterior crystals inside the buccinator, muscle fibres were gently separated. The initial distance between the anterior and posterior crystals with the muscle at rest was 12-34 mm. The superficial (lateral) and deep (medial) crystals were intended to measure muscle thickness and were placed under the skin (superficial crystal) and deep to the mucosa (deep crystal), in the mediolateral (transverse) plane, about midway between the anterior and posterior crystals. The crystals used in these locations each had two loops which were used to suture them to the underside of the skin or to the deep surface of the mucosa. The initial (resting) thickness was 4-7 mm. The incisions were sutured and the cables connected. EMG electrodes were placed as in the daily recordings. Procedures were then repeated on the other side.

Animals were given analgesics (ketorolac, 1 mg/kg and buprenorphine, 0.01 mg/kg, both IM) and allowed to awaken. Foods of different consistency were offered as during the previous recordings of normal mastication. Pressures were recorded along with the EMG data in Acqknowledge III. Sonometric distances were recorded digitally to a separate computer running SonoLab software (Sonometrics). One or more distances were simultaneously recorded in Acqknowledge III in order to associate EMG data with the dimensional and pressure changes of the buccinator. However, accurate quantification of dimensions was only possible in SonoLab, where information on the side of chewing was absent.

After the mastication data were acquired (about 30 min), the pigs were anaesthetized again and placed prone on the table. The buccinator was stimulated through the fine-wire electrodes (5 ms pulses, 55 pulses/s, 600 ms trains, 0.5 trains/s) whilst pressure, length and thickness were recorded.

2.4. Buccinator histology

Anaesthetized pigs were euthanized with pentobarbital at the end of the terminal experiment. The cheeks were removed and immersed in fixative (Prefer, Anatech, Battle Creek, MI). After about 20 days of fixation, the facial skin and subcutaneous fat were removed and the specimens embedded in paraffin wax. Parasagittal sections (7 μ m) were cut, mounted on glass slides, stained with haematoxylin–eosin and cover slipped.

2.5. Data analysis and statistical procedures

The EMG and pressure signals were filtered (highpass of 250 Hz for EMG and lowpass of 30 Hz for pressure). EMG was analysed qualitatively to assess the timing of buccinator and orbicularis

oris activity relative to that of the jaw-closing and -opening muscles. For pressure and dimensional changes, 10–20 consecutive chewing cycles from each type of food were quantified using SonoView (Sonometrics) and Acqknowledge III. Baseline was considered to be the lowest pressure, greatest anteroposterior length (L_o) and least thickness (T_o) for each cycle, and peak values were the highest pressure, shortest length and greatest thickness. Measurements of cyclic changes were made by subtracting the baseline from the peak values for each chewing cycle. Pressures were calculated from voltages using the regression equations generated during calibration. Strains were calculated from dimensional measurements as the peak change per cycle divided by the initial distance, i.e. $\Delta L/L_o$ for length and $\Delta T/T_o$ for thickness. Descriptive statistics were calculated using SPSS 13.0 for Windows.

3. Results

3.1. Feeding behaviour and EMG activity

The food preference for each pig varied, and no animal ate all the foods, except for pig chow. Only one animal (#416) drank

liquid (orange juice) during the sessions. Drinking, as in other studies,^{20–22} occurred by suction. As shown in Fig. 2, the buccinator muscles were symmetrically and phasically active, as were the orbicularis oris muscles, although these pairs of muscles were out of phase with each other. Jaw-closer activity was negligible.

As in other minipig studies,^{21,23} feeding behaviour on solid food consisted of periods of food collection (0.5 to several seconds) alternating with about 20 consecutive masticatory cycles. Food gathering featured very rapid (4-6/s) cycles in which the tongue picked up food particles and was easily distinguished from the slower mastication (2-3/s). Food gathering showed similar EMG patterns for all types of food (Fig. 3). The jaw closers exhibited brief bursts of activity. The buccinator was tonically active at a low level, and superimposed on this baseline activity were low to moderate bursts towards the end of the opening phase of each cycle. In contrast to the closers and the buccinator, the orbicularis oris and digastric were strongly active when the pigs were gathering food, with symmetrical phasic activity in the right and left sides. However, their timing differed. The orbicularis oris activity corresponded to the contraction of the jaw closers (Fig. 3), whilst the digastric was active in the opening phase (digastric data not shown).



Fig. 2 – EMG for drinking (orange juice) compared to mastication (orange slices) in #416, the only pig that drank. The 2-s segments are from the same file and have the same scale. The jaw closers have minimal or no activity during drinking, whilst the orbicularis oris presents stronger activity than during mastication. Right and left temporalis (RT and LT); right and left masseter (RM and LM); right and left buccinator (RBuc and LBuc); right and left orbicularis oris (ROO and LOO). Scale bar 400 mV.



Fig. 3 – EMG during a continuous sequence of food gathering and mastication of pig chow (#421). The working side is identified as right (R) or left (L). During food gathering, the orbicularis oris muscles are strongly active and the buccinators show weak irregular activity. As the pig initiates mastication, the buccinator becomes phasically active with the burst occurring just prior to the jaw-closer burst. As the chewing becomes regular, a distinction between working and balancing side activity develops in the buccinator, as shown in the boxed blow-up. The balancing side (open arrows) is active about 100 ms before the working side (solid arrows), which presents higher amplitude and a shorter burst. Muscle abbreviations as in Fig. 2. Scale bar 200 mV.

Compared to food gathering, jaw closer EMG during mastication was stronger, more prolonged, and more asymmetric because of the alternation of working and balancing sides. The working side was discerned from the delayed offset of the working side masseter and balancing side temporalis EMG activity.²¹ As the chewing sequence commenced, rhythmic activity increased in the buccinator as well as the jaw-closing muscles, with the buccinator peak preceding that of the jaw closers and occurring right after the digastric peak (Figs. 3 and 4). In contrast to the buccinator, the orbicularis oris was less active during mastication than during food gathering, and when bursts were seen they coincided more often with the jaw closers than with the buccinator.

For pig chow, the buccinator pattern became more regular as the pigs chewed, with distinctive differences developing between working and balancing side activity. Because buccinator activity immediate preceded jaw-closer activity, "working side" refers to the upcoming chewing stroke; in most cases the balancing side was the working side in the previous cycle. The EMG duration of the balancing side buccinator was longer, lower in amplitude, and started sooner (about 100 ms); the working side activity was shorter, later and had higher amplitude (Fig. 3). Low activity often continued on both sides throughout the jaw-closer burst (Fig. 4, left, particularly LBuc). This well-defined pattern was observed in all 6 animals studied during mastication of pig chow and was also seen in 3 other pigs previously evaluated (data from laboratory archives). When the pigs were chewing other foods (orange slices, apples, almonds or pistachios with shells), the buccinator EMG pattern was less clear and regular (Figs. 2 and 4), although the working/balancing side distinction was often visible. A major reason for the less regular pattern for non-pig chow foods was the presence of additional strong bursts of buccinator activity, often occurring as the jaw-closer burst was ending (Fig. 4).

EMG patterns were the same during the terminal experiment as those observed during the daily recording (Fig. 5). Therefore, the surgery and implantations did not disrupt buccinator activity.

3.2. Length and thickness of the buccinator (ultrasound crystals) during feeding

The ultrasound crystals were generally stable during the feeding sessions as indicated by consistency of the baseline dimensions.

The single animal that drank during the daily sessions (#416) also drank during the final recording. Dimensional changes during drinking were very small. With each burst of EMG, anteroposterior length was slightly (3%) reduced on both right and left sides. Changes of thickness were negligible (1–2%), irregular, and not consistently correlated with EMG or length.

During food gathering, the buccinator muscle showed regular changes in dimension corresponding to the rapid movement cycles of the jaw (Fig. 5, left). Although much more distinct than during drinking, dimensional changes during food gathering were less than half (average 47–48%) of the values seen during mastication. Anteroposterior shortening and mediolateral thickening were initiated just before the



Fig. 4 – Comparison of masticatory EMG for pig chow (#424) and apple with skin (#423). The working side is identified (R or L) above each masseter burst. Buccinator activity is higher but less regular with apple, and the difference between working and balancing activity is less clear. During apple mastication, the buccinator on both sides shows an additional peak of activity coinciding with the end of the jaw-closer burst (arrows). Right and left digastric, RD and LD. Other muscle abbreviations as in Fig. 2. Scale bar 200 mV.



Fig. 5 – EMG activity (RBuc), pressure (RPress), anteroposterior length (RLength) and mediolateral width (RThick) of the right buccinator during gathering and mastication of pig chow (#418). Pressure and dimensional changes are larger and more regular in mastication than in food gathering. Changes are similar regardless of the side of mastication (identified above the masseter burst). Roughly coincident with the maximum buccinator peak (shaded regions), pressure begins to rise, length decreases, and the muscle thickens. Minimum length and maximum thickness and pressure are typically reached during the jaw-closer burst, illustrated by the right masseter (RM). Scale bars are 400 mV for EMG, 0.5 kPa for pressure, 2.0 mm for length, and 0.2 mm for thickness.

jaw-closing muscles were active (when buccinator EMG bursts were most often seen) and continued during the brief contractions of the jaw closers, with minimum length and maximum thickness reached just after the jaw-closing bursts. Because buccinator EMG was often irregular, correspondence between EMG and dimensional changes was poor during food gathering.

During mastication dimensional changes of the buccinator were much more rhythmic and dramatic and were clearly associated with EMG. As shown in Table 1, length strains averaged $-16.6 \pm 3.6\%$ (n = 12, range -12 to -22%) and thickness strains averaged $8.4 \pm 2.7\%$ (n = 11, range 3–11%). Thickness strains were roughly half as large as length strains, i.e. the Poisson ratio was about 0.5 (calculated from Table 1). As illustrated in Fig. 5 (right side), anteroposterior length was maximal in the opening phase of the masticatory cycle. Shortening began with the buccinator EMG burst at the end of the opening phase, and minimum length was reached in the middle of the jaw-closer burst; thus shortening accompanied early closing. Buccinator thickness presented a roughly inverse relationship with length; as the buccinator shortened, it became thicker. However, the relationship was not exact. In the example illustrated (Fig. 5), elevated thickness was maintained for the full duration of the power stroke, dropping only after cessation of the jaw-closer activity, but this was not always the case. Regardless of the details for individual pigs, the patterns appeared the same on working and balancing sides, and rough measurement of the waveforms in Acqknowledge III indicated no quantitative difference between working and balancing sides (p = 0.14 for length and 0.45 for thickness, paired t-tests). Because of this, and because working and balancing sides could not be distinguished in the sonometric files, chewing sides are combined in Table 1.

Differences amongst foods were difficult to assess, owing to varying sample size. Even pig chow was only eaten by 5 of the 6 animals, apple by 4, and the other foods were eaten only by 2-3 (Table 2). Nevertheless, these data suggest that food texture/size may matter. Considering only the 4 animals that ate at least three foods, a pattern emerges if these data are surveyed in terms of which foods elicited the largest changes in length and thickness and which the smallest. For both length and thickness, apple most often produced the greatest strains. Apricot appeared to be comparable, but here sample size was very poor (2-3 sides of 1-2 pigs). At the other end of the spectrum, the two kinds of nuts (unshelled pistachios and shelled almonds) were most often associated with the smallest muscle strains. Pig chow and orange produced intermediate results.

3.3. Loading of the buccinator attachment (pressure transducers) during feeding

Negative pressure (i.e. tension) was never recorded during the experiments. During feeding, baseline loading approximated 0 in all animals, and changes from this level were always positive, i.e. pressure against the mandibular attachment area.

No distinct pressure variations were seen during drinking in the single individual that performed this behaviour. During feeding activities with larger jaw movements, however, pressure variations were in phase with dimensional changes (Fig. 5). Peaks of pressure corresponded with minimal

Table 1 – Dimensi	onal and pressu	re changes of th	ie buccinator m	uscle during n	nastication."					
Pig #	R ΔLei	ngth ^b	R AThic	kness	R APressure	τ Διε	ngth	L ΔThi	ckness	L APressure
	Mean ± SD ^c (mm)	Strain (mm/mm)	Mean ± SD (mm)	Strain (mm/mm)	Mean ± SD (kPa)	Mean ± SD (mm)	Strain (mm/mm)	$\begin{array}{l} \text{Mean}\pm\text{SD}\\ \text{(mm)}\end{array}$	Strain (mm/mm)	Mean ± SD (kPa)
416	-3.28 ± 1.24	-0.19	0.26 ± 0.02	0.05	3.39 ± 0.91	-3.63 ± 0.77	-0.20	0.63 ± 0.18	0.11	1.03 ± 0.35
418	-3.44 ± 1.80	-0.12	$\textbf{0.59}\pm\textbf{0.02}$	0.07	0.56 ± 0.13	-2.00 ± 0.26	-0.12	$\textbf{0.65}\pm\textbf{0.06}$	0.09	$\textbf{0.88}\pm\textbf{0.32}$
419	-1.63 ± 0.34	-0.13	0.16 ± 0.05	0.03	0.51 ± 0.31	-3.47 ± 0.71	-0.13	0.43 ± 0.09	0.08	0.96 ± 0.31
421	-5.44 ± 0.16	-0.16	$\textbf{0.38}\pm\textbf{0.06}$	0.07	0.49 ± 0.24	-3.79 ± 0.41	-0.17	ND ^d	QN	1.08 ± 0.36
423	-3.70 ± 0.17	-0.16	0.59 ± 0.13	0.10	0.48 ± 0.19	-5.74 ± 0.50	-0.17	$\textbf{0.45}\pm\textbf{0.05}$	0.09	1.09 ± 0.15
424	-5.88 ± 0.36	-0.22	0.66 ± 0.20	0.11	3.04 ± 0.15	-5.50 ± 1.25	-0.22	$\textbf{0.55}\pm\textbf{0.35}$	0.12	1.00 ± 0.19
Grand mean \pm SD		-0.16 ± 0.04		$\textbf{0.07}\pm\textbf{0.03}$	1.41 ± 1.40		-0.17 ± 0.04		$\textbf{0.10}\pm\textbf{0.02}$	1.01 ± 0.08
^a For length and thick cvcles were averaged	tness, 10 cycles for (for each pig. and th	each food were ave lese were for pig ch	eraged, resulting in	totals of 20 cycle c #424. which did	es for #418, 30 cycl not eat pig chow	es for #418, #419 (p during the final rec	ig chow only), #421 cording. Working a	l, and #424, and 50 nd balancing side:	cycles for #423. I swere unknown	or pressure, 30 for dimensions

and were combined for pressure (see text). $^{\rm b}$ R, right buccinator; L, left buccinator; A, change from baseline. $^{\rm c}$ SD, standard deviation. $^{\rm d}$ ND, no data.

Table	2 – Bi	iccinato	r muscle :	strains f	or differer	nt foods.ª									
Pig #	Side	Average change in anteroposterior length (%)							Average change in mediolateral thickness (%)						
		Chow	Orange	Apple	Almond	Pistachio	Apricot	Chow	Orange	Apple	Almond	Pistachio	Apricot		
416	L	<u>17.1</u>	19.1	25.4	-	-	-	<u>9.5</u>	<u>9.6</u>	15.3	-	-	-		
	R	<u>12.1</u>	18.7	26.5	-	-	-	5.6	<u>5.5</u>	<u>4.9</u>	-	-	-		
418	L	13.3	11.1	-	-	-	-	8.5	9.7	-	-	-	-		
	R	17.0	7.8	-	-	-	-	6.0	5.8	-	-	-	-		
419	L	13.9	-	-	-	-	-	7.2	-	-	-	-	-		
	R	14.4	-	-	-	-	-	3.1	-	-	-	-	-		
421	L	17.0	-	19.0	<u>15.0</u>	-	-	-	-	-	-	-	-		
	R	<u>15.0</u>	-	16.0	16.0	-	-	<u>5.0</u>	-	7.0	8.0	-	-		
423	L	20.0	-	17.0	18.0	<u>16.0</u>	18.0	-	-	-	8.0	9.0	10.0		
	R	17.0	-	16.0	<u>15.0</u>	17.0	17.0	9.0	-	14.0	11.0	8.0	12.0		
424	L	-	-	27.0	20.0	<u>18.0</u>	-	-	-	16.0	<u>3.0</u>	14.0	-		
	R	-	-	24.0	22.0	<u>21.0</u>	25.0	-	-	-	-	11.0	-		

^a Data are averages of 10 masticatory cycles per each food eaten. For pigs that ate at least three foods, maximum strains are bolded and minimum strains are underlined.

anteroposterior lengths and maximal thickness both in food gathering and in mastication. Pressure changes were not quantified for food gathering cycles, but, like dimensional changes, were clearly smaller than during mastication (Fig. 5).

In mastication, pressure began to rise just after the major burst of buccinator EMG in the majority of animals; pressure peaked whilst the jaw closers were still active (Fig. 5). Peak pressure changes varied quite a bit amongst animals (especially on the right side, Table 1) but within animals were consistent. The overall mean was 1.21 ± 0.97 kPa (n = 12, range 0.49–3.39 kPa). The low values and high variability suggest that the transducers did not function optimally during mastication, as discussed further below. There was no relation between the chewing side and pressure values (p = 0.16, paired t-test), so chewing sides are combined in Table 1. Comparison of different foods showed the same general (but weak) trend as the dimensional data, with apple and possibly apricot associated with higher pressures and nuts associated with lower pressures (data not shown).

3.4. Length, thickness and loading of attachment area during buccinator stimulation

Anaesthetized animals in the prone position consistently showed buccinator resting lengths and pressures that differed from the baseline dimensions and pressures of awake, feeding

Table 3 – Stimula	tion o	of the bucc	inator in a	naesthetized j	pigs, resting	vs. contrac	ted dimension	s and pressu	re. ^a
Pig #		Resting length (mm)	Length ∆ ^b (mm)	Length strain (mm/mm)	Resting thickness (mm)	Thickness Δ (mm)	Thickness strain (mm/mm)	Resting pressure (kPa)	Pressure Δ (kPa)
416	R ^c	17.9	-1.6	-0.09	3.2	0.2	0.06	1.28	11.60
	L	16.4	1.0	0.06	5.1	2.0	0.39	0.65	3.88
418	R	26.1	0.5	0.02	8.0	ND ^d	ND	0.45	7.41
	L	15.5	0.5	0.03	7.6	0.3	0.04	0.84	6.05
419	R	11.0	1.0	0.09	5.4	0	0	0.44	8.21
	L	23.9	0.8	0.03	5.8	-0.7	-0.12	0.37	9.55
421	R	34.4	-6.2	-0.18	ND	ND	ND	0.84	10.16
	L	20.8	0.9	0.04	ND	ND	ND	0.68	8.67
423	R	ND	ND	ND	ND	ND	ND	0.28	4.91
	L	ND	ND	ND	ND	ND	ND	0.35	7.48
424	R	21.4	2.5	0.11	3.15	0	0	0.88	11.96
	L	28.7	-6.9	-0.24	4.06	0.3	0.07	0.59	5.93
Grand mean \pm SD				-0.01 ± 0.12			$\textbf{0.06} \pm \textbf{0.16}$	$\textbf{0.64}\pm\textbf{0.29}$	$\textbf{7.98} \pm \textbf{2.55}$

^a Length and thickness are maximal values from the stimulation procedures, whereas pressures are averages.

 $^{\rm b}$ $\Delta,$ difference between resting and contracted values.

^c R, right buccinator; L, left buccinator.

^d ND, no data.

animals. Buccinator anteroposterior length was an average of 1.3 mm shorter when pigs were anaesthetized than when they were awake (p = 0.009), although mediolateral thickness did not change significantly. Concurrently, resting pressure at the mandibular attachment of the buccinator was higher in anaesthetized animals, averaging 0.64 ± 0.29 kPa (data from Table 3) in contrast to 0.02 ± 0.02 kPa at feeding baseline (p < 0.0001).

The purpose of the buccinator stimulation was to clarify the effect of muscle activity on muscle dimensions and loading at the attachment area by inducing maximum contraction. This procedure was, however, not reliable for dimensional changes, which varied widely in direction as well as in amount when the buccinator was tetanized (Table 3). In some pigs the muscle thickened and shortened as in mastication (Fig. 6) but in other cases the opposite changes were observed or both dimensions increased when the muscle was stimulated. Because the corner of the mouth always retracted, which implies that anteroposterior shortening occurred, the inconsistency in ultrasound measurements was probably an artefact produced by irregular buckling of the cheek.

In contrast to muscle dimensions, pressure at the alveolar attachment changed consistently and dramatically when the buccinator was stimulated. Tetanic pressure increases at the attachment area (7.98 ± 2.55 kPa, data in Table 3) were almost an order of magnitude higher than mastication peaks (1.21 ± 0.97 kPa, combined left and right from Table 1) (p < 0.001). These elevated tetanic pressures corresponded to the contraction of the muscle and were sustained until the end of each tetanus (Fig. 6).



Fig. 6 – Stimulated tetanus of the left buccinator. Stimulation produced much higher pressures (LPress) on the alveolar bone than did mastication in all pigs. Dimensional changes were variable, probably because of distortions within the muscle. In this animal (#416) dimensional changes were similar in direction (but not magnitude) to those of mastication, anteroposterior (LLength) shortening, and mediolateral (LThick) thickening. LBuc is the stimulus recorded through EMG electrodes in the left buccinator. Scale bars are 2.0 kPa for pressure and 2.0 mm for length and thickness.

3.5. Buccinator anatomy

The anterior part of the buccinator was very superficial and the most superficial fibres were vertically oriented. Dissection was difficult, because fibres attached to the skin and blended



Fig. 7 – Parasagittal sections through the buccinator muscle; rostral (anterior) is to the left. (A) Low power view of #422 (scale bar 1 mm) showing the generally horizontal orientation of the deeper fibres, becoming interwoven near the oral commissure (OC). Salivary tissue (S) can be seen in the lower portion of the section. (B) Same specimen, a slightly more inferior (ventral) region, showing multiple buccal salivary glands (S) interwoven with fibre bundles. Scale bar 500 μm. (C). A similar area from #418 shows muscle fibres that appear attached to the gland capsule (arrows). Scale bar 500 μm.

with those of the orbicularis oris. After removal of the masseter, the posterior, deeper part of the buccinator could be visualized extending to the back of the oral cavity; most of these fibres were horizontally oriented. Although the muscle was attached to the alveolar bone of the maxilla and mandible, the fibres did not seem to originate from these structures but rather were bonded by connective tissue to the thin mucosa that covered the bone.

The parasagittal histological sections revealed a surprisingly complex arrangement of buccinator fibres. Most ran anteroposteriorly, especially in the middle part of the muscle, but vertical and mediolateral fibres were also apparent (Fig. 7A). In the anterior portion of the muscle, near the oral commissure, the anteroposterior fibres interdigitated with the vertical fibres and orbicularis oris in a basket-weave pattern. Salivary tissue was found throughout most sections, even between the buccinator fibres (Fig. 7B). Some areas seemed to show a direct attachment of buccinator fibres to these minor salivary glands (Fig. 7C).

4. Discussion

4.1. EMG activity of the buccinator in relationship to other muscles

EMG recording of the buccinator is challenging due to its proximity to other facial muscles, especially orbicularis oris, and the potential for crosstalk from the much larger masseter.¹⁷ Fine-wire electrodes are a necessity, and specificity must be confirmed by comparison with neighbouring muscles. In the present study, crosstalk from the masseter was not a problem because the buccinator was consistently activated earlier than the masseter in every pig evaluated and the pattern was identical for all of them. Furthermore, the activities of the buccinator and orbicularis oris were quite different. During food gathering orbicularis oris was more active and more rhythmic, and during mastication the reverse was true. Moreover, these two facial muscles were mostly out of phase.

The different activity patterns of the buccinator and orbicularis oris clearly relate to their different roles during feeding in pigs. The orbicularis oris is especially important during drinking, which occurs by suction through tightly pursed lips. Similarly, the lips, especially the lower, are involved in picking up particulate food as it is gathered, aiding the tongue.^{20,21} However, the lips and orbicularis oris are apparently not essential for mastication, judging from the irregular activity seen in the present study. The relatively low-amplitude bursts of the buccinator during food gathering may indicate that the muscle participates in the transport of particles to the cheek tooth row, but strong activity in the buccinator is reserved for mastication, discussed further below.

The independence of orbicularis oris and buccinator activity patterns further indicates that despite the apparent continuity of their fibre bundles at the oral commissure, these two muscles in pigs are neurologically distinct. This conclusion is in accord with the clear separation of buccinator and orbicularis oris motor pools within the facial nucleus.²⁴ Independence of buccinator and orbicularis oris EMG patterns is also seen in rabbits⁷ but not in humans.⁶ In long-snouted animals the cheek tooth row is so distant from the lips that orbicularis oris activity would not aid in controlling the bolus; thus no coordination between buccinator and orbicularis may be necessary in minipigs. The shortening of the snout in higher primates has presumably brought about a greater functional continuity of these muscles, such that they work together as a total facial mask³ to control ingested food.

During mastication, the timing of the buccinator EMG burst was just before the jaw-closer burst, as has also been reported for humans.⁶ (Ingervall and Thüer¹⁷ found simultaneous buccinator and masseter activity, but ascribed this to cross-talk. They also saw low activity from the buccinator before the masseteric burst, and this was probably genuine.) In pigs this timing corresponds with the initiation of jaw closure²⁵ and therefore suggests the buccinator is controlling the placement of the bolus on the occlusal table as the jaw closes, an interpretation also supported by videofluorographic studies of bolus position in humans.⁹

A very interesting aspect of our buccinator EMG results was the difference between the working side and balancing side (Fig. 3). It is not immediately obvious why the balancing side buccinator should be active at all, but conceivably the balancing side cheek aids the tongue as it twists to brace the bolus against the teeth from the lingual side.²⁶ The greater amplitude of the buccinator burst on the working side was expected and has been observed in humans as well.^{4,6} However, the early, prolonged burst of the balancing buccinator vs. the short sharp burst of the working buccinator was not expected. One possibility is that this unusual pattern is related to the alternating chewing style of pigs. Because the side of chewing is reversed with every stroke, the early balancing activity is likely related to the transfer of the bolus to the other side.

A second interesting EMG finding was the variability introduced by different food consistencies (Fig. 4). Conceivably, pigs chewed more stereotypically with chow simply because they were most accustomed to this foodstuff. More likely, however, pig chow, which is an agglomerate of smaller particles, formed a more predictable and cohesive bolus than any of our more natural dietary offerings. With the possible exception of dried apricot, all of the natural foods we used would either produce juice or would fracture (or both) when crushed. Unpredictable bolus fracture would make transfer from balancing to working side more variable and also would necessitate additional closing-phase activity as the cheeks attempt to control the position of fragments during the power stroke.

Of the three species from which detailed buccinator EMG data are now available, pigs resemble humans⁶ more than rabbits.⁷ The principal difference is the greater difference of timing between the sides in rabbits, with very late activity in the working-side buccinator, corresponding more to the power stroke than to the initiation of closing. Rabbits have a much broader transverse movement during the power stroke than either humans or pigs, and the jaws are so anisognathic that there is no tooth contact at all on the balancing side.²⁷ This probably accounts for the almost opposite activity patterns of the two buccinators in rabbits,

with the balancing side aiding the tongue in moving the bolus to the working side, just as in humans and pigs, but the working side functioning to ensure that the bolus stays with the lower molars as they sweep lingually across the uppers.

4.2. Dimensional changes and pressure during mastication and stimulation

A primary goal of this study was to establish whether and how the buccinator thickens the cheek. Direct measurement was difficult because the buccinator is thin. The initial measured thickness (between the two central crystals) of 4-7 mm included some cheek connective tissue in addition to the buccinator. The results are nevertheless valid as relative, if not absolute, values because the buccinator is the only contractile element in the cheek. Similarly, although the anteroposterior measurement did not include the most posterior part of the buccinator, there is no reason to believe that this part would have strained any differently. As might have been expected, the sonomicrometric methodology was highly reliable in revealing changes in muscle dimensions in awake animals, but essentially useless for semi-isometric contractions that were induced by tetanizing the muscle in anaesthetized animals.

The dimensional changes observed during function indicate that when the buccinator contracts at the beginning of jaw closing, the primary change is an anteroposterior shortening, which further causes a retraction of the corner of the mouth and, most importantly, an increase in the thickness of the cheek. This causality is supported by the fact that length and thickness changes were always in opposite directions (Fig. 5 and Table 1), and by the tendency for tough foods such as apple with skin to produce larger changes in both dimensions (Table 2). Thus the buccinator does behave as a muscular hydrostat, altering the shape of the cheek by muscle contraction. Cheek thickening early in the closing movement is the most likely physical mechanism by which the bolus is manipulated from the buccal side. Dimensional changes were similar on the working and balancing sides, suggesting that the higher amplitude of the working side EMG activity compensated for the longer duration of the balancing side burst.

In contrast to the ultrasound crystals, the pressure transducers on the alveolar bone gave much higher readings during isometric tetany than during mastication in awake animals. The high values for stimulated contraction probably reflect not only the increased force produced by the tetanized muscle, but also more stable recording conditions. In previous studies with these instruments^{28,29} we have noted that if the transducer is not absolutely flat against the bone and immobilized, the measured pressure is underestimated. Even with careful suturing, immobility was probably rarely achieved during mastication as the mandible opened and closed. For this reason, we believe that the "outlying" high values of 3.0 and 3.4 kPa (Table 1) are a more accurate estimate of the true alveolar pressure than the overall average of 1.2 kPa. With the absence of movement during buccinator stimulation, this difficulty vanished, and the average value of 8.0 kPa (Table 3) is likely a good estimate of the maximum pressure that the buccinator can exert on its mandibular

attachment. The very different technologies employed in measuring pressure in the human oral cavity have produced a wide variety of values,^{10,16,18} but available estimates for mastication are reasonably close to ours, 3.2–3.7 kPa for the buccal surfaces of mandibular and maxillary molars^{17,18} and 4.4–5.6 kPa for the maxillary alveolar bone above and slightly anterior to the upper first molar, increasing to 8.6 kPa for swallowing.¹⁷

Underestimated or not, it is clear that the mechanical environment of the mandibular bone at the buccinator's attachment is pressure, not tension as sometimes proposed. Furthermore, our results show clearly that contraction of the buccinator is the cause of pressure against the alveolar process. First, in mastication pressure rose in conjunction with buccinator EMG as the jaw closed, not as the jaw opened, as would be the case if passive stretching brought the cheek into contact with the alveolar bone. Second, higher pressures for tough foods were generally associated with greater dimensional changes. Third, even when the muscle was passive, the higher resting pressure seen in prone anaesthetized animals compared to the standing awake baseline was correlated with a shorter anteroposterior length of the buccinator. Fourth, the highest pressures observed occurred during full tetanus of the buccinator. Insofar as one can extrapolate from miniature pigs to humans, these results suggest that the use of buccal shields to relieve pressure on the dental arches from active buccinator contraction is valid.

4.3. Anatomy

As in humans, the predominant orientation of buccinator fibres is anteroposterior with a crossing of upper and lower fibres at the oral commissure. The only vertical fibres were extremely superficial and anteriorly located. We could not confirm that any fibres truly originated from the alveolar attachments, which may explain why tension was never observed here. Nevertheless, as also reported for humans,³⁰ in histological sections fibre orientation is highly complicated. The interweaving of buccinator fibres in three planes suggests that the thickening of the muscle during contraction is not necessarily due to the increasing diameter of shortening anteroposterior fibres, but also may involve additional threedimensional shape changes.

Small salivary glands were interspersed within the buccinator in intimate relationship to the muscle fibres. This proximity raises the possibility of a functional relationship. Conceivably, buccinator contraction could "milk" the salivary glands, expressing saliva to aid bolus cohesiveness. This situation is reminiscent of the attachment of buccinator fibres to the parotid duct in humans, which Kang et al. argued would dilate the terminal portion of the duct and promote secretion.²

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