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Ontogeny of bone strain: the zygomatic arch in pigs

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Summary

At the time of weaning, infant animals have little experience with hard food, and thus their skulls are not likely to be epigenetically adapted for the loads imposed by mastication. We examined bone strain in the zygomatic arch of 4-week-old weanling piglets. Functional strains in piglets differed from those previously reported for older pigs in that the squamosal bone was not bent in the horizontal plane and the principal tensile strain on the zygomatic bone did not correspond to the direction of masseter muscle pull. Strain patterns were more variable in piglets than in older pigs. In older pigs, masticatory strains can be reproduced by stimulating the masseter muscles. When the piglet masseter was stimulated, strain patterns were more similar to those of older pigs, but shear strain magnitudes were the largest yet recorded from mammalian skull bones, up to 4000 με. To put these findings in the context of skeletal adaptation, 45 dry skulls, including some animals from the strain study, were measured. Reduced major axis regressions indicated that the infant arch was rounder in cross section and straighter than that of older animals. With growth, the arch became dorsoventrally higher, while mediolateral thickness decreased in the squamosal bone. Overall, these changes should make strain more predictable, explaining the lower variability in older animals. Other factors likely to be important in causing unique strain regimes in piglets include (1) unfamiliarity with hard food, (2) greater importance of muscles other than the same-side masseter and (3) greater proximity of molariform teeth to the arch. Collectively, these data indicate that the skeleton is not pre-adapted for specific functional loads.

Key words: bone strain, skull, mastication, weaning, pig.

Introduction

The shapes of all mammalian and avian bones change during ontogeny. Because the primary function of bones is mechanical, and because mechanical loads stimulate bone growth and remodeling, many authors have looked for mechanical causes, or at least correlates, for these ontogenetic changes (Carter and Beaupré, 2001). However, there is little information on functional loading in infant bones, and the initial state of skeletal adaptation at the beginning of mechanical function is unknown.

One explanation for ontogenetic shape changes in bone is that the young skeleton is less mineralized than the mature skeleton and that infant bones compensate by being geometrically more robust. Especially in limb bones, this idea is buttressed by the common ontogenetic pattern among mammals and birds of an initially stout bone becoming relatively more slender (Carrier, 1983; Carrier and Leon, 1990; Main and Biewener, 2004). The result of this process would be to preserve relatively constant strain magnitudes at any given location, which has, in fact, been reported in growing chicks and rats (Biewener et al., 1986; Keller and Spengler, 1989). Like long bones, the cranium typically becomes more elongated and less round during ontogeny. Morphological analyses have suggested that these changes could maintain relatively constant strain environments at given sites (Biknevicius and Leigh, 1997; Vinyard and Ravosa, 1998; Thompson et al., 2003).

An additional consideration is that even though infant muscles are comparatively small and forceful behaviors are relatively rare, their motor behavior is sometimes clumsy (Herring, 1985). Thus, variable, unpredictable and potentially large bone strains could be encountered. The fact that infant limb bones are typically straighter and rounder than adult bones (Biewener and Bertram, 1994; Main and Biewener, 2004; Skedros et al., 2004) also suggests a less predictable loading environment (Bertram and Biewener, 1988). Higher variability in strain of younger animal limb bones has, in fact, been documented (Main and Biewener, 2004). Although the magnitude of these strains would be moderated by the increased robustness of infant bones (Heinrich et al., 1999), the strain pattern should be more variable than in older animals, as suggested by two studies on the cranium, one on anesthetized monkeys (Iwasaki, 1989) and one on *ex vivo* pig heads (Fisher et al., 1976). Interestingly, the latter study applied loads to different-aged skulls in a highly uniform manner intended to mimic masticatory muscle contraction; thus, the variability of strain in the infant skulls did not result from awkward movement but from the skulls themselves. Such a finding (if real) implies that the young skulls were not yet adapted to the simulated masticatory loads placed on them and that the older skulls reflected growth changes that specifically adapted them for function.

The mammalian skull provides an opportunity to examine the influence of function on ontogenetic changes in bone strain, because the feeding mechanism of infants (suckling) is very different from that of adults (chewing). At the feeding transition, there must be a radical change in both the magnitude and pattern of loading. If skull shape during infancy is adapted for suckling, then it is unlikely to be optimized for chewing. These considerations led us to focus on the mammalian head at the time of weaning. In particular, we examined the zygomatic arch, an element that is closely associated with the masseter muscle both phylogenetically and mechanically. The masseter is the predominant jaw-closing muscle of ungulate mammals (Turnbull, 1970), including our study species, the pig *Sus scrofa* (L.). The masseter has a strong aponeurotic origin from the zygomatic bone (Fig. 1) but affects the squamosal only indirectly (Rafferty et al., 2000; Herring et al., 2001). Compared to its activity during suckling, the contractions of the piglet masseter during chewing are longer in duration and asymmetrical (Herring and Wineski, 1986). Strong electromyographic (EMG) activity can be produced in either feeding mode (Herring and Wineski, 1986; Huang et al., 1994).

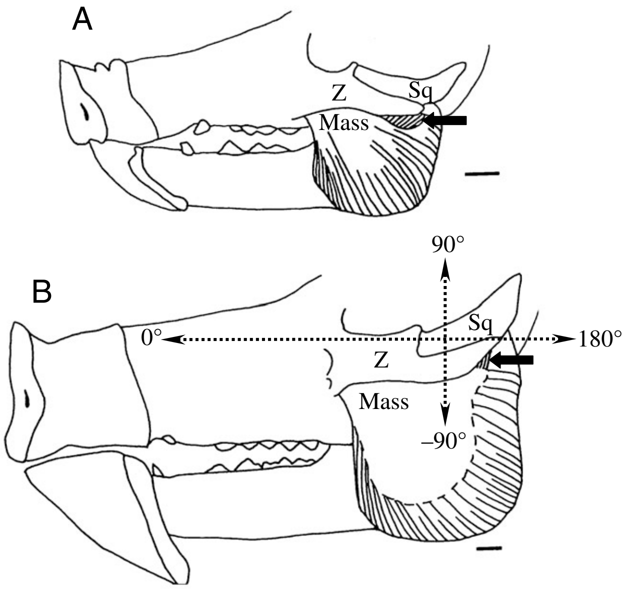

Fig. 1. The zygomatic arch, consisting of zygomatic (Z) and squamosal (Sq) bones, and the masseter muscle (Mass) in (A) a 3.5-week-old piglet and (B) a 3.5-month-old juvenile pig, both domestic farm animals. The masseter originates mostly aponeurotically from the zygomatic bone. In the piglet (A), the aponeurosis is so thin posteriorly that the underlying zygomaticomandibularis muscle (arrow) is clearly visible. In the juvenile (B) the aponeurosis is thicker and shows clearer margins (denoted by a dashed line), and only a small corner of the zygomaticomandibularis (arrow) is visible. The scale bar indicates 1 cm. The dotted lines in B show the reference axes for plotting the orientation of tensile strain. The 0–180° line is parallel to the occlusal plane. Modified from Herring and Wineski (1986).

In part because its superficial location makes it accessible, a rich database for strain in the zygomatic arch is available for the pig (Herring and Mucci, 1991; Herring et al., 1996; Rafferty et al., 2000) and other species (Iwasaki, 1989; Hylander and Johnson, 1997). The prior pig data were derived from subadult animals, i.e. animals that were still growing but relatively mature in muscle anatomy and in the performance of mastication (Herring and Wineski, 1986). The orientation of masticatory strains in the arch was mimicked by stimulating the ipsilateral masseter muscle in anesthetized animals (Herring et al., 1996). The squamosal bone typically experienced greater strain magnitudes (shear strain averaging 974 $\mu\epsilon$ for masseter stimulation and 411 $\mu\epsilon$ for mastication) than the zygomatic bone (473 $\mu\epsilon$ for stimulation and 298 $\mu\epsilon$ for mastication). Principal strains on the two bones were approximately orthogonal to each other, with zygomatic tension being oriented anterodorsal to posteroventral (the direction of masseteric pull) and squamosal tension anteroventral to posterodorsal. Furthermore, the lateral zygomatic surface showed a pattern of dorsal compression and ventral tension, indicating bending in the parasagittal plane with the lower border becoming more convex (Herring et al., 1996). By contrast, the major bending plane of the squamosal was horizontal (medial-lateral), with the lateral surface tensed (becoming more convex) and the medial surface compressed (Rafferty et al., 2000).

In the present study, we investigated whether this masseter-dominated pattern of strain would be apparent in piglets that had not yet been fully weaned to a hard-food diet. We hypothesized (1) that the magnitude of bone strain on infant arches would not differ from that of older pigs and (2) that strain on infant arches would vary more than that in older skulls, especially in orientation but possibly in magnitude as well. The first hypothesis implies that bones in infant animals are pre-adapted for their functional environment, but only in a general sense, so that they would be strong enough not to break under a variety of loading possibilities. The second hypothesis implies that the bones become more adapted to their specific functions by a process of differential growth.

Materials and methods

All procedures were reviewed and approved by the University of Washington Institutional Animal Care and Use Committee. Experimental studies were performed using ten 4-week-old Hanford-strain miniature pigs (Charles River, Wilmington, MA, USA) (Table 1). This age corresponds to

Table 1. *Subjects and strain gage locations*

Pig	Sex	Weight (kg)	Left squamosal bone	Left zygomatic bone
135	Male	3.1	Strip	Rosette
136	Male	2.5	Strip	Rosette
137	Female	3.8	Strip	Rosette
138	Female	3.4	Rosette	Strip
141	Male	4.1	Strip	Strip
142	Male	4.2	Rosette	Rosette
143	Female	3.6	Strip	Strip
144	Female	2.7	Rosette	Rosette
145	Female	3.8	Rosette	Strip
146	Female	4.1	Rosette	Rosette

weaning in many commercial swine facilities, but natural weaning is typically after 8 weeks (Pond and Houpt, 1978). The piglets were acclimated to the laboratory for approximately one week, during which time they were offered milk formula, softened pig chow pellets and unsoftened pellets. At the time of the experiment, all accepted the hard food, but none were fully weaned. A previous report described feeding behavior in these animals using EMG and video recording (Huang et al., 1994).

The procedures followed were identical to those used on older juvenile pigs (Herring et al., 1996). After halothane/nitrous oxide anesthesia, the skin and periosteum overlying the left zygomatic arch were incised and reflected to expose the lateral surface of the zygomatic and squamosal bones. The center of each bone surface was cauterized, sanded, degreased and dried. Either a three-element stacked rosette strain gage (SA-06-030WR-120; Measurements Group, Raleigh, NC, USA) or a strip gage with two or three parallel elements (SA or EA-06-031ME-120) was affixed to each prepared surface (Fig. 2A; Table 1) using cyanoacrylate glue. Rosette gages give the orientation and magnitude of the principal strains (tension and compression) at a single location. Strip gages do not give such information but do indicate bending in the parasagittal plane. Strip gages were roughly parallel to the occlusal plane and the middle elements of rosette gages were perpendicular to the occlusal plane (Fig. 2A). Lead wires were connected to strain conditioner/amplifiers (2120A;

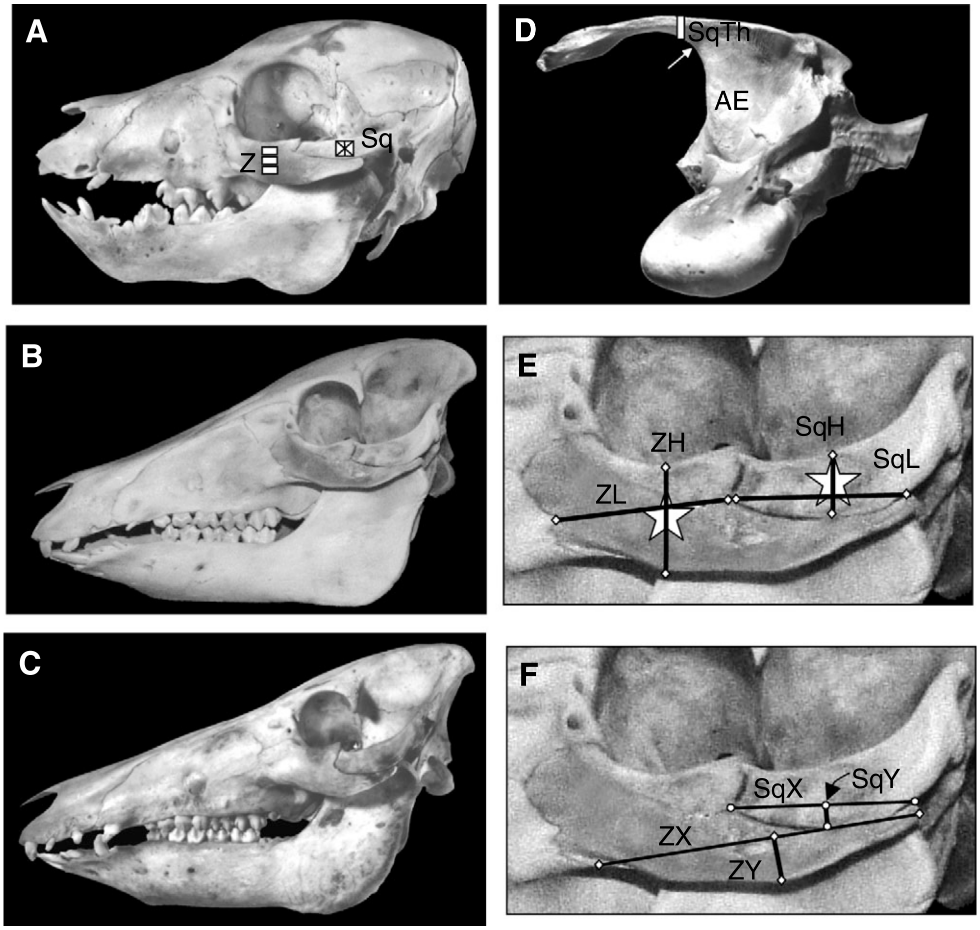

Fig. 2. Examples of skulls, strain gage locations, and measurements. (A) Skull of a piglet of the age used in the present study; condylobasal length approximately 100 mm. A strip gage, consisting of three parallel anterior-posteriorly oriented single-element strain gages, is illustrated on the zygomatic bone (Z), and a stacked rosette gage is shown on the squamosal bone (Sq). (B) Skull of a juvenile animal, a sibling of one used in the previous study (Herring et al., 1996); condylobasal length ~200 mm. (C) Skull from a young adult (dentition complete except for third molars); condylobasal length approximately 270 mm. Note the progressive deepening and curving of the zygomatic arch with increasing age. (D) Ventral view of a disarticulated left squamosal bone, showing the articular eminence (AE) with the medial shelf (arrow) that sometimes continued to the level of the gage site, where squamosal thickness (SqTh) was measured. (E) Bone dimensions. Zygomatic body length (ZL) was measured from the most posterior part of the maxillary-zygomatic suture to the corner of the zygomatic-squamosal suture. Squamosal bone length (SqL) was measured from the zygomatic-squamosal suture corner to the posterior extent of the suture. Bone height (ZH and SqH) was measured vertically (relative to the occlusal plane) at the gage sites. Medial-lateral bone thickness (at the stars) was also measured at the gage sites. (F) Method for estimating ventral curvature. A straightedge was laid across the most ventral parts of the sutures (ZX and SqX). From these lines, the heights of the maximum perpendiculars (ZY and SqY) were measured. Curvature was estimated as ZY/ZX and SqY/SqX.

Measurements Group) and the periosteum and skin were separately sutured. EMG electrodes were inserted as previously described (Huang et al., 1994). An analgesic (buprenorphine; 0.02 mg kg^{-1} i.m.) was administered and the animals were allowed to recover from anesthesia (5–20 min). Strain gage data were recorded during drinking of powdered milk formula and mastication of commercial pig chow either softened with milk (soft) or unsoftened (hard). Signals were recorded on magnetic tape (HP recorder with FM modules, DC-1250 Hz) for off-line analysis.

To determine which, if any, aspects of masticatory strain could be ascribed to masseter muscle contraction, the animals were re-anesthetized and placed prone with the teeth in occlusion. Needle electrodes were placed bilaterally in the posterodorsal and anteroventral aspects of each masseter muscle. Tetani were produced using 450 ms trains of 3 ms pulses delivered at 60 pulses s^{-1} (Grass Model S48 and SIU; East Warwick, RI, USA). Stimulation voltage was administered to provide a supramaximal contraction of the masseter and zygomaticomandibularis (deep masseter) muscles without spread to adjacent muscles. Typically, 60–70 V was used for bilateral stimulation and 30–40 V was used for unilateral stimulation. Strain signals were displayed on a calibrated storage oscilloscope and photographed. At the termination of these procedures, animals were euthanized using intracardiac injections of sodium pentobarbital. After sacrifice, gages were inspected to verify their condition and to document their position.

Bone strains during mastication were digitized and analyzed using AcqKnowlege (Biopac Systems, Goleta, CA, USA) software. Rosette data were used to calculate the peak principal strains for each feeding cycle. The angle of the maximum principal strain (tension) was expressed relative to the occlusal plane (0°=180°) with positive values measured clockwise from the left and negative values measured counterclockwise from the left (Fig. 1B). Highly variable individual differences in angle presented a problem for analysis. We decided to express values as either positive (e.g. 107°) or negative (e.g. –73°) in order to minimize variation of the sample as a whole. Strip gage data were scored qualitatively by comparing the peak values of each element in the array. Higher compression in the dorsal element than in the ventral element indicates bending such that the dorsal surface of the bone becomes more concave. Conversely, if strain in the dorsal element is more tensile than that in the ventral element, the bone is bent such that its dorsal surface becomes more convex. Strains from stimulations were measured directly from the scaled photographs. Data were organized using Excel spreadsheets, and principal strains were calculated following the formulae provided by Rosette-Plus (Measurements Group).

For morphometric comparisons, 45 skulls of Hanford pigs of known age (Fig. 2A–C), including five from the present sample and three from the previous study of juveniles (Herring et al., 1996), were measured using digital calipers (±0.01 mm). Condylobasal length was used as an index of skull size. In addition to antero-posterior lengths of arch components, medio-lateral thicknesses and dorso-ventral heights of the zygomatic and squamosal bones were measured at the level of the strain gages (Fig. 2D,E). As a rough measure of curvature in the parasagittal plane, the deviation of the most ventral point from a straightedge placed along each bone's long axis was measured and expressed as a percentage of antero-posterior length (Fig. 2F). Measurements on 11 of the skulls were repeated two weeks later to test for measurement error, calculated as $\sqrt{\Sigma d^2/2N}$, where d is the difference between the first and the second measurement and N is the number of subjects. Paired t-tests indicated no systematic error, and measurement error ranged from 0.15 to 0.65 mm (mean, 0.32±0.13 mm). Proportions (thickness and height relative to length, curvature) were calculated and transformed with the arcsin function in order to spread out the distribution (Snedecor and Cochran, 1967). Reduced major axis regressions against condylobasal length were performed using RMA 1.17 software (http://www.bio.sdsu.edu/pub/andy/RMA.html) to assess whether the arch becomes less robust with age and to explore its changes in shape. The software calculated confidence intervals by bootstrapping over cases (20 000 replicates) using random sampling with replacement. Parameters were considered to differ significantly if 95% confidence intervals did not overlap.

Results

Feeding

For feeding, the peak principal strains derived from rosette gages are listed in Table 2. Ipsilateral chewing caused slightly higher but otherwise similar strains than contralateral chewing. For both bones, the magnitudes of maximum and minimum principal strains (tension and compression, respectively) were similar. Mean strain magnitudes increased with increasing food hardness (Fig. 3A) but, owing to the large standard deviations, most comparisons were not statistically significant. Comparison of the two bones also suggested a tendency for strain magnitudes to be larger in the squamosal than in the zygomatic bone, but high variability precluded any statistical significance. The orientation of tensile strain did not change with foodstuff but did differ between the bones (Fig. 3A), with the mean orientation being approximately 110° for the squamosal bone but approximately 160° (or –20°) for the zygomatic bone (data in Table 2, $P<0.05$ for a two-sample t-test).

During drinking, and to a lesser extent feeding on soft chow, low strains were observed in the strip gages, with no clear evidence of bending in the parasagittal plane. However, when piglets were masticating hard chow, the strip gages showed obvious bending in most bones (Table 3). Patterns of bending were surprisingly variable among pigs, and some animals also showed different patterns for ipsilateral and contralateral chewing. For the squamosal bone, most piglets showed the same pattern regardless of chewing side but, of these, two evinced concave-downward bending, one showed concave-upward bending and one showed no bending with all areas under tension. One animal showed concave-downward bending for ipsilateral cycles but concave-upward bending for contralateral cycles. For the zygomatic bone, three animals showed concave-downward bending (with one changing to no bending with compression for contralateral chewing), while the fourth always had concave-upward bending.

Table 2. *Rosette strain gages during feeding: peak principal strain magnitude and orientation (relative to occlusal plane) in the zygomatic arch of piglets*

Pig, side of chewing (number of cycles)	Squamosal tension ($\mu\epsilon$)	Squamosal compression ($\mu\epsilon$)	Orientation of sq. tension (deg.)	Zygomatic tension ($\mu\epsilon$)	Zygomatic compression ($\mu\epsilon$)	Orientation of zyg. tension (deg.)
Hard chow						
135 Ipsilateral (10)	–	–	–	158±46	–392±121	–73±1
Contralateral (9)	–	–	–	81±41	–159±47	–72±3
136 Ipsilateral (12)	–	–	–	117±19	–86±11	10±2
Contralateral (24)	–	–	–	156±32	–105±27	8±2
137 Ipsilateral (13)	–	–	–	246±88	–190±112	–23±24
Contralateral (27)	–	–	–	209±66	–130±78	–10±34
138 Ipsilateral (17)	246±155	–127±87	125±39	–	–	–
Contralateral (18)	157±95	–314±182	173±38	–	–	–
142 Ipsilateral (13)	227±79	–101±63	87±64	–	–	–
Contralateral (18)	166±36	–155±38	66±10	–	–	–
144 Ipsilateral (9)	246±136	–404±197	113±41	185±64	–267±107	–12±60
Contralateral (6)	276±61	–395±319	138±48	181±59	–231±75	–40±6
145 Ipsilateral (20)	385±161	–542±259	44±20	–	–	–
Contralateral (23)	349±118	–367±216	56±35	–	–	–
146 Ipsilateral (22)	791±142	–458±91	143±3	398±66	–223±55	4±12
Contralateral (19)	418±86	–184±49	151±2	388±77	–234±26	11±9
Ipsilateral mean	379±239	–326±200	102±38	221±110	–231±112	–19±33
Contralateral mean	273±114	–283±108	116±52	203±114	–171±59	–21±35
Soft chow						
135 Ipsilateral (9)	–	–	–	53±26	–115±70	–72±4
Contralateral (15)	–	–	–	82±44	–157±107	–74±3
136 Ipsilateral (15)	–	–	–	65±49	–62±38	10±23
Contralateral (12)	–	–	–	72±54	–53±28	6±25
137 Ipsilateral (19)	–	–	–	27±11	–22±9	26±27
Contralateral (3)	–	–	–	30±33	–29±34	38±9
138 Ipsilateral (10)	25±16	–44±19	138±37	–	–	–
Contralateral (9)	57±39	–46±24	12±43	–	–	–
144 Ipsilateral (13)	140±116	–367±304	90±2	122±121	–226±200	–50±6
Contralateral (14)	189±260	–302±317	107±33	150±139	–247±227	–46±3
145 Ipsilateral (4)	35±3	–56±23	41±18	–	–	–
Contralateral (6)	32±32	–65±68	34±24	–	–	–
146 Ipsilateral (15)	629±375	–420±223	160±39	343±165	–138±34	15±34
Contralateral (16)	231±284	–201±155	16±46	197±89	–138±67	–1±16
Ipsilateral mean	207±286	–221±199	107±53	122±128	–113±78	–14±44
Contralateral mean	127±98	–153±121	42±44	106±67	–125±87	–15±44
Drinking						
135 (13)	–	–	–	27±12	–37±21	–74±3
136 (5)	–	–	–	41±21	–27±10	–53±33
137 (14)	–	–	–	16±7	–19±8	–29±45
138 (19)	52±11	–44±12	175±11	–	–	–
142 (18)	22±9	–24±15	141±62	–	–	–
144 (10)	463±328	–769±694	121±43	125±137	–103±87	1±45
145 (7)	122±70	–110±69	172±63	–	–	–
Mean	164±203	–236±356	152±26	52±50	–46±38	–38±32

Values are means ± S.D.

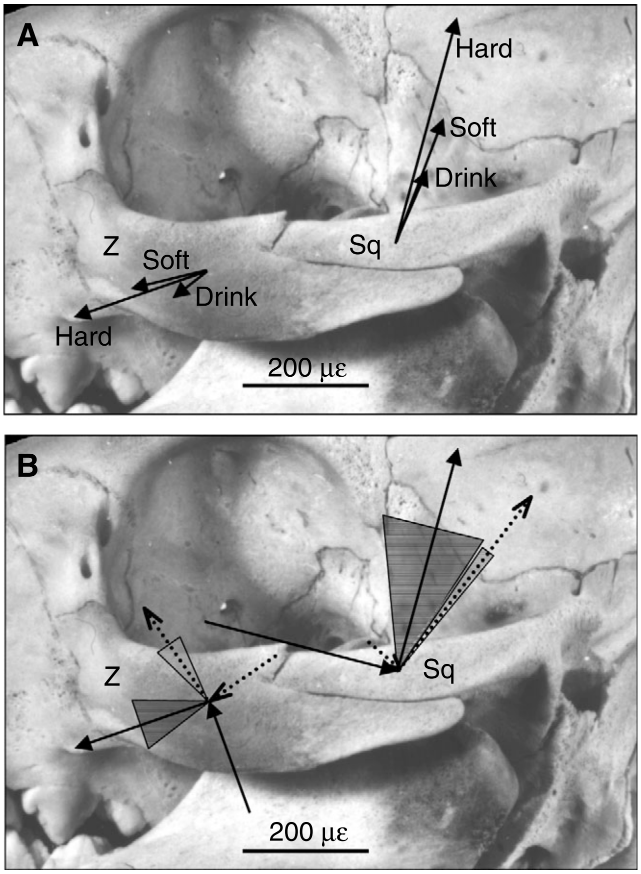

Fig. 3. Piglet left zygomatic arch, showing magnitude and orientation of bone strain during feeding, based on rosette data from Tables 2, 7. Peak maximum principal strains (tension) are shown as arrows headed away from the gage sites, and peak minimum principal strains (compression) are shown as arrows headed towards the gage sites. (A) Comparison of food types in piglets. Mean peak tensile strains recorded during the ipsilateral mastication of hard and soft chow and during milk drinking are represented on the left zygomatic arch. Compressive strains were roughly equal to tensile strains and perpendicular to them. Strain magnitude tended to be higher for harder foods and to be higher for the squamosal (Sq) than for the zygomatic (Z) bone. (B) Comparison of piglet (solid arrows) and juvenile pigs (dotted arrows) for hard chow. The triangles superimposed on the tensile strain indicate one standard deviation in magnitude (height of triangle) and in orientation (angle at apex). Strain orientations tend to be more variable in piglets. In contrast to piglets, juvenile pigs have negligible compressive strain on the squamosal. Zygomatic strain orientations indicate an opposite direction of torsion in piglets *vs* juveniles.

Masseter stimulation

Rosette strain gage data for muscle stimulations are presented in Table 4 and illustrated in Fig. 4A. Intra-individual variation was negligible for stimulated tetanus, thus standard deviations are presented only for the sample as a whole. When the ipsilateral masseter (with or without the contralateral masseter) was contracting, the magnitudes of the principal strains were strikingly larger than for mastication (Table 2), typically 3-fold higher (all comparisons between bilateral

Table 3. *Strip gages: patterns of bending during mastication of hard chow*

	Left squamosal bone		Left zygomatic bone	
Subject	Left chews	Right chews	Left chews	Right chews
135	∩	∩		
136	∪	∪		
137	∩	∩		
138			∩	– (compression)
141	– (tension)	– (tension)	∪	∪
143	∩	∪	∩	∩
145			∩	∩

∩ Bending such that the ventral edge becomes relatively concave (more tensile in dorsal element and/or more compressive in the ventral element).

∪ Bending such that the dorsal edge becomes relatively concave (more compressive in dorsal element and/or more tensile in the ventral element).

– No in-plane bending (similar strains in dorsal and ventral elements); the polarity of strain is indicated in parentheses.

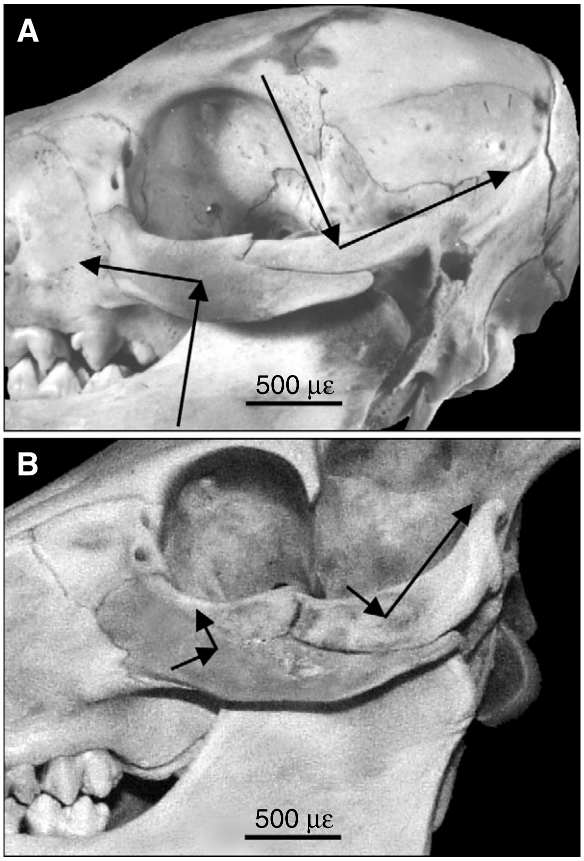

Fig. 4. Magnitude and orientation of zygomatic arch strain during tetanus of the ipsilateral masseter muscle, based on rosette strain gage data from Tables 4, 8. Conventions as in Fig. 3. (A) Piglet. (B) Juvenile. Except for squamosal tension, strain magnitudes are larger in piglets than in juveniles.

Table 4. *Rosette strain gages during masseter muscle stimulation: principal strain magnitude and orientation (relative to occlusal plane) in the zygomatic arch of piglets*

Pig	Squamosal tension (με)	Squamosal compression (με)	Orientation of sq. tension (deg.)	Zygomatic tension (με)	Zygomatic compression (με)	Orientation of zyg. compression (deg.)
Bilateral masseter stimulation						
135	–	–	–	714	–2114	23
136	–	–	–	876	–1176	–54
137	–	–	–	864	–764	40
138	1266	–2766	139	–	–	–
142	1192	–642	144	–	–	–
144	1203	–2003	88	545	–445	–23
145	539	–539	124	–	–	–
146	1695	–2345	129	2332	–532	33
Mean ± S.D.	1179±414	–1659±999	125±22	1066±720	–1006±681	4±41
Ipsilateral masseter stimulation						
135	–	–	–	323	–1423	33
136	–	–	–	805	–1105	–52
137	–	–	–	600	–400	90
138	1126	–1501	148	–	–	–
142	2336	–1586	144	–	–	–
144	400	–600	90	860	–710	–26
145	618	–568	121	–	–	–
146	1242	–1742	128	553	–253	4
Mean ± S.D.	1144±752	–1199±568	126±23	628±215	–778±486	10±55
Contralateral masseter stimulation						
135	–	–	–	331	–431	12
136	–	–	–	251	–201	17
137	–	–	–	324	–124	13
138	52	–302	157	–	–	–
142	468	–418	217	–	–	–
144	728	–728	172	262	–162	23
145*	0	0	–	–	–	–
146	250	–250	225	442	–142	15
Mean ± S.D.	374±290	–424±214	193±33	322±76	–212±126	16±4

*This gage produced no measurable strain during contralateral stimulation. The values are not included in the means.

stimulation and ipsilateral mastication statistically significant at P<0.01–0.05). Even stimulations of the opposite-side masseter resulted in strains that were usually higher (although not significantly different) than during mastication. Orientations of the mean principal strains were rotated somewhat clockwise relative to those of mastication (as seen from the left, compare Figs 3A and 4A) but were not statistically different from those of mastication.

The strip gages indicated that bending in the parasagittal plane was more consistent for muscle stimulations than for mastication (Table 5). Ipsilateral masseter and bilateral contractions produced concave-downward bending patterns in four of five squamosal bones and concave-upward bending patterns in three of four zygomatic bones. Contralateral masseter contractions resulted in low, predominantly tensile, strains on both bones with little indication of bending.

Morphometrics

Before examining shape changes within bones, the dimensions were compared with skull (condylobasal) length to assess overall allometry. The lengths of the individual bones increased approximately isometrically with skull length for both the squamosal [slope=0.15 (0.98 for logged data), r^2=0.96] and zygomatic [slope=0.14 (0.84 for logged data), r^2=0.97]. Zygomatic height was almost isometric with skull length (slope=0.12, r^2=0.93), but squamosal height showed a significantly lower slope (0.07, r^2=0.86). Thickness was also negatively allometric, more so for the squamosal (slope=0.001, r^2=0.18) than for the zygomatic bone (slope=0.003, r^2=0.67). Thus, bone length and zygomatic height keep pace with skull length, while bone thickness and squamosal height do not.

Bone shape was assessed by plotting height and thickness relative to bone length against total skull length. Greater relative height and thickness were considered to indicate the robustness of the arch bones. In both bones, relative height was found to increase with increasing skull length, but much more dramatically for the zygomatic than for the squamosal bone (Fig. 5A; Table 6). Zygomatic height was approximately 40%

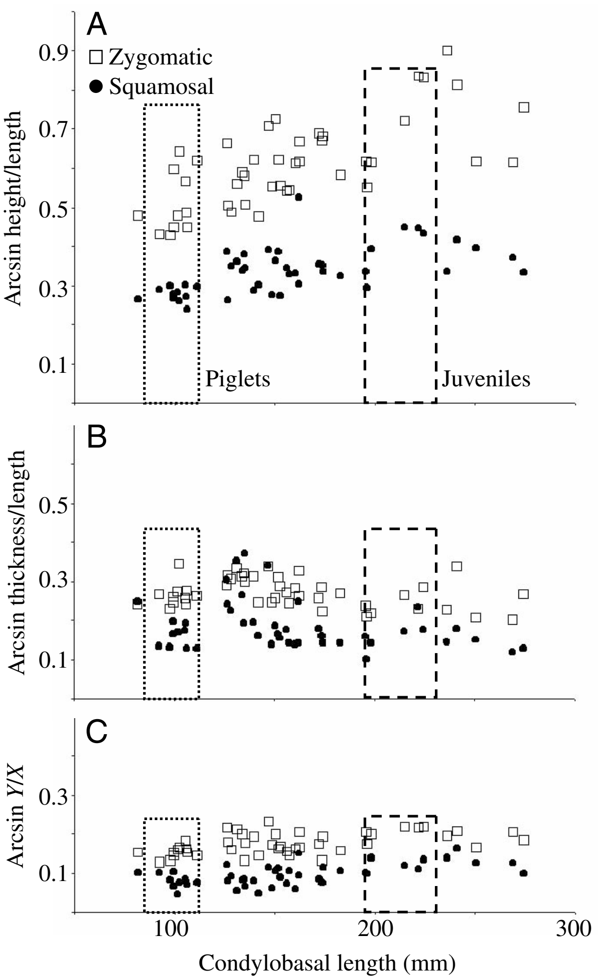

Fig. 5. Changes in bone proportions and shape with increasing skull size. Scatter plots are based on 45 skulls of Hanford strain pigs. Reduced major axis parameters are presented in Table 6. The two boxed areas indicate the size ranges of the piglets used in the present study (92–106 mm condylobasal length) and the juveniles from the previous study (Herring et al., 1996; 196–222 mm condylobasal length). Arch dimensions measured are illustrated in Fig. 2D–F. (A) Bone height relative to bone length increased for both zygomatic and squamosal bones, but more so for the former. (B) Bone thickness relative to bone length decreased significantly for the squamosal but not for the zygomatic bone. (C) Curvature of the ventral border increased in both bones.

of zygomatic length in the smallest animals, increasing to 80% in the largest, whereas squamosal height increased from approximately 25% to approximately 40% of squamosal length. The difference in reduced major axis slope between the bones was statistically significant at $P<0.01$. By contrast, relative bone thickness slightly decreased in the squamosal (Fig. 5B) from ~25% to ~15% of bone length. This decrease can be ascribed in part to the medial shelf of the squamosal bone's articular eminence, which often extended to the strain gage location in the smaller but not the larger skulls (Fig. 2D). In the zygomatic bone, relative thickness remained roughly constant at approximately 30% of bone length. Despite the fact that the squamosal bone showed a significantly negative slope and the zygomatic bone did not, the slopes of the two bones did not differ at the 0.05 level. In summary, the smallest pigs had zygomatic arches with the roundest cross sections and the largest pigs had arches elongated vertically. With growth, the arch became more 'robust' in the parasagittal plane but not in the horizontal plane. These changes were more marked in the zygomatic than in the squamosal bone.

Table 5. *Strip gages: patterns of bending during masseter muscle stimulation*

	Squamosal bone		Zygomatic bone	
Subject	Ipsi- or bilateral masseter	Contralateral masseter	Ipsi- or bilateral masseter	Contralateral masseter
135	∩	∪		
136	∩	∩		
137	∩	–		
138			∩	–
141	∪	–	∪	–
143	∩	–	∪	–
145			∪	∩

∩ Bending such that the ventral edge becomes relatively concave (more tension in dorsal element, more compression in the ventral element).

∪ Bending such that the dorsal edge becomes relatively concave (more compression in dorsal element, more tension in the ventral element).

– No in-plane bending (similar strains in dorsal and ventral elements); in most cases strain magnitudes were negligible.

Reduced major axis analysis of relative curvature in the parasagittal plane (Fig. 2F), with or without arcsin transformation of the proportional data, indicated that the zygomatic bone was more curved than the squamosal bone, which actually had an intercept near zero (i.e. no curvature). However, both bones became significantly more curved with age, with no difference in slope (Fig. 5C; Table 6).

Discussion

Food and feeding: performance variability

We anticipated that harder foodstuffs would result in greater muscle activity and hence higher strains on the zygomatic arch. This was generally the case, although somewhat obscured by high variability. In addition to the increasing strain magnitudes from drinking to soft chow to hard chow, bending of the bones in the parasagittal plane could only be discerned for hard chow mastication. As bending patterns could be reliably produced by muscle stimulations, their absence from drinking and chewing of soft food is best interpreted as indicating low muscle activity.

The differences between foods of varying hardness paled in comparison to the variability within each food. Inaccurate reporting could have accounted for some of the variability – we considered cycles to be 'drinking' if the animal had its snout in the bowl of liquid, but occasionally a bolus of chow

Table 6. *Reduced major axis regressions of relative bone height, thickness and curvature (arcsin transformed data)* vs *condylobasal skull length (*N=*45 dry skulls)*

	Intercept (95% C.I.)	Slope (95% C.I.)	r^2 ± S.E.M.
D–V height/A–P length			
Squamosal	0.15 (0.09–0.19)*	0.0012 (0.0009–0.0016)*	0.29±0.13
Zygomatic	0.25 (0.17–0.33)*	0.0024 (0.0018–0.0028)*	0.49±0.11
M–L thickness/A–P length			
Squamosal	0.38 (0.30–0.47)	–0.00124 (–0.00177 to –0.00078)	0.06±0.05
Zygomatic	0.39 (0.16–0.43)	–0.00076 (–0.00097 to –0.00075)†	0.06±0.09†
Relative curvature			
Squamosal	0.012 (–0.013–0.031)*,†	0.00053 (0.00042–0.00070)	0.34±0.11
Zygomatic	0.087 (0.062–0.104)*	0.00056 (0.00045–0.00074)	0.23±0.11

*No overlap of 95% confidence intervals for squamosal and zygomatic bones.

†Not significantly different from 0.

A, anterior; D, dorsal; L, lateral; M, medial; P, posterior; V, ventral.

could have been in the mouth at the time. For the most part, however, the variability appears to reflect real differences in the performance of feeding. Inter-individual variability was most obvious in the parasagittal bending direction as deduced from the strip gages (Table 3) and was much greater than in juvenile pigs (Herring et al., 1996). Inter-individual variation was also substantial for rosette data, but in this case older animals were variable too. *F*-tests comparing the variances from the present study with those of the juveniles were not significant for strain magnitude, but squamosal strain orientation was more variable in the piglets ($P<0.001$) and zygomatic strain orientation tended in the same direction ($P=0.10$). The rosette data also showed remarkably high intra-individual variability in piglets. When individuals were analyzed separately, piglets were far less consistent in their performance than older animals, with individual coefficients of variation in the 40–60% range for strain magnitude in five out of the eight piglets (calculated from Table 2), in contrast to 20–30% for individual older animals (Herring et al., 1996). These same five piglets had standard deviations of 20–64° for orientation, in contrast to less than 15° for older animals (Herring et al., 1996) and the remaining three piglets. The variability within individuals was equally high for all foodstuffs.

High inter- and intra-individual variability suggests that none of these feeding behaviors was fully hardwired and that the young animals had not yet learned a stereotyped motor program. In particular, piglets were not adept at dealing with the hard food. Our previous analysis of mastication in these infant animals (Huang et al., 1994) revealed that, compared with older animals, they chewed more slowly with longer and more variable bursts of closing muscle activity, used a larger number of cycles to process a bolus, were less regular in alternation of side and had more equal working to balancing side muscle activity. Because the piglets had just one pair of occluding molars, bolus position and hence loading might have been unpredictable, producing an unstable masticatory stroke. More variable and inefficient feeding behavior in younger individuals has also been described in other mammalian species (Binder and Van Valkenburgh, 2000).

Whatever the cause, these data indicate that, like young limb bones (Main and Biewener, 2004), the skulls of infant animals show variable, even erratic strains. Thus, they do not appear adapted to the functional loads produced by mastication.

Strain magnitude and pattern in comparison with older animals

Tables 7 and 8 compare rosette data from the piglets with those of the previous study on juveniles in which the gages

Table 7. *Comparison of peak principal masticatory strains from the zygomatic arch of pigs of different ages, ipsilateral and contralateral cycles combined*

	Squamosal			Zygomatic		
Age	Tension (με)	Compression (με)	Angle (deg.)	Tension (με)	Compression (με)	Angle (deg.)
4 weeks[1]	327±177 (5)	–309±128* (5)	109±44 (5)	212±108 (5)	–203±76 (5)	–20±33* (5)
3–7 months[2]	349±187 (5)	–62±76* (5)	126±5 (5)	174±91 (9)	–124±106 (9)	54±24* (9)

[1] Present study.

[2] Herring et al. (1996). Gage locations were exactly the same as in the present study.

*Principal compressive strain on the squamosal was larger in the piglets than in the 3–7-month sample ($P<0.02$), and principal tensile strain was differently oriented ($P<0.001$). No other comparison produced statistical significance.

Values are means ± S.D. Number of animals is given in parentheses.

Table 8. *Comparison of masseter stimulation strains from the zygomatic arch of pigs of different ages: principal strain magnitudes and orientation of tensile strain relative to the occlusal plane*

	Bilateral masseters			Ipsilateral masseter			Contralateral masseter		
Age	Squamosal tension (με)	Squamosal compression (με)	Squamosal angle (deg.)	Squamosal tension (με)	Squamosal compression (με)	Squamosal angle (deg.)	Squamosal tension (με)	Squamosal compression (με)	Squamosal angle (deg.)
4 weeks[1]	1179±414* (5)	−1659±999* (5)	125±22 (5)	1144±752 (5)	−1199±568* (5)	126±23 (5)	374±290* (5)	−424±214* (5)	13±33 (5)
3–7 months[2]	519±95* (5)	−190±58* (5)	118±5 (5)	715±106 (5)	−259±79* (5)	118±3 (5)	62±31* (5)	−19±28* (5)	7±16 (5)
	Zygomatic tension (με)	Zygomatic compression (με)	Zygomatic angle (deg.)	Zygomatic tension (με)	Zygomatic compression (με)	Zygomatic angle (deg.)	Zygomatic tension (με)	Zygomatic compression (με)	Zygomatic angle (deg.)
4 weeks[1]	1066±720* (5)	−1006±681* (5)	4±41* (5)	628±215* (5)	−778±486* (5)	10±55* (5)	322±76* (5)	−212±126* (5)	16±4 (5)
3–7 months[2]	354±191* (7)	−369±254* (7)	68±18* (7)	214±127* (12)	−259±193* (12)	70±37* (12)	79±47* (9)	−46±36* (9)	51±36 (9)

[1] Present study.

[2] Herring et al. (1996). Gage locations were exactly the same as in the present study.

*Difference between the 4-week and the 3–7-month samples statistically significant at $P<0.05$ or better.

Values are means ± S.D. Number of animals is given in parentheses.

were in equivalent locations (Herring et al., 1996). Data from other locations in juveniles (Liu and Herring, 2000; Rafferty et al., 2000) indicate that there are (opposite) anterior–posterior gradients of strain magnitude in both arch bones; this restricts statistical comparison to the 1996 study. During mastication, piglets and juveniles had similar strain magnitudes (Table 7; Fig. 3B). This similarity actually suggests that the arch is less stiff in the piglets, because although EMG levels are comparable (Huang et al., 1994), the piglet masseter muscles are relatively smaller and less forceful than those of juveniles (Herring and Wineski, 1986; Anapol and Herring, 1989). Low stiffness of the piglet arch under masseteric contraction is even more clearly implied by the results of muscle stimulation. During same-side masseter tetanus, piglets evinced vastly higher strains than juveniles (Table 8; Fig. 4). As far as we can determine, these strains, ranging up to 4000 με shear strain (maximum minus minimum principal strain), are the highest ever recorded from the mammalian skull and rival those recently reported for biting alligators (Metzger et al., 2005). Assuming that ultimate compressive strain for the zygomatic arch is in the order of 2% (Skedros et al., 2003), the safety factor for full recruitment of the masseter would be less than 10. Even stimulation of the opposite-side masseter produced strains in the same order of magnitude as did chewing (except for #145; Table 4). By contrast, in the juvenile arch, masseter tetanus seldom produced strains more than twofold the magnitude of masticatory strain, and stimulation of the opposite masseter produced only negligible strain (Table 8). These findings indicate that the only reason that masticatory bone strain magnitudes are similar in piglets and in juveniles is that the piglets recruit proportionately much less muscle force. This agrees with the *ex vivo* simulation experiments of Fisher et al. (1976) showing that equivalent muscle contraction resulted in higher strains in younger skulls.

Beyond magnitude, there are three major differences of strain pattern between piglets and juveniles (Figs 3B, 4). First, in piglets, tensile and compressive strains were roughly coequal on both bones, but in older animals the squamosal showed little or no compression on its lateral surface (Herring et al., 1996; Liu and Herring, 2000; Rafferty et al., 2000). Therefore, the bending in the horizontal plane that characterizes the older squamosal does not occur in piglets. Instead, the infant squamosal is either twisted such that its anterior ventral border moves medially, or sheared such that its anterior extremity is forced ventrally (Fig. 6). Both of these loads could arise from the ventral and medial pull of the masseter on the zygomatic, transferred to the squamosal at the suture (Herring et al., 1996; Hylander and Johnson, 1997; Rafferty et al., 2000), but this does not explain why bending in the horizontal plane did not occur in the piglets. This problem is addressed further below. Interestingly, the infant strain pattern is the same as that reported for the posterior arch (i.e. squamosal bone) of *Macaca* (Iwasaki, 1989; Hylander and Johnson, 1997); thus, in this regard piglets more closely resemble higher primates than they do older pigs.

The second age difference in strain pattern is the orientation

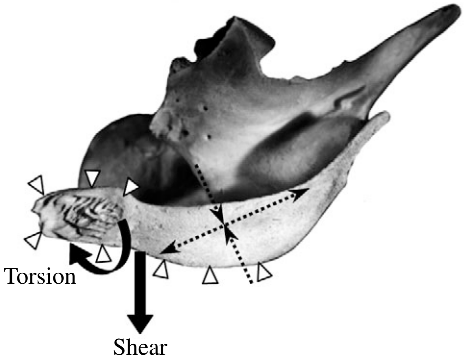

Fig. 6. Disarticulated left squamosal bone, shown in a lateral and slightly dorsal view. The sutural contact areas for the zygomatic bone (open arrowheads), where masseteric loads would be transferred to the squamosal, are on the anterior section of the lateral surface and the ventral edge. Acting through the zygomatic bone at the suture, masseteric loading could give rise to the observed strain pattern (dotted lines; compare with Fig. 4A) by either twisting the lower edge of the squamosal medially (torsion) or pulling it ventrally (shear).

of principal strains on the zygomatic. During chewing, tensile strain was directed anteriorly and ventrally in infants but anteriorly and dorsally in juveniles (Fig. 3B), with a mean difference of 74° (Table 7). In this case, it is the juvenile pigs that are similar to macaques (Iwasaki, 1989; Hylander and Johnson, 1997). Masseter stimulation tended to alter the piglet orientation to an intermediate, slightly superior, orientation, but it was still significantly more anterior by ~60° (Table 8) than that of older animals, which showed no difference in orientation between mastication and stimulation (Figs 3B, 4B). In the older animals, this orientation was interpreted as a direct consequence of masseteric pull (Herring et al., 2001). This explanation may still be relevant for stimulation, because infant masseter muscle fibers have a more anterior mean inclination than older masseter muscles (Fig. 1), but is not persuasive because the age difference in mean muscle angle is much less than 60° (Herring and Wineski, 1986). In any case, the consistently ventral component of zygomatic tension observed during functional behaviors (Fig. 3A) cannot possibly be due to the masseter. Rather, the zygomatic bone must be loaded from another source during feeding, resulting in twisting or shear in a direction opposite to that which the masseter should cause. Transmission of muscle forces from distant locations is a distinct possibility, given the fact that contralateral masseter contraction produced large strains on the arch. However, the most likely source of loading is probably the occlusion. As can be seen in Fig. 2A–C, the cheek teeth are horizontally and vertically closer to the zygomatic bone in piglets than in older animals. A medial torque on the maxillary molariform teeth could conceivably give rise to the anterior–ventral orientation of tension in the piglet zygomatic bone.

The third difference in strain pattern between infant and juvenile pigs is that in the infants, neither the squamosal nor the zygomatic bone showed consistent bending in the parasagittal plane during feeding. Masseter stimulation, however, usually bent the squamosal concave downward and the zygomatic concave upward, the same patterns seen in juveniles during both feeding and stimulation. Thus, the muscle does cause parasagittal bending, but the strong contractions required are not produced under normal functional conditions. As argued for older pigs (Herring et al., 1996), the concave-upward zygomatic bending is most likely a result of the masseter's ventral pull on the zygomatic bone, which is supported at its anterior and posterior ends by sutures with the maxillary and squamosal bones, basically a three-point bending load. The concave-downward bending of the squamosal is explained by the existence of only two important loads, the ventrad force of the zygomatic bone anteriorly and the dorsad joint force posteriorly (Herring et al., 1996).

All of these findings strongly suggest that, unlike juveniles, feeding strains on the zygomatic arch of weanling pigs are not dominated by the masseter muscle. Previous work indicates that the temporalis is relatively more important and the masseter less important in very young than in older animals (Herring, 1977). Less forceful chewing, alternate muscle use and a larger role for the more proximate occlusion all probably contributed to the patterns observed as well as their variability.

Although it is agreed that different skeletal sites are under unique strain regimes (Biewener et al., 1986; Hylander and Johnson, 1997), the long bone literature suggests that, for a given site, both the pattern and the magnitude of strain are maintained at a relatively constant level by bone modeling (Biewener et al., 1986; Keller and Spengler, 1989; Biewener and Bertram, 1993). Similarly, measurements of the mandibular symphysis of *Macaca* indicated that postnatal growth could maintain functional equivalence in bone strain (Vinyard and Ravosa, 1998). By contrast, our findings in piglets clearly show that the pattern of strain was not constant but changed with growth. Specifically, squamosal compression was elevated and zygomatic strain was differently oriented in infants as compared with juveniles. The difference in strain pattern was not due to the changeover from liquid to solid food, because the strain patterns were identical for all foods offered. These data indicate that functional equivalence in the pig zygomatic arch does not extend to strain pattern.

Ontogenetic shape changes in the zygomatic arch

The exceptionally high strains that were produced by stimulation of relatively small (Herring and Wineski, 1986) muscles suggest that, like infant long bones (Carrier and Leon, 1990; Heinrich et al., 1999; Skedros et al., 2004), piglet arches have low stiffness. Indeed, the entire piglet skull is probably relatively compliant, as indicated by the substantial strains produced on the arch by a distant load, the contralateral masseter. Unlike long bones, however (Main and Biewener, 2004), infant zygomatic arches were not more robust overall than those of older animals. Although there was a slight age decrease in relative transverse dimension, the relative vertical dimension increased strongly (Fig. 5), making older animals better buttressed for bending in the parasagittal plane. Thus, piglet arches were weak in geometric as well as in material properties, at least for dorsal–ventral loading. Functional strain magnitudes that were age-invariant resulted from low muscle activity in the piglets, not bone adaptation.

Like infant limb bones (Biewener and Bertram, 1994; Main and Biewener, 2004; Skedros et al., 2004), piglet zygomatic arches were straighter and rounder than those of juveniles. This geometry undoubtedly contributed to the high intra- and inter-individual variability we observed in bone strain, because the bending direction of a straight cylinder is more sensitive to slight variations in loading direction than that of a curved cylinder or a beam (Bertram and Biewener, 1988). At a more detailed level, the shapes of the two bones were different at the start and changed differently during growth. At birth, the zygomatic bone was relatively taller than the squamosal and this difference became more accentuated with age. The piglet zygomatic bone was also slightly thicker and more curved than the squamosal, whereas the squamosal was the rounder, straighter bone. These differences were maintained.

In long bones and the mandible, ovate cross sections and curvatures develop in relation to mechanical loading from muscle contraction and weight bearing (Lanyon, 1980; Hall and Herring, 1990; Biewener and Bertram, 1994; Skedros et al., 2004). The growth changes in the zygomatic bone are clearly adaptive for the parasagittal-plane bending that predominated in older animals but was not seen during function in the piglets. The dramatically increased height creates a beam-like structure that would strongly resist both the ventral pull of the masseter and the dorsal shear imposed by occlusal force. The increasing curvature is in the same (concave-upward) direction as the parasagittal bending, which should greatly increase the predictability of deformation. Interestingly, these geometric changes do not predict altered strain magnitudes on the zygomatic bone. Although deepening of the zygomatic bone would be expected to make it stiffer and decrease strain magnitudes, its increasing curvature should increase bending and strain magnitudes. Because maximal (stimulation) strains decreased with age, the stiffening effect may have been the more important one. In summary, the zygomatic bone shows some pre-adaptation for its eventual loading regime but becomes much better adapted to it with age, both in terms of robustness and predictability.

The squamosal bone of piglets was not bent in the horizontal plane, in contrast to older animals, even when the masseter was stimulated. This absence is surprising, given the fact that otherwise the strain pattern of the squamosal in response to the masseter was similar to that of older animals. One likely explanation is the presence of the medial shelf (Fig. 2D), which buttresses the squamosal in the medial-lateral direction. Our strain gage position for piglets was usually just opposite this shelf. During growth, the elongation of the squamosal is not accompanied by elongation of the medial shelf, and therefore the gage position in the older sample was not opposite to it. In addition, the longer expanse of unsupported squamosal bone would be more subject to cantilever bending. Thus, the diminishing presence of the medial shelf should make the older squamosal more prone than the younger to bending in the horizontal plane. Casual observations on *Macaca* skulls suggest that a medial shelf is present, which would explain why piglets were more similar in their squamosal strain pattern to these primates than to older pigs. The pig squamosal showed no growth adaptation that would strengthen it against bending in the horizontal plane; in fact, decreasing thickness would weaken it in this direction. However, the disappearance of the shelf from most of the medial surface would make horizontal-plane bending more predictable.

In the parasagittal plane, bending of the squamosal was inconsistent during piglet function, but even in older pigs this mode of deformation was less important than for the zygomatic (Herring et al., 1996). The growth adaptations of the squamosal in the parasagittal plane were less striking than those of the zygomatic. Both the modest increase in height (Fig. 5A) and the increase in upward concavity (which is the reverse of the concave-downward bending caused by the masseter) would tend to stiffen the squamosal against parasagittal-plane bending. In short, for its major strain regime, bending in the horizontal plane, the squamosal bone resembles limb bones in that it is more robust in infants and more predictable in older animals. For its minor regime of bending in the parasagittal plane, it becomes more robust and possibly less predictable.

Testing of hypotheses

We had hypothesized that strain levels in weanling pigs would be roughly comparable to those in older animals because of compensating factors. In particular, we expected the newly increased muscle loads and the poorly mineralized tissue to be countered by more robust geometry of the bones. The hypothesis was supported in that, during mastication, piglet strain levels were about the same as those of juveniles. However, the reasons for this were not as anticipated. When the muscles were supramaximally stimulated, the piglet strain levels were very much higher than those of the juveniles. Therefore, the similar masticatory strain levels were not primarily due to the robust geometry of the bones but to the fact that the piglets do not recruit muscles as fully as do juveniles during feeding. Indeed, we found that the piglet zygomatic arches were less geometrically robust than those of older animals, at least for bending in the parasagittal plane.

We also hypothesized that piglet strain patterns would be more variable than those of juveniles, in part because of a less adaptive bone geometry that would result in unpredictable deformation. This hypothesis was strongly supported. Intra-individual variation during piglet feeding was especially high, and the bones of the arch were straighter and rounder in younger pigs. Changes in geometry, in particular the increasing curvature of the bones in the parasagittal plane and their relative increase in height but not thickness, would all contribute to greater predictability of bone deformation in older pigs. However, bone geometry was not the only cause of strain variability in the infants. Other important contributing factors included less stereotyped chewing behavior with less reliance on the masseter muscle and greater proximity of the occluding molars to the zygomatic bone compared with older animals.

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