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SHORT COMMUNICATION

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Dietary consistency and the midline sutures in growing pigs

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Structured Abstract

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Objectives – The purpose of this study was to investigate the effects of reduced masticatory function on midline suture growth and morphology in growing pigs.

Setting and Sample Population – The sample was 20 pigs separated into two dietary groups and raised at the Department of Anthropology, Harvard University. Midline suture specimens were analyzed at the Department of Orthodontics, University of Washington.

Materials and Methods – Ten farm pigs and 10 minipigs, all male, were randomly assigned to hard (n = 9) and soft-diet (n = 11) groups. Fluorochromic mineral labels were administered to document bone apposition, and the animals were killed after 12 weeks. Undecalcified sections of the interfrontal, interparietal, internasal, and intermaxillary sutures were evaluated for bone quantity and sutural thickness, interdigitation ratio and growth rate.

Results – Soft-diet pigs were characterized by a slower rate of weight gain and less bone than their hard-diet counterparts. Even after correction for weight gain, soft-diet pigs had reduced suture growth rate and thickness. However, no difference in interdigitation ratio was detected between dietary groups.

Conclusions – Restriction to a soft diet reduces midline suture growth and bone apposition in the growing pig.

Key words: dietary consistency; pig; skull; soft diet; suture growth

Introduction

Sutures separate rigid bones and absorb physical strain incurred during normal function (1). In the skull, sutures are subjected to both the static expansion of growing soft tissues and dynamic masticatory forces (2), and their growth and morphology are thought to reflect these compressive and tensile loads (3–5). Sutural interdigitation increases the surface area available for collagen fiber attachment (6) and provides the architecture suitable for oblique fiber orientation characteristic of compression resistance (5). Conversely, 'butt-ended' sutures with perpendicular or cruciate collagen fibers are associated with tension (7).

Diet is a determinant of masticatory strain magnitude. Modern humans in industrialized nations consume a more processed, softer diet than pre-industrial and pre-agricultural humans. The transition from a harder

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to a softer diet has been accompanied by decreases in mandibular length and alveolar growth, as well as increased incidence of malocclusion (8–10). To reproduce this historical reduction of masticatory function experimentally, restriction of animals to a soft diet has proved to be a valuable strategy. Controlled studies comparing hard vs. soft diet in several species have supported the finding that the jaws are typically narrower in animals fed a soft diet (11–22) and show decreased bone density (23).

The most extensive studies on the effects of decreased masticatory function, and one of the few to examine craniofacial sutures, used rats (17, 24). A surprising finding of these studies was osteogenic obliteration of the internasal suture in many soft-diet animals. This fusion of a midline suture would clearly restrict subsequent transverse facial growth. Thus, it is possible that the narrow upper jaws that characterize soft-diet animals are partially a consequence of premature sutural synostosis. However, because the internasal area of the rat skull is dominated by the ever-growing incisors, it is not clear that non-rodents would show the same effect. Furthermore, rats and mice are not ideal models for human sutural biology in that their sutures normally remain patent throughout life, except for the posterior interfrontal, which fuses very early. For these reasons, several research groups have studied larger animals with sutural biology more comparable to humans, chiefly the pig (2, 13, 19).

The current study provides a systematic examination of the midline sutures in a pig model of decreased masticatory function. Based on prior findings, we hypothesized that growing pigs fed a soft diet would demonstrate decreased rates of midline suture growth, simpler suture morphology, and potentially localized synostosis.

Materials and methods

Using procedures approved by the Institutional Animal Care and Use Committee at Harvard University, 21 pigs (*Sus scrofa)* were randomly assigned to either a soft-diet group or a hard-diet control. In total, 11 (six soft, five hard diet) of the animals were 6-week-old farm pigs (Yorkshire strain), and 10 (five soft, five hard diet) were 8.5-week-old minipigs (Hanford strain). All were male except one farm pig in the hard-diet group. The single female was much smaller than the males and failed to thrive. It was therefore omitted from the present study.

For the duration of the 12-week experiment, the farm pigs were fed a mixture of hard or water-softened pig chow (Grower Diet; Purina, St. Louis, MO, USA) and a corn additive (Azteca Milling, Irving, TX, USA); dried corn for the hard diet, and masa flour for the soft diet. The minipigs were fed hard or water-softened pig chow (Rumilab, Richmond, IN, USA). The diets were available *ad libitum*. Animals were weighed at the beginning and at the end of the 12-week period.

The farm pigs were given approximately 30 mg/kg of calcein (Sigma, St. Louis, MO, USA) intermaxillary (IM) at the start of the study and every 2 weeks thereafter for 6 weeks. They were killed 2 weeks after the final label. The minipigs received calcein (20 mg/kg) when the experiment began and tetracycline (50 mg/kg; Sigma) at week 6. Animals were killed while under anesthesia at the age of 18 weeks (farm pigs) or 20.5 weeks (minipigs).

The skulls were de-fleshed in a dermestid beetle tank, degreased in a solution of ammonium hydroxide 28–30% and hydrogen peroxide 40% v/v, and fumigated with paradichlorabenzene (Sigma). Midline sutural samples, full thickness encompassing approximately 2 cm × 2.5 cm of the ectocranial surface, were removed using a Dremel® (Racine, WI, USA) tool and surgical carbide bur (Fig. 1A, B).

The specimens were dehydrated in an ethanol series, infiltrated with Micro-bed embedding resin solution (EMS; Fort Washington, PA, USA) overnight under vacuum, and then cured in cooled plastic wells under ultraviolet light at 365 nm for 2–3 days. A total of 6–8 sections of each sample were cut in the coronal plane at a thickness of 50–60 μm using a circular saw microtome (Leica SP1600; Leica Microsystems, Wetzlar, Germany). Half of these sections were dried flat at 37°C and mounted unstained on slides using Di-n-butyl Phthalate in Xylene (DPX) medium (Fluka, Buchs, Switzerland). The other half were stained with a 1% v/v toluidine blue (Sigma) solution for 5 s, rinsed with distilled water, and dried flat at 37°C prior to mounting.

The toluidine-stained sections were used to evaluate morphology. The unstained sections were used to visualize the fluorescent labels to determine sutural growth rate. Low power (1–2×) images (Spot Camera; Diagnostic Instruments, Inc., Chantilly VA, USA; Nikon Eclipse 400 Microscope; Nikon Imaging, Tokyo, Japan) recorded the full thickness of the suture, from

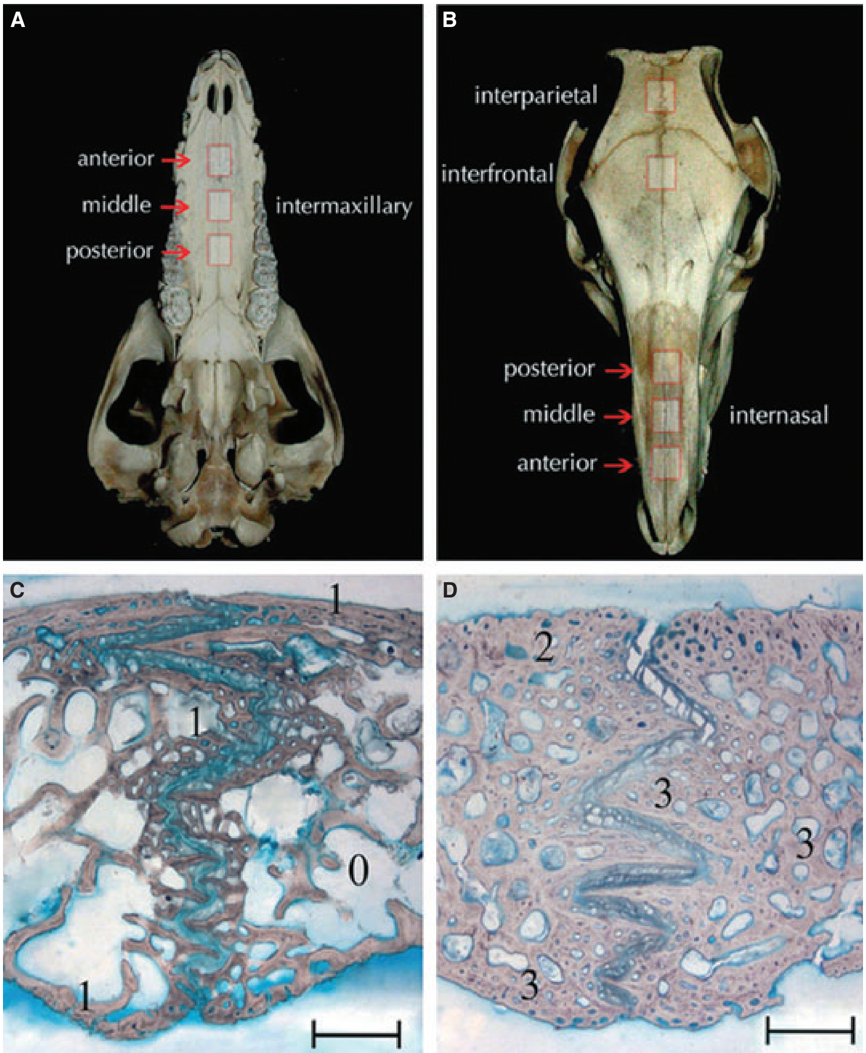

Fig. 1. (A) Ventral and (B) dorsal views of a pig skull, showing locations and dimensions of sutural samples. C and D are stained sections illustrating bone quantity scores from anterior internasal sutures from representative (C) soft and (D) hard-diet animals. The ectocranial side is toward the top, and the endocranial (nasal) side is toward the bottom of the figures. Values were assigned on a 0–3 scale at four locations for each specimen: ectocranial cortex, endocranial cortex, sutural cortex, and trabeculae. Calibration bars = 1 mm.

endocranial to ectocranial surface. Photoshop 7.0 (Adobe Photosystems, Mountainview, CA, USA) was used to merge multiple images for specimens too large to be captured in a single field of view. The tetracycline label of the minipigs was not successful, and the previous calcein label was greatly remodeled because of the time elapsed between administration and termination (12 weeks), so fluorescent images were obtained for the farm pigs only. Images were analyzed using the software package METAVUE (Universal Imaging Corp, Downingtown, PA, USA). All measurements were made by one investigator (AB), after blinding to specimen identity. Intra-examiner error for each variable was estimated by re-measurement of five slides on five non-consecutive dates.

Parameters evaluated included:

Bone Quantity: Bone quantity was graded at four locations within each suture specimen: ectocranial cortex, endocranial cortex, sutural cortex, and trabeculae. Representative anterior internasal specimens are shown in Fig. 1 for (C) soft and (D) hard-diet minipigs. A score from 0–3 was given for each location:

0 = very porous, primarily marrow space.
1 = sparse, thin trabeculae or cortex and large marrow spaces.
2 = moderate, medium trabeculae and marrow spaces.
3 = dense, small marrow spaces only.

Although subjective, this scoring system was found to be as repeatable as measurements of sutural marginal length (see results).

Sutural Thickness: Length in μm from endocranial to ectocranial surface (Fig. 2A). This measure is equivalent to the thickness of the skull at the suture.

Interdigitation Ratio (IR): An estimate of sutural complexity (5), equal to the true length in μm of the left

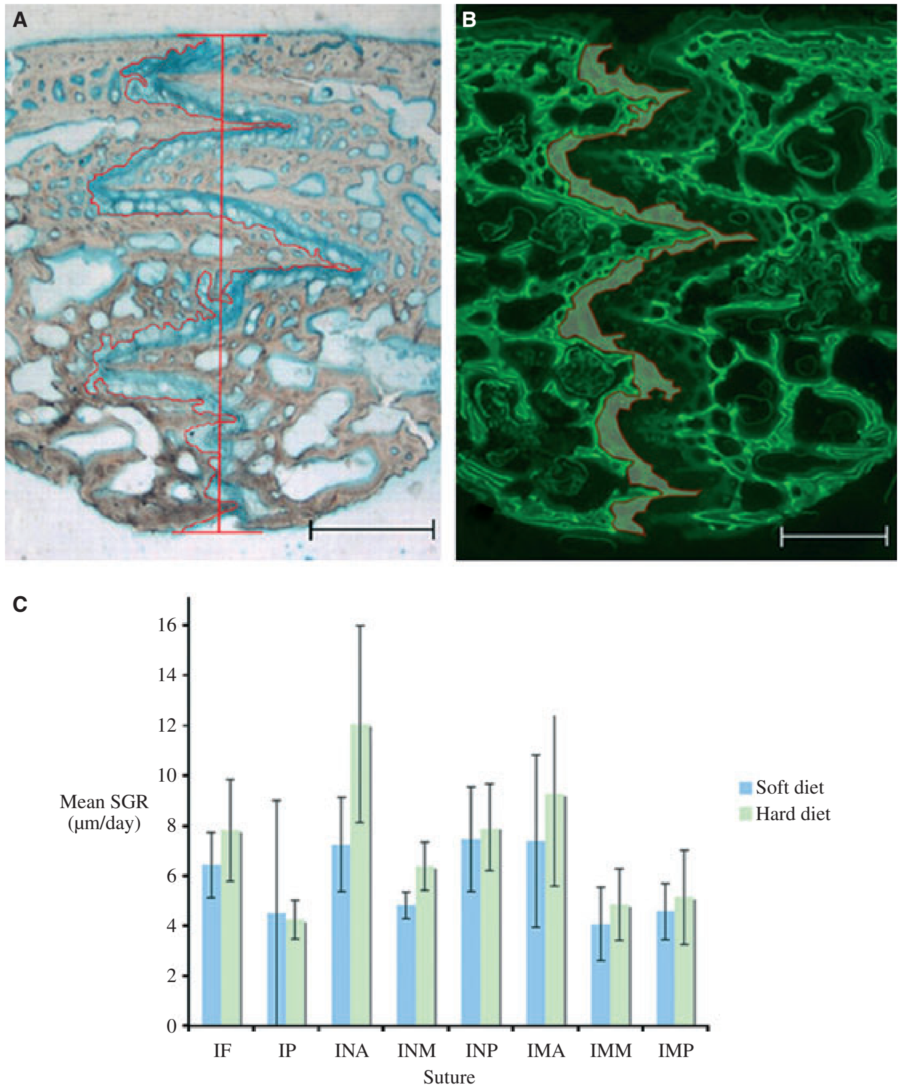

Fig. 2. (A) Toluidine-stained and (B) unstained coronal sections of an internasal suture illustrate the evaluation of morphology and suture growth rate. Suture thickness is represented by the vertical line in (A), while interdigitation ratio is the length of the left suture margin (wavy line) divided by the thickness. Suture Growth Rate (B) was calculated as the area between the label margin and bone front margin (shaded area) divided by the average length of the two margins and then divided by the number of days since the last label (μ/day). Calibration bars = 1 mm. Suture growth rate (SGR) for farm pigs is depicted in (C). SGR was significantly decreased in soft-diet animals when all locations were considered together ($p = 0.002$). Locations: IF, interfrontal; IP, interparietal; IN, internasal; IM, intermaxillary (A-anterior, M-middle, P-posterior).

(arbitrarily chosen) suture margin, divided by sutural thickness (Fig. 2A). More interdigitated sutures have higher values.

Suture Growth Rate (SGR) (farm pigs only): Because of the uncertainty in distinguishing among all six labels, SGR was calculated only for the period of time between the last label and termination. The average distance between this last label's margin and the margin of the bone apposition front was calculated on the left (arbitrarily chosen) half of the suture. This was accomplished by tracing both the label and bone front margins and dividing the area between them by the average length of the two (Fig. 2B). This gives a measurement of suture growth since the last label, which was divided by the number of days since the last label to give SGR in μm/day (2).

Statistical analyses

Weight gain for hard vs. soft-diet groups was evaluated using Student's *t*- tests assuming unequal variances. Mean and standard deviation were calculated for sutural thickness, IR, and SGR for each location in each group. Because IR is a ratio, these data were normalized with an arcsine transformation. Linear regression models were constructed to evaluate dietary and breed effects on suture thickness and IR at each suture location, after correction for differences in weight gain. A similar linear regression model, omitting breed, was constructed for SGR. Linear regression models were conducted to assess the significance of dietary and breed effects on suture thickness, arcsine-transformed IR, and SGR after pooling across locations (i.e. all sutures were considered together) and adjusting for the effect of location and its interaction with diet and breed. Bone quantity scores were evaluated using Wilcoxon Rank-Sum tests, because they do not satisfy the parametric distributional assumptions of linear regression. Results were deemed statistically significant at $p < 0.05$.

Results

Measurement error was estimated to be 6–7% for bone quantity score, 1–2% for suture thickness, and 5–6% for length of suture margin.

Animals restricted to a soft diet were characterized by slower rate of weight gain, significantly for farm pigs ($p = 0.03$) but not minipigs ($p = 0.13$), although the average difference was 0.6 kg/week in both breeds. No synostoses were observed in any specimen. Mean data for sutural thickness, IR, and bone quantity are presented in Table 1.

Table 1 illustrates the diverse nature of sutural morphology, with thickness varying from 3000–4000 μm (e.g. anterior IM) to well over 10 000 μm (e.g. interparietal). Similarly, IR varied from a low near 1–2 (interparietal) to a high of 6–7 (IM). These location differences are clear in both breeds of pigs and both diet regimes.

Taken individually, no single suture location showed significant effects of either pig breed or diet on thickness, IR, or SGR, although the internasal suture approached significance (thinner anterior internasal, $p = 0.08$, and less interdigitated posterior internasal, $p = 0.07$) in soft-diet animals using a linear regression model. However, when all the suture locations were pooled, suture thickness was significantly greater in hard diet than in soft-diet animals ($p = 0.0002$) and greater in farm than in minipigs ($p < 0.0001$) (Table 2). There was significant variation in thickness among the suture locations ($p < 0.0001$) and significant interactions between diet and location ($p = 0.02$) and between breed and location ($p < 0.0001$) (Table 2). Sutural growth rate considered collectively was also significantly greater in hard-diet animals ($p = 0.002$) with significant variation among locations ($p < 0.0001$) (Table 2, Fig. 2C). In general, cranial sutures were less interdigitated than facial sutures ($p < 0.0001$) (Table 1). IR, however, was not significantly associated with either breed or diet, although there was a significant interaction between diet and location ($p = 0.01$) (Table 2). Soft-diet pigs of both breeds had significantly less bone quantity when locations were combined ($p < 0.001$) (Table 1).

Discussion

In addition to addressing the basic question of the extent to which dietary consistency alters growth and morphology of the midline sutures, the results shed light on breed differences and differences among the suture locations.

Effect of diet

Soft-diet pigs were distinguished by slower weight gain, less abundant bone (Fig. 1), thinner sutures, and reduced sutural growth rate. It is important to note that the findings of thinner sutures and decreased SGR remained highly significant even after accounting for differences in weight gain, especially considering the modest sample size and typical levels of individual variability in the parameters measured. Thus, the effect of diet is a strong one.

Other investigators have usually found increased weight gain in hard-diet animals compared to those fed a soft diet (11, 13, 17, 25). The reasons for the difference are unclear. One likely factor may be increased weight of the skull itself. Hard-diet craniofacial bones are both thicker and denser than those of soft-diet animals, and thus the skulls were probably heavier. However, this factor is insufficient to account for the average difference of 6–8 kg in weight gain between dietary groups. Another factor is consumption. Both groups in the present study were fed *ad libitum*, but it is possible that the proprioceptive stimulation provided by mastication of the harder food increases its appeal.

The decreased bone quantity and sutural thickness observed for soft-diet animals in this study concurs with reports of decreased bone mineral density, cortical apposition, and trabecular volume in rats (17, 23, 26) and rabbits (27) on a soft diet. The reduction in sutural growth found in soft-diet pigs in the present study also supports findings in soft-diet rats of decreased mineral apposition rate at the IN, naso-premaxillary, and interpremaxillary sutures (25).

Table 1. **Descriptive statistics for sutural thickness, interdigitation ratio, sutural growth rate, and bone quantity**

				Internasal			Intermaxillary		
	N	Interfrontal	Interparietal	Anterior	Middle	Posterior	Anterior	Middle	Posterior
Sutural thickness in μm, mean (SD)									
Farm pigs									
Soft diet	6	7771 (2323)	15902 (3078)	5739 (443)	4568 (486)	6084 (770)	3789 (1109)	4195 (778)	4309 (443)
Hard diet	4	9259 (1062)	19747 (2430)	5891 (455)	4935 (559)	7056 (7810	4216 (655)	4440 (513)	5145 (567)
Minipigs									
Soft diet	5	6438 (1782)	10234 (2939)	4050 (569)	3249 (459)	3999 (920)	3151 (568)	3820 (1476)	4551 (1023)
Hard diet	5	7492 (887)	13871 (3221)	4453 (511)	4541 (1183)	4683 (1047)	3438 (573)	3724 (410)	5240 (1451)
Interdigitation ratio, mean (SD)									
Farm pigs									
Soft diet	6	2.57 (0.37)	1.19 (0.1)	2.14 (0.63)	3.35 (0.44)	2.74 (0.32)	5.08 (1.75)	6.88 (1.54)	7.66 (1.62)
Hard diet	4	2.62 (0.38)	1.12 (0.02)	2.15 (0.16)	3.13 (0.44)	2.53 (0.53)	3.61 (0.69)	6.43 (1.3)	7.60 (1.17)
Minipigs									
Soft diet	5	1.04 (0.13)	1.46 (0.48)	2.64 (0.54)	3.62 (0.43)	2.80 (0.49)	3.23 (1.42)	6.34 (2.16)	4.77 (1.44)
Hard diet	5	2.05 (0.89)	1.13 (0.15)	2.72 (0.58)	3.57 (0.63)	3.35 (0.78)	6.70 (1.24)	5.40 (0.71)	6.69 (1.41)
Suture growth rate in μm/day, mean (SD)									
Soft diet	6	6.42 (1.30)	4.50 (1.77)	7.24 (1.88)	4.81 (0.53)	7.45 (2.09)	7.37 (3.44)	4.07 (1.47)	4.57 (1.12)
Hard diet	4	7.81 (2.03)	4.25 (0.77)	12.01 (3.89)	6.38 (0.96)	7.94 (1.73)	9.26 (3.67)	4.85 (1.43)	5.13 (1.87)
Bone quantity, mean score (SD)									
Farm pigs									
Soft diet	6	1.83 (0.6)	1.67 (0.3)	1.38 (1.0)	1.63 (0.7)	1.46 (0.2)	2.08 (0.5)	1.96 (0.2)	1.92 (0.2)
Hard diet	4	2.25 (0)	2.25 (0.1)	2.5 (0.4)	2.00 (0.30)	1.50 (0.3)	2.25 (0.3)	2.20 (0.4)	2.00 (0.5)
Wilcoxon	*p*	0.07	0.02	0.11	0.33	0.06	0.28	0.32	0.74
							Combined locations		$\boldsymbol{p < 0.001}$
Minipigs									
Soft diet	5	2.13 (0.32)	2.00 (0.66)	1.90 (0.29)	1.90 (0.14)	1.70 (0.37)	2.15 (0.22)	1.65 (0.29)	2.10 (0.29)
Hard diet	5	2.7 (0.41)	2.55 (0.2)	2.45 (0.51)	1.90 (0.60)	2.10 (0.45)	2.35 (0.29)	2.30 (0.21)	2.30 (0.21)
Wilcoxon	*p*	0.10	0.13	0.10	0.83	0.20	0.01	0.44	0.28
							Combined locations		$\boldsymbol{p < 0.001}$

The non-parametric Wilcoxon ranked-sum test was used to compare bone quantity, which was evaluated subjectively. The other variables satisfied the parametric requirements for linear regression, the results of which are found in Table 2. Statistical significance indicated in bold.

Taken together, these findings point to slower overall apposition in soft-diet animals and thus imply that functional loading because of mastication is a significant factor in osteogenesis at suture fronts and overall in the craniofacial skeleton. Indeed, strain gauge measurements of the interparietal, interfrontal, and internasal sutures of pigs show that all these midline sutures undergo extensive deformation during mastication of a hard diet (2, 5, 7). The interparietal and interfrontal sutures are usually tensed (2, 7), whereas the internasal is compressed (5). Sutural strains are distinctly smaller when pigs are eating softer food items (unpublished results). Thus, it seems probable that the soft diet removed a large fraction of the functional loading on the sutures.

Interdigitation ratio was not decreased in the soft-diet pigs, except possibly for the posterior internasal suture ($p = 0.08$). The lack of significant effect on IR may be because soft-diet mastication does not alter the polarity (tension vs. compression) of functional strain. A model that eliminates or reverses the polarity of functional loading would be required to evaluate this aspect of suture morphology.

Table 2. **Linear Regressions evaluating the effects and interactions of diet, breed, and location on thickness, IR and SGR**

	Thickness		IR		SGR	
Linear regression (pooled locations)	*F*	*p*	*F*	*p*	*F*	*p*
Diet	14.55	**0.0002**	1.18	0.28	10.44	**0.002**
Breed	54.95	**<0.0001**	0.95	0.33	N/A	N/A
Location	136.08	**<0.0001**	101.19	**<0.0001**	8.97	**<0.0001**
Diet × Breed	0.02	0.88	0.14	0.71	N/A	N/A
Diet × Location	2.49	**0.02**	2.67	**0.01**	1.19	0.33
Breed × Location	8.35	**<0.0001**	1.75	0.10	N/A	N/A
Diet × Breed × Location	0.12	0.99	3.44	**0.002**	N/A	N/A

Sutural locations were pooled, and the results were adjusted for the differential in weight gain between dietary groups. Statistical significance indicated in bold.
IR, interdigitation ratio; SGR, suture growth rate.

Effect of body size

Although body size was accounted for by our statistical methods, differences remained. Specifically, weight-corrected sutural thickness was significantly greater in farm pigs than in minipigs. This difference points to the conclusion that farm pigs are not just larger versions of minipigs.

Difference in suture locations

Generalized narrowing and localized synostosis of the ectocranial portion of the internasal suture typifies rats fed a soft diet (17, 18). Decreased apposition of bone at the internasal suture has also been demonstrated in the same model (25). In the present study, soft-diet animals tended to have thinner anterior internasal and less interdigitated posterior internasal sutures after adjustment for weight gain. The internasal suture may be particularly affected by alterations in dietary consistency in these long-snouted species, a finding that cannot be extrapolated to humans.

Regardless of diet, the cranial sutures (interfrontal and interparietal) were thicker and less interdigitated than the facial sutures (internasal and IM) (Table 1). Within the internasal and IM sutures, the posterior locations were usually thicker than the anterior and middle locations. Interdigitation also varied within as well as between sutures. The internasal suture was most interdigitated in the middle section, intermediate in the posterior, and lowest in the anterior portion, as reported previously in a study that correlated compressive strain polarity with the interdigitated structure (5). The interparietal and interfrontal sutures, characterized by tension (5, 7), were less interdigitated than the internasal suture, supporting their contention that strain polarity and sutural complexity are related. Masticatory stain in the IM suture was speculated to be tensile based on recording in anesthetized animals (28). Nevertheless, the present findings on interdigitation in the IM suture show that it is, on average, considerably more interdigitated even than the internasal suture, leading to a prediction that this suture should be strongly compressed during function.

Considering the differences between pigs and rats in size, cranial anatomy, and feeding mechanisms, it is remarkable that our findings are similar in showing growth reduction in the sutures as well as decreased bone generally. However, we did not see premature obliteration of the internasal or any other sutures. This may be a function of the longer growth period in pigs than in rats. The animals in our study were still actively growing at the time of termination (18–21 weeks of age). Nevertheless, because growth reductions were prominent features of the midline sutures in these disparate species, it is likely that a soft diet would have the same effect in humans. Specifically, individuals with soft-diet preferences, or reduced functional loading from any cause, would likely show an overall reduction in craniofacial bone and a decreased rate of midline suture growth. This tendency may lead to potentially reduced transverse jaw width, and the orthodontic problems associated therewith.

In conclusion, in this large animal model of reduced masticatory function, midline sutures had a reduced rate of growth, and the adjacent bone fronts were thinner and contained less bone. Sutural morphology was unaffected by diet hardness.

Clinical relevance

Suture biology is of importance in clinical orthodontics, as many malocclusions are treated by modification of suture growth. Decades of research and clinical experience have shown that sutures are dynamic structures whose development is linked to their biomechanical environment. This study shows that restriction to a soft diet decreases midline suture growth and bone apposition in growing pigs, presumably through reduced craniofacial strain during mastication. It is likely that a soft diet would have a similar effect in humans and may result in a decreased rate of midline suture growth and potentially reduced transverse growth of one or both jaws.

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