Enlargement of the temporalis muscle and alterations in the lateral cranial vault

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Synopsis The purpose of this study was to test the hypothesis that increased masticatory muscle accompanied morphologic changes in the temporal bone and squamosal suture. Ten mice deficient for the protein myostatin (Mstn/C0/C0) had significantly increased skeletal muscle mass and were compared with nine controls (Mstn+/+). Variables measured include linear and areal metrics describing temporal size and temporal bone shape as well as the extent of the area of the squamosal suture that overlaps, or bevels, with parietal bones. Mstn−/− mice showed significantly larger temporalis muscles. Their temporal bones showed significantly decreased size as well as decreased beveling of the squamosal suture. These decreases were absolute as well as relative and were not restricted to either vertical or horizontal axes. The increased masticatory musculature of Myostatin-null mice had a shrinking effect on the temporal aspect of the cranium. These results are inconsistent with the interpretation that increased temporalis mass induces morphologic changes in temporal bone that compensate for putative increases in compressive forces transduced at this region. Rather than increase in the area of overlap between two calvarial bones, potential increase in biomechanical loading along the temporal squama led to a smaller bevel which would presumably weaken this joint. It is unclear why this is so. Either compressive forces are not anabolic to suture beveling or they do upregulate growth of the suture bevel, with compression not being the primary loading regime at this suture.

Introduction

It is generally accepted that there is an interaction between the neuromuscular system and bone formation. In this study, the effects of enlarged temporalis muscles on adjacent bone were studied. The authors used a hypermuscular mouse model lacking myostatin (Mstn). Myostatin, a member of the transforming growth factor β superfamily of secreted growth and differentiation factors (GDF-8), is a negative regulator of the growth of skeletal muscle. Mice and cattle deficient for myostatin (Mstn−/−) show a marked increase of muscle mass compared to normal animals (McPherron and Lee 1997; McPherron et al. 1997; Lee and McPherron 1999). While Mstn expression is predominantly found in muscle, it has also been observed as an early stage within the bone fracture and healing cascade (Cho 2002). Additionally, the deletion of Mstn decreases adipogenesis suggesting a role in fat metabolism (Lin 2002). Previous studies by Byron et al. (2004) showed the myostatin deficient (or knockout) mice with bite forces that were on average 33% greater than the wild type. Previous studies utilizing this mouse model have evaluated the quantitative and qualitative effects of this increased muscle on bone in the humerus, temporomandibular joint (TMJ), mandible, and cranial sutures (Hamrick et al. 2000; Hamrick, 2003; Hamrick et al. 2003; Byron et al. 2004; Nicholson et al. 2006; Ravosa et al. 2007; Vecchione et al. 2007). In general, increased muscle mass altered the morphology of bone such that it was anabolic to bone growth.

The temporal bone

The temporal bones are irregularly shaped components of the bilateral cranial vault. From a lateral perspective they are bounded by the parietals superiorly, the occipital posteriorly, and the sphenoid and zygomas anteriorly. The morphology of the sutures at each of these margins is complex but the anterosuperior and anteroinferior borders are of an elaborately beveled type. These bevels reflect where the temporal bone overlaps the adjacent parietal and sphenoid bones. Figure 1 demonstrates this skeletal...
region, including the associated musculature and their loading vectors in both humans and mice.

Early experiments by Moss (1957), using a mouse ontogenetic model, transplanted beveled (coronal) sutures into intracerebral locations in order for them to develop without extrinsic calvarial muscle forces. Additionally, ligatures were tied around end-to-end (butt joint) sutures to artificially load them in compression. Interestingly, the coronal suture did not develop its beveled morphology (i.e., it retained an end-to-end design) while the sagittal suture, when artificially loaded in compression, did develop an overlapping joint. These findings led Moss to reason that the butt-joint suture reflects the intrinsic morphology of most cranial sutures while the overlapping nature of sutures, like the temporal squama, is a secondary phenomena imposed by extrinsic mechanical forces such as occur during mastication. If this hypothesis is correct, then the beveling of temporal squama should be positively related to extrinsic mechanical loading.

The function of suture beveling has been attributed to better controlling movements, or reducing stress, between bones (Moss 1957; Herring 1972) when compared to end-to-end sutures. Additionally, it has been proposed that beveling helps expedite fast growth of bone (Koskinen 1976). In either case, beveling is a response to mechanical loading most likely coming from the upward forcing of the temporal bone against the parietal during compression of the temporomandibular joint (Herring 1972). The prediction that compressive strains are manifested along beveled sutures is further supported by the arrangement of compression-resistant collagen
fibers found in beveled sutures (Herring and Mucci 1991; Rafferty and Herring 1999). In light of this evidence, it stands to reason that as masticatory forces increase, suture overlapping may grow in regions where compression defines the loading milieu.

In this study the temporal bone was selected as a region of interest because it is found deep to the temporalis muscle. Furthermore, the squamosal suture is covered by, and oriented perpendicular to, the axis of temporalis myofibers making compressive stress, and not tension, the likely loading regime at this suture during contraction. The authors here hypothesize that mice deficient in myostatin (exhibiting enhanced masticatory muscles) would show features that compensate for increased compressive forces along the lateral sides of the cranial vault. Specifically it was predicted that there would be enlargement of the temporal bones as well as greater beveling (overlapping) of the squamosal sutures with the parietals.

**Materials and methods**

Byron et al. (2005) has previously shown that this mouse genotype can produce greater bite force due to its increased temporalis muscle. Nineteen total mice (Mstn \(-/\)- versus Mstn \(+/+\)) were raised under the same conditions with access to pelleted feed *ad libitum*. These animals were reared and euthanized in compliance with Institutional Animal Care and Use Committee guidelines at the Medical College of Georgia. Four-month-old animals were chosen for these analyses because mice at this age represent a mature musculoskeletal phenotype and thus reflect differences in mechanical loading during development. Temporal bones were isolated from the cranium through potassium hydroxide (KOH) digestion of fibrous connective tissues and then stained using alazarin red for bone according to Wassersug (1976). The temporal bones were imaged using a high-resolution digital scanner and desktop computer interface. Metric variables were collected from these digital scans using SigmaScan Pro 4.0. The following variables were measured (Table 1, Fig. 2): length, height, perimeter, and area of the temporal bone, and area of overlap between the temporal and the anterosuperior parietal and sphenoid bones (squamosal suture bevel). From these

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Metric and ratiometric variables relating to the temporal bone</th>
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<td>Temporal Perimeter</td>
<td>Linear distance along outer margin, mm (Fig. 2)</td>
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<tr>
<td>Temporal Length</td>
<td>Linear distance, ( \mu )m (Fig. 2)</td>
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<tr>
<td>Temporal Height</td>
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<td>Temporal Area</td>
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<td>Bevel Area</td>
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<td>( \left( \frac{\text{Bevel Area}}{\text{Temporal Area}} \right) \times 100 )</td>
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<td>Temporal Mass</td>
<td>Mass of temporalis muscle freshly dissected, ( \mu )g</td>
</tr>
<tr>
<td>Relative Temporal Mass (% Body Mass)</td>
<td>( \left( \frac{\text{Temporal Mass}}{\text{Body Mass}} \right) \times 100 )</td>
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Fig. 2 This figure depicts a standard temporal bone from a control individual. Various aspects of its morphology are highlighted in order to demonstrate morphometric variables that are compared in this article. \( L = \) length of the temporal bone, \( H = \) height of the temporal bone, and \( B = \) temporal bone beveling, or light gray area of overlap with cranial vault bones. Other variables that are not explicitly labeled, but observed here, are the perimeter length (outside margin of bone) of the temporal bone and the area (region inside the temporal bone margin) of the temporal bone.
measurements ratiometric variables are computed that account for relative differences in size between experimental genotypes.

**Statistical analysis**

All quantitative variables were entered into Statgraphics Centurion XV in order to carry out independent samples t-tests with pooled variances. Twelve dependent variables (Table 1) were analyzed for differences between genotypes (grouping variable) as seen in Table 2. T-scores for each comparison are reported along with the P-value of that t-score. A final column, “Adjusted Significance”, compares this P-value to the critical value needed in order for that dependent variable to achieve table-wide significance at the 95% confidence interval. This method is a modified version (sliding-scale type) of the Bonferroni post-hoc adjustment for multiple tests (Rice 1989).

**Results**

Overall body mass did not show significant differences between mouse genotypes. Alternatively, temporalis muscle mass did show a significant increase in Mstn−/− individuals. The total area of the temporal bone, however, was larger in the control mice (Table 2 and Fig. 3). This reflects the fact that the temporal bone showed significant increases in length, height, and perimeter. Temporal bevel size also showed a decrease in Mstn−/− mice. Interestingly, when the bevel area is subtracted from the overall temporal area and then size compared, there were no significant differences between mouse models in non-bevel temporal area.

Potential differences in body size represent a challenge for morphological analysis because any other differences must be interpreted through the lens of this overall size discrepancy. Thus, several ratiometric variables were computed such as Relative Temporal Bevel Area (see formula in Table 1). The knockout mice (Mstn−/−) with larger musculature had smaller relative bevel areas but this was not a significant decrease given a table wide alpha of 0.05. The significant decrease in non-size corrected temporal beveling was not due to decreases in beveling per unit length or height (Relative Bevel Area as % Length or Height is non-significant) (Table 2). Decreasing bevel area was accompanied by equivalent decreases in area of the temporal bone in hypermuscular mice. The non-bevel temporal areas were the same in these experimental groups and it was only in the region of squamosal overlap that differences between groups were observed. This observation is contrary to a component of the hypothesis outlined above that masticatory muscle mass will be positively related to compression-resisting increases in temporal bones and their beveling. Here, the relationship between the size of the temporalis muscle and squamosal beveling was actually negative. If compression within the squamosal suture does occur in vivo, then overlapping of the temporal bone with parietal and sphenoid bones should not be considered in positive association with greater compressive loading. These results are discussed below.

**Discussion and conclusions**

The results of this study suggest that the temporalis muscle was not anabolic to the temporal bone or the
area of overlap along the anterosuperior temporal squama. In specimens with larger temporalis muscles the temporal bone appeared to be “compressed” in length and height. The decreased size of the temporal bone was more a factor of this squamosal area of overlap and less a factor of non-beveling regions of the temporal bone. This relationship may have resulted from compressive strains that acted to restrict growth on the anatomic region studied here (see below). Alternatively, large masseter muscles (accompanying Mstn−/− phenotype) may have had a stress-shielding effect on the actions of the temporalis muscle. If true, then increased contractile force that accompanied larger temporalis muscles were possibly negated by similar increased force operating on the opposite side of the zygoma. The result may have been lower strain environments within the Mstn−/− sutures. Further experiments isolating increases in the masseter versus the temporalis may elucidate this problem.

These results are inconsistent with the interpretation that increased temporalis mass induces morphologic changes in temporal bone that compensate for putative increases in compressive forces transduced at this region. Rather than increasing the area of overlap between two calvarial bones, increased biomechanical loading along the temporal squama leads to a smaller bevel that would presumably weaken this joint. It is unclear why this is so. Either compressive forces are not anabolic to suture beveling or they do upregulate growth of the suture bevel with compression not being the primary loading regime at this suture. In either case, global changes in cranial morphology may result from growth allometries such that fundamental differences in the shape of the head exist between mouse models.

**Do muscles used in chewing restrict cranial growth?**

If compressive forces are not anabolic to growth of sutures as stated above, an important question becomes “Do they restrict sutural growth?” If the answer is “yes” then one can reason that, overall, the cranial bones would become smaller if appositional growth around the perimeter slows. Thus, one should expect to see increased cranial growth with masticatory muscle gracilization. Stedman et al. (2004) outlined a related rationale to explain a myosin gene mutation unique to *Homo sapiens* that causes a decrease in the size of the temporalis and masseter. These authors posit that masticatory gracilization may have been an important mechanism during human evolution that led to increases in the size of the brain. Their hypothesis suggests that larger chewing-muscles act to suppress growth of bone and brain tissues. New evidence by Cray et al. (2008) demonstrates that Mstn−/− mice have equivalent brain sizes compared to Mstn++/+ controls. This suggests that maximum brain size is not restricted by increased masticatory musculature. If compression in the cranial sutures restricts bone growth it apparently does not affect endocranial volume. The MYH16 mutation and chewing-muscle reduction observed in humans to the exclusion of other primates may indeed release cranial margins to grow more. It is unlikely, however, that this is causative to significant increases in brain size observed during Plio-Pleistocene human evolution, as pointed out by McCollum et al. (2006).

Of interest in relation to our data are similar findings by Vecchione et al. (2007) in which masticatory hypermuscularity resulted in significantly
The craniofacial morphology of hominins is associated with mechanical loading due to masticatory forces. Their study utilized Mstn−/− mice and demonstrated that the cranial vault was shorter and the mandible longer and more “rocker-shaped” as compared to Mstn+/+ mice. In light of our data, this shortening of the cranial vault could be linked to reductions in beveling of the temporal bone since non-beveled temporal bone is equivalent between these genotypes. Using the same mouse model Ravosa et al. (2007) and Nicholson et al. (2006) found increased bone plasticity with hypermuscularity at the mandibular symphysis, along the corpus, and at the TMJ. These changes were detected using Micro-CT and thus relate to internal structural differences between mouse genotypes. In summary, there are differential effects of muscle robusticity on the cranium and mandible. While bones of the cranial vault appear to decrease in size, the size of the mandibles increase.

If sutural growth on the cranial vault is restricted such that braincases are shorter and brain volume remains equivalent, one may expect to find excessive growth in an orthogonally axis. In the case of the data presented here, Mstn−/− mice are predicted to have taller or perhaps wider cranial vaults. In this case, reduction in length along the anteroposterior axis is compensated for in the mediolateral or dorsoventral axes. Byron et al. 2004 demonstrated that the sagittal suture (interparietal) shows differential trajectories of growth in Mstn−/− mice compared to controls. Hypermuscular mice showed increases in sagittal suture complexity that was accomplished through new bone formation along convex surfaces. Opposing concave bone surfaces were later identified as regions of active bone resorption via osteoclasts (Byron 2006). This combined remodeling activity leads to increased bone growth in sutures primarily loaded in tension. This would compensate for vault growth restrictions along sutures loaded in compression. The combination of these processes should lead to shorter and wider skulls with a large cephalic index (i.e., brachycephalic). Future directions include investigating parietal bone size and shape and exploring whether brachycephalism is associated with masticatory robusticity in mice and other comparative mammalian taxa.

What about suture beveling in robust hominins?

Discrete differences between hominin species in the size of the masticatory apparatus during the African Plio-Pleistocene are commonly observed. Both gracile and robust forms occur sympatrically and appear to have occupied separate ecological feeding niches. Robust hominins possess morphological traits in the craniofacial skeleton presumed to be associated with feeding on exceptionally hard, tough, or otherwise obdurate foods. On the other hand gracile hominins do not possess these pronounced architectural features. For a recent review of these highly derived robust hominin traits see Wood and Constantino (2007).

The robust Paranthropus species possess large and tall mandibles with large molar occlusal surfaces. In addition to these characters, Rak (1978) and Rak and Kimbel (1991, 1993) have identified an additional trait that differs between robust and gracile forms. They include an extensive overlap along a narrow region of the posterosuperior temporal squama as a component of the robust phenotype with respect to P. aethiopicus (KNM-WT 17000). This large bevel is aligned with what they interpret would have been the most robust region of an overlying temporalis muscle. This morphological feature in hyper-robust P. aethiopicus contrasts with the squamosal suture as observed in other robust hominins like P. boisei and this is purportedly due to flaring zygomatic arches in the latter species (Rak and Kimbel 1991, 1993).

Future experiments utilizing finite element modeling of hominin skulls will address the strain dampening effects of these craniofacial structures and will be able to test the relationship between beveling and mechanical loading. These studies could also directly test the hypotheses that P. aethiopicus suture beveling helped accommodate for increased masticatory forces and that P. boisei accomplished this same biomechanical outcome through laterally protruding zygomatic arches. It is accepted that the stress and strain orientations in the mouse cranium will differ somewhat from hominins since rodent masticatory morphology is highly derived relative to primates. Nevertheless, strict relationships between compression acting on cranial suture connective tissues and anabolic growth of bevels to absorb strain are not supported by these data.

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