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Intracranial and hierarchical perspective on dietary plasticity in mammals☆

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ABSTRACT

The effect of dietary properties on craniofacial form has been the focus of numerous functional studies, with increasingly more work dedicated to the importance of phenotypic plasticity. As bone is a dynamic tissue, morphological variation related to differential loading is well established for many masticatory structures. However, the adaptive osteogenic response of several cranial sites across multiple levels of bony organization remains to be investigated. Here, rabbits were obtained at weaning and raised for 48 weeks until adulthood in order to address the naturalistic influence of altered loading on the long-term development of masticatory and non-masticatory elements. Longitudinal data from micro-computed tomography (μCT) scans were used to test the hypothesis that variation in cortical bone formation and biomineralization in masticatory structures is linked to increased stresses during oral processing of mechanically challenging foods. It was also hypothesized that similar parameters for neurocranial structures would be minimally affected by varying loads as this area is characterized by low strains during mastication and reduced hard-tissue mechanosensitivity. Hypotheses were supported regarding bone formation for maxillomandibular and neurocranial elements, though biomineralization trends of masticatory structures did not mirror macroscale findings. Varying osteogenic responses in masticatory elements suggest that physiological adaptation, and corresponding variation in skeletal performance, may reside differentially at one level of bony architecture, potentially affecting the accuracy of behavioral and in silico reconstructions. Together, these findings underscore the complexity of bone adaptation and highlight functional and developmental variation in determinants of skull form.

1. Introduction

Bone is a dynamic, hierarchically organized tissue capable of sensing and responding to mechanical loads in order to produce a structure better equipped to withstand routine loads. This capability implies that bone is a plastic structure whereby altered external conditions can initiate an osteogenic cascade that modulates anatomical structure. Generally, these environmentally induced, postnatal responses reflect the functional or adaptive nature of an element or system of interest (Gotthard and Nylin, 1995; Agrawal, 2001; West-Eberhard, 2003). By fine-tuning the link between form and behavior, an organism can achieve a phenotype better matched to its surroundings. Adaptive plasticity in skeletal form is related to functional adaptation, or the dynamic coordinated series of cellular, tissue, and molecular processes of skeletal modeling and remodeling that maintain a sufficient safety factor for routine peak and cyclical loads (Bouvier and Hylander, 1981, 1996a, 1996b; Lanyon and Rubin, 1985; Biewener, 1993). Given increasing evidence that safety factors vary across the vertebrate skeleton (Hylander et al., 1991a, 1991b; Ravosa et al., 2000a, 2010a), it is particularly important to evaluate if patterns of functional adaptation also differ regionally.

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Among mammals, the plasticity of masticatory elements to altered loading conditions has been well studied in lagomorphs, rodents, carnivorans, suids, hyracoids, and primates (Beecher and Corruccini, 1981; Bouvier and Hylander, 1981, 1982, 1984, 1996a, 1996b; Beecher et al., 1983; Bouvier, 1988; Yamada and Kimmel, 1991; Ciochon et al., 1997; He and Kiliaridis, 2003; Lieberman et al., 2004; Larsson et al., 2005; Nicholson et al., 2006; Ravosa et al., 2007, 2008a, 2008b, 2010b, 2016; Menegaz et al., 2009, 2010; Scott et al., 2014a, 2014b; Menegaz and Ravosa, 2017). These prior studies, though limited primarily to the feeding apparatus, have generally demonstrated a significant response to elevated loading conditions in various skeletal parameters such as external dimensions, cortical bone thickness, cross-sectional area, and tissue mineral density. Limited research has been conducted on the plasticity of sites less directly involved with oral processing, though gross proportions of the neurocranium and calvarial cross-sectional thickness have been investigated in lagomorphs (Menegaz et al., 2010; Franks et al., 2016), with results suggesting a lack of a significant plastic response.

As stated above, previous studies of diet-induced plasticity were largely focused on a single masticatory element or functional region or, to a lesser degree, the hard- and soft-tissue responses that maintain the integrity of composite structures such as the mandibular symphysis or temporomandibular joint (Ravosa et al., 2007, 2008a, 2016; Ravosa and Kane, 2017). As such, a comprehensive regional analysis of adaptive plasticity of hard tissues across multiple craniomandibular sites in the same specimens is lacking. This is of critical importance as within-element variation in limb responses (Hsieh et al., 2001; Hamrick et al., 2006) suggests that similar regional variation may exist in the skull. It is also becoming increasingly evident that varying skeletal parameters respond differently to a given loading pattern (Kohn et al., 2009; Wallace et al., 2009; Scott et al., 2014a; Ravosa et al., 2015b, 2016) and that the hierarchical nature of bone allows it to respond adaptively to mechanical stimuli at multiple organizational levels. To this end, the performance of skeletal elements is dictated by a number of factors including bone quality, bone quantity, and bone distribution and there are multiple mechanisms for increasing bone strength. Thus, it is possible that a functional signal may be differentially represented at one level of organization vs. another level, potentially posing an issue for accurate behavioral and functional characterizations. For example, in a prior study of the rabbit mandibular symphysis, it was demonstrated that an internal dimension, cortical bone thickness, exhibited a greater disparity vs. gross external dimensions (Ravosa et al., 2007, 2008a). Furthermore, the disparity in cortical bone biomineralization between rabbit dietary groups was shown to be lower than that for cortical bone thickness (Ravosa et al., 2007, 2008b). These findings are of critical importance because gross skeletal dimensions are more commonly used to track diet-related variation in morphological studies (e.g., Ravosa, 1991, 1996; Ravosa and Hogue, 2004; Wright, 2005; Friscia et al., 2007) and it is unlikely that the singular use of external dimensions will furnish the requisite evidence for meaningful paleobiological reconstructions (Ravosa et al., 2016). Additionally, it further underscores the complexity of bony organization and adaptation.

Currently, there is a significant gap in our understanding of the effects of varying diets on regional and hierarchical variation in hard tissues of the developing skull and feeding apparatus. To complement prior analyses, we report the results of a long-term diet manipulation experiment conducted using an animal model (white rabbit) that examined adaptive plasticity at various levels of bony organization at multiple bony sites across the skull vis-à-vis variation in food mechanical properties. More specifically, we probed the relationship between masticatory loading and morphological plasticity in a number of representative bony regions in order to ascertain the effect of elevated loading on the *overall* craniomandibular unit. Moreover, to investigate how altered loads differentially affect varying levels of bony organization, each region was assessed on a macro- and microscale to document the dynamic cascade of coordinated adaptive events.

We test the hypothesis that cortical bone formation and bone quality in the developing skull adapt postnatally to increased masticatory loading and the resulting elevated stresses via correlated osteogenic processes. Given that maxillomandibular bone strain levels are higher than elsewhere along the mammalian skull during routine feeding behaviors (e.g., Hylander et al., 1991a, 1991b; Ravosa et al., 2006, 2010a) and that neurocranial osteoblasts exhibit reduced mechanosensitivity vs. elsewhere in the skeleton (Rawlinson et al., 1995; Ravosa et al., 2015b), it is first predicted that masticatory regions will exhibit a more pronounced response to elevated loading, indicating the presence of regional variation in diet-induced plasticity. Second, it is predicted that long-term increased masticatory stresses will result in skeletal elements of the feeding complex with more robust proportions and increased bone quality. Hard tissues in the masticatory region of rabbits raised on a more challenging diet should develop increased cortical bone thicknesses and elevated biomineralization. In contrast, non-masticatory regions (i.e., the neurocranium) should display a minimal plasticity response at both levels of analysis given that these regions experience lower strains during oral processing.

2. Materials and methods

2.1. Animal model and experimental design

To evaluate the long-term plasticity of cranial elements vis-à-vis altered loading levels, 20 genetically similar male New Zealand white rabbits (*Oryctolagus cuniculus*) were obtained at weaning (five weeks old) from Harlan Laboratories and housed at the University of Notre Dame's animal care facility, Freimann Life Science Center. Both institutions are USDA-licensed and AAALAC-accredited and subject to periodic inspections. Day-to-day care of the animals, including periodic health evaluations, was handled by trained veterinary staff. Additionally, all procedures were approved by the University of Notre Dame's Institutional Animal Care and Use Committee (IACUC).

The animals were raised for 48 weeks immediately following weaning, making them 53 weeks old at the conclusion of the experimental period. In white rabbits weaning typically occurs at 4–5 weeks of age, while skeletal and sexual maturity are reached at ∼26 weeks of age (Masoud et al., 1986; Isaksson et al., 2010).

Previous experimental work has established that the masticatory apparatus of growing rabbits is sensitive to variation in food mechanical properties (Ravosa et al., 2007, 2008a, 2010b; Menegaz et al., 2009; Scott et al., 2014a, 2014b), exhibiting levels of phenotypic diversity between treatment groups that mirror evolutionary variation between closely related species with different diets (Ravosa et al., 2016). Importantly, white rabbits resemble other mammalian herbivores in key features of the masticatory apparatus and bone biology that make them a suitable model organism. These features include: (i) the configuration of the skull, which is characterized by a vertically deep facial skeleton, tall mandibular ramus, and a temporomandibular joint (TMJ) situated high above the occlusal plane; (ii) mandibular kinematics, with a TMJ capable of rotational and translational movements, and transverse jaw movements during mastication; (iii) intracortical bone remodeling; and (iv) well-characterized patterns of covariation among dietary properties, jaw-adductor muscle activity, and jaw-loading regimes (Weijs and de Jongh, 1977; Weijs and Dantuma,