Bone remodeling and cyclical loading in maxillae of New Zealand white rabbits (*Oryctolagus cuniculus*)

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**Abstract**
Mammalian feeding behaviors are altered when mechanically challenging (e.g., tough, stiff) foods require large bite forces or prolonged mastication. Bony responses to high bite forces are well-documented for the mammalian skull, but osteogenesis due to cyclical loading, caused by repetitive chewing, is more poorly understood. Previous studies demonstrate that cyclical loading results in greater bone formation in the rabbit masticatory apparatus and in substantial Haversian remodeling in primate postcrania. Here we assess the relationship between cyclical loading and remodeling in the rabbit maxilla. Twenty male New Zealand white rabbits (*Oryctolagus cuniculus*) were raised on either an overuse or control diet (10 per group) for 48 weeks, beginning at weaning onset. The control group was raised on a diet of rabbit pellets (*E* = 29 MPa, *R* = 1031 J/m²), whereas the overuse group ate rabbit pellets and hay, which has high stiffness (*E* = 3336 MPa) and toughness (*R* = 2760 J/m²) properties. Hay requires greater chewing investment (475 chews/g) and longer chewing durations (568 s/g) than pellets (161 chews/g and 173 s/g), therefore causing cyclical loading of the jaws. Remodeling was measured as osteon population density (OPD), percent Haversian bone (%HAV), and osteon cross-sectional area (On.Ar). The only significant difference found was greater On. Ar in the alveolar region of the maxilla (*p* < 0.001) in the overuse group. The hypothesis that cyclical loading engenders Haversian remodeling in the developing maxilla is not supported. The continuation of modeling throughout the experimental duration may negate the need for remodeling as newly laid bone tends to be more compliant and resistant to crack propagation.

**KEYWORDS**
food material properties, Haversian system, mastication, microdamage, osteon

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**1 | INTRODUCTION**
Bone is an adaptive tissue that responds to the stresses and strains imposed on the skeleton during the development of an organism. This adaptive ability allows the skeleton to maintain structural integrity throughout life under varying loading environments (Huiskes & van Rietbergen, 2005; Martin et al., 2015b). The bony response to mechanical...
loading has long been of interest to biologists, paleontologists, and anthropologists for its potential to elucidate form–function relationships in the fossil record. However, the exact nature of osteogenic responses to specific loading parameters is still being explored. In particular, the extent of the relationships (e.g., strain magnitude, rate, or mode) each contribute to skeletal formation is not completely understood. The following is an investigation of the relationship between one mode of bone adaptation, Haversian remodeling, and repetitive loading in an understudied aspect of the mammalian masticatory apparatus.

Haversian remodeling (also referred to as intracortical bone remodeling and, herein, simply remodeling) is a process by which existing cortical bone is resorbed and replaced though the coordinated actions of osteoclasts and osteoblasts. The process results in the formation of cylindrical structures called secondary osteons (not to be confused with primary osteons, which form during bone modeling and not following resorption (Maggiano, 2012). Secondary osteons are visible in cross-section because of their concentric lamellae and surrounding cement line. The resorption phase of remodeling releases mineral stores, aiding mineral homeostasis (Enlow, 1962), but occurs in response to mechanical deformation and the formation of microcracks (Burr, 2002; Burr & Martin, 1993; Burr et al., 1985; Mori & Burr, 1993). Microcracks have been associated with both large mechanical deformations (i.e., high strain) (Bouvier & Hylander, 1996; Mullender & Huiskes, 1995; Rubin & Lanyon, 1985; Turner & Pavalko, 1998) and repetitive (cyclical) loading (Carter & Hayes, 1977a). Thus, high incidences of remodeling are expected to occur in regions of the skeleton where loading conditions are more severe because those regions should accrue greater microdamage. However, it is less clear whether elevated remodeling can be attributed to high strain or cyclical loading when the load case is unknown (e.g., if it is not possible to apply in vivo strain gages or load case cannot be estimated from behavior).

In Old World monkeys, Lad et al., (2016) found that the mandibular symphysis, a region of very high strain, exhibited lower remodeling, whereas the mandibular corpus, which has relatively low strain, had a greater incidence. Furthermore, they found that taxa with higher chewing frequencies tended to have a greater incidence of remodeling in the corpus than species with lower mastication frequencies, which include the sooty mangabey, a monkey that engages in very powerful chewing to open hard seeds. Analysis of the postcranial skeleton in the same monkeys (Lad et al., 2019), however, had less definitive findings. Femora having experienced high frequencies of propulsive leaping had greater incidences of remodeling than species that leap infrequently. Interestingly, humeri of all species had more remodeling than femora despite greater peak loads in the hindlimbs compared to forelimbs. In an attempt to parse the effects of strain magnitude and cyclical loading on remodeling across the skeleton, Lad et al., (2019) found the highest incidence of remodeling in the ribs, which experience cyclical loading but very low strains compared to the limbs. Combined, these findings suggest that high strain may not be necessary for substantial remodeling to occur and that cyclical loading may be more likely to result in elevated remodeling.

The present study tests the hypothesis that cyclical loading engenders remodeling in the absence of high strain using an experimental approach in which the loading conditions can be closely controlled. Although the remodeling response to loading in mammalian jaws is not well-studied, there is a more substantial body of work regarding the modeling response to loading in the masticatory apparatus of diverse mammals (e.g., Bouvier & Hylander, 1981; He & Kiliaridis, 2003; Lieberman et al., 2004; Ravosa et al., 2007; Yamada & Kimmel, 1991). Modeling refers to changes in the amount, size, and shape of bone via the resorption or addition of bone de novo during ontogeny, whereas remodeling is the resorption of existing bone and formation of new bone at the same site. Tough and stiff foods require substantial chewing effort to process in terms of both the number of chews it takes to process a gram of food (chewing investment) and the duration of a chewing bout (chewing duration) (Nett et al., 2021; Ravosa et al., 2015). Chewing investment and duration determine the number of load cycles in the masticatory apparatus. Thus, the jaws undergo cyclical, or repeated, loading in rabbits that chew a great deal.

Rabbits raised on diets that engender cyclical loading have the following osteogenic responses compared to those raised on less mechanically challenging diets: (a) larger palatal cross-sectional area, thicker cortical bone along the oral lamina, and anterior palate in rabbits raised to subadulthood (15 weeks) (Menegaz et al., 2009); (b) increased palatal, symphyseal, and coronal cross-sectional areas in rabbits raised to adulthood (48 weeks) (Scott et al., 2014a); (c) greater cortical thickness in the lateral corpus, lateral symphysis, and hard palate in rabbits raised to adulthood (48 weeks) (Franks et al., 2017); and (d) higher bone volume fraction along the condylar articular surface (Terhune et al., 2020). Combined, these results provide a fairly clear picture of the bony adaptations of the masticatory apparatus to cyclical loading in terms of modeling.

Here, we compare the maxillary remodeling response in rabbits raised to adulthood on a stiff/tough diet (i.e., high chewing investment and duration; elevated cyclical loading) with that of rabbits raised on a diet of
low stiffness and toughness (i.e., low chewing investment and duration; reduced cyclical loading). We also report the modeling response in the lateral alveolus, because those data have yet to be published. Our ultimate aim is to better understand the relationships between mechanical loading and bone adaptation in order to make connections between bone form and behavior in extant animals so that behavioral interpretations in the fossil record may be possible. Another benefit of our analyses is they characterize remodeling in a poorly known part of the skull, thus contributing to our understanding of skeleton-wide patterns of load-induced bone formation.

2 METHODS

2.1 Sample

New Zealand white rabbits were used as the experimental animal model because, unlike rodent models, they exhibit Haversian remodeling and because they share several key characteristics of the masticatory apparatus with many other mammals: (a) tall mandibular ramus; (b) vertically deep facial skeleton; (c) high temporomandibular joint relative to the occlusal plane, capable of rotational and translational movements in two planes; and (d) well-characterized patterns of covariation among dietary properties, jaw muscle activity, and jaw loading regimes (Weijs et al., 1989; Weijs & Dantuma, 1981; Weijs & de Jongh, 1977).

Our feeding experiments employed white rabbits where masticatory behaviors were manipulated postnatally by modulating food type. The sample consisted of 20 males obtained at the onset of weaning (4 weeks of age) from Harlan Laboratories (www.harlan.com) and housed in the University of Notre Dame’s animal care facility, Freimann Life Science Center. The experimental period began at 5 weeks of age and lasted 48 weeks; thus, the rabbits were 53 weeks of age at the end of the experiment. The onset of weaning marks when juvenile rabbits begin to eat solid food and adopt adult chewing behaviors (Herring, 1985; Weijs et al., 1989). Previous work has established that the growing rabbit masticatory apparatus is sensitive to variation in dietary mechanical properties (Menegaz et al., 2009; Ravosa et al., 2007; Ravosa et al., 2010; Ravosa et al., 2008; Scott et al., 2014b). Skeletal maturity occurs around 26 weeks of age (Isaksson et al., 2010; Masoud et al., 1986).

2.2 Dietary manipulation

The 20 rabbit cohort was divided into two treatment groups of 10 each for the duration of the experimental period: “control” and “overuse.” The control rabbits were raised on a diet of 175 g of Purina rabbit pellets per day for the duration of the experiment while the overuse rabbits were fed three hay cubes (~3.2 × 1.9 × 1.9 cm) per day in addition to 175 g of pellets. Hay is both stiffer and tougher than pellets (Ravosa et al., 2007), resulting in greater chewing investment (number of chews per gram of food) by rabbits (Table 1) (Ravosa et al., 2015). Specifically, the greater chewing investment required for hay equates to approximately three times the number of masticatory load cycles and three times the chewing duration needed to process pellets (Ravosa et al., 2015). Thus, adding hay cubes to the overuse group diet causes the rabbits to engage in prolonged cyclical loading, whereas the control group rabbits have significantly fewer load cycles per day. Mastication of hay versus pellets does not, however, alter mean peak strains along the chewing-side mandibular corpus (Table 1) (Weijs & de Jongh, 1977), so the treatment effect studied here is purely that of cyclical loading, whereas the control group rabbits have significantly fewer load cycles per day. Mastication of hay versus pellets does not, however, alter mean peak strains along the chewing-side mandibular corpus (Table 1) (Weijs & de Jongh, 1977), so the treatment effect studied here is purely that of cyclical loading, and not strain magnitude. Furthermore, mean peak strain in the corpus during chewing of these foods is low compared to peak functional strains in mammalian limbs (Rubin & Lanyon, 1984, see table 6 in ref 32) and in vitro mean peak strains in the macaque lingual mandibular symphysis (Hylander, 1984).

<table>
<thead>
<tr>
<th>Food</th>
<th>Mean elastic modulus (E, MPa)a</th>
<th>Mean toughness (R, J/m²)a</th>
<th>Mean chewing investment (chews/g)b</th>
<th>Mean chewing duration (s)b</th>
<th>WS corpus mean peak strain (γ)c</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hay (dry)</td>
<td>3335.6</td>
<td>2759.8</td>
<td>474.9</td>
<td>568</td>
<td>604</td>
</tr>
<tr>
<td>Pellet</td>
<td>29.2</td>
<td>1030.6</td>
<td>161.0</td>
<td>173</td>
<td>590</td>
</tr>
</tbody>
</table>

Note: Hay is both tougher and stiffer than pellets, requiring greater chewing investment and chewing duration than pellets. Peak working-side corpus strain is not different between rabbits chewing hay versus pellets, which eliminates strain magnitude as a compounding factor when assessing the effects of cyclical loading.

aRavosa et al. (2007).
bRavosa et al. (2015).
cWeijs and de Jongh (1977).

*Statistically significant difference between foods.
2.3 | Sectioning and histology

The alveolar portion (P2 to M3) of the left maxilla from all 20 rabbits and the hard palate (Figure 1a; mostly maxilla but also including a small portion of the inferior palatine) from 14 rabbits were removed using a bone saw (Stryker, Kalamazoo, MI). Six of the rabbits no longer had intact hard palates due to destructive sampling for another study. The bone was embedded in epoxy resin (Buehler EpoThin 2) and allowed to cure. Most secondary osteons are visible in cross section (Figure 1b) in the coronal plane in the alveolar portion of the maxilla and in the sagittal plane in the hard palate, so sectioning was performed in these planes, respectively. Thin sections were cut to 100 μm in thickness using a Buehler Isomet low speed saw equipped with a 4 × 0.012 × 0.5 in diamond wafering blade (Illinois Tool Works, Lake Bluff, IL). The thin sections were polished to remove debris using a Buehler Phoenix BETA grinder/polisher and microlcloth polishing cloth, and then stained with Toluidine Blue O solution following the methods of Osborne and Curtis (2005) in order to improve visibility of secondary osteons. The thin sections were then dried under static pressure to prevent warping and mounted to microscope slides using Cytoseal 60 (Thermo Scientific) and covered with a xylene-dipped slipcover.

2.4 | Histological data collection and analysis

Thin sections were photographed at 100x magnification with a QIClick CCD Camera (QImaging, Surrey, BC) mounted onto a brightfield microscope. The photographs from each section were stitched together in PTGui Photo Stitching Software to create a composite image of the entire thin section (Figure 1c). ImageJ image processing and analysis software (Abramoff et al., 2004) was used to take the following measurements from each thin section: osteon population density (OPD), percent Haversian bone (%HAV), and osteon cross-sectional area (On.Ar). OPD is the number of intact and fragmentary secondary osteons per mm² of cortical bone. %HAV is the percentage of total cortical area composed of intact and fragmentary secondary osteons (area contained within cement lines). On.Ar is the area contained within the cement line of an individual intact osteon. Fragmentary osteons are remnants of older secondary osteons that have been partially resorbed. They are identifiable by the presence of a cement line. Mann–Whitney U tests (α = .05), which avoid assumptions of normality, were performed to compare OPD, %HAV, and On.Ar among control and overuse groups in both the hard palate and alveolar portions of the maxilla. Bonferroni correction (α value of .05 divided by six comparisons) was used to reduce
chances of type I error; thus, the \( p \)-value threshold for significance was .0083.

### 2.5 | Micro-computed tomography data collection

All 20 rabbit heads were imaged with micro-computed tomography (microCT) (Bioscan/Mediso X-CT, Budapest, Hungary; settings: 70 kVp, 100 mA, with 71 mm reconstructed isometric voxel size). Alveolar cross-sectional area (CSA) and five cortical thickness measures were taken from coronal microCT slices at depths corresponding to each of the five maxillary cheek teeth (P3 to M3) using ImageJ. CSA area is defined as the total area (mm\(^2\)) of cortical bone forming the left buccal alveolus, excluding the zygomatic arch and lingual alveolus, as measurements of those regions for this sample have been reported elsewhere (Franks et al., 2016; Franks et al., 2017). Cortical thickness was obtained at five sites spanning the superoinferior length of the left lateral alveolus: superior-most, superior-middle, middle, inferior-middle, and inferior-most. All cortical thickness data were size-controlled by dividing by cranial length (a size proxy measured from the posterior-most point on the neurocranium to the anterior-most point between the maxillary central incisors, in the mid-sagittal plane) and CSA data were divided by the square of cranial length, before all data were compared between group means for each site using \( t \) tests. Bonferroni correction (a value of .05 divided by 30 comparisons) requires a \( p \)-value of .0017.

### 3 | RESULTS

Summaries of the histology data and results can be found in Tables 2 and 3. The were no significant differences in OPD or \%HAV in either the hard palate or alveolar portions of the maxilla. Alveolar On.Ar is significantly greater (\( p < .001 \)) in the overuse group compared to the control (Figure 2). Mean On.Ar is 0.024 mm\(^2\) in the overuse group, ranging from 0.005–0.075 mm\(^2\). Mean On. Ar is 0.016 mm\(^2\) and ranging from 0.002 to 0.115 mm\(^2\) in the control group. On.Ar was not significantly different between dietary groups in the hard palate. No cortical thickness or cross-sectional area measurements were statistically significant.

### 4 | DISCUSSION

The hypothesis that cyclical loading engenders high degrees of remodeling is not supported by the data presented here. Rabbits that process an overuse diet that includes tough and stiff hay do not have more secondary osteons or more secondary bone than conspecifics raised on a control diet of only pellets. This is surprising given that previous studies (Lad et al., 2016; Lad et al., 2019) suggest that substantial remodeling is generated by repetitive loading in the absence of high strain.

The overuse group has larger osteons than the control group in the maxillary alveolus. Osteon size is often measured in the context of differing strain modes (e.g., tension vs. compression) (Skedros, 2012; Skedros et al., 2013; Skedros et al., 1994; van Oers et al., 2008). Thus, the reason for the larger osteons in the overuse group is not immediately evident because there is no apparent difference in strain magnitude or mode between the groups. One potential explanation is that while the incidence of microcracks, the instigators of remodeling, is not greater in the overuse group, the size of the microcracks may still be greater. In other words, cyclical loading might not have caused more microdamage in terms of total number, but it may have caused longer or wider cracks. If larger microcracks require a larger resorption area, then larger osteons would theoretically form. Current research aims to quantify microdamage accumulation and crack size in rabbit

| TABLE 2 | Bone histology data summary: mean, range, and SD of osteon population density (OPD), percent Haversian bone (%HAV), and osteon cross-sectional area (On.Ar.) data for control and overuse groups in alveolar and hard palate regions of the maxilla |
|----------|---------------------------------|---------------------------------|---------------------------------|---------------------------------|
|          | OPD Mean | Range | SD | %HAV Mean | Range | SD | On.Ar (mm\(^2\)) Mean | Range | SD |
| Alveolar maxilla | | | | | | | | |
| Control | 0.942 | 0.383–1.836 | 0.4642 | 1.450 | 0.45–2.740 | 0.6471 | 0.016 | 0.002–0.115 | 0.0125 |
| Overuse | 1.131 | 0.482–1.707 | 0.4561 | 2.740 | 1.20–5.950 | 1.3428 | 0.024 | 0.005–0.075 | 0.0122 |
| Hard palate | | | | | | | | |
| Control | 1.477 | 0.231–3.859 | 1.3648 | 2.201 | 0.335–6.188 | 2.1847 | 0.014 | 0.002–0.045 | 0.0075 |
| Overuse | 1.343 | 0.225–2.724 | 0.8857 | 1.927 | 0.595–3.087 | 1.0854 | 0.015 | 0.003–0.060 | 0.0083 |
jaw to test this hypothesis. However, a caveat to be considered here is that On.Ar may be distorted for some osteons, as not all osteons run in the same plane. Bone was sectioned in the plane that displayed the most osteons in transverse cross-section but some visible osteons were slightly out of this plane, thus the On.Ar measurements of those osteons were not orthogonal to the osteon long axis and resulted in enlarged area measurements. Whether this affected the area comparison between groups is unknown.

The question of interest now becomes, Why are there not more osteons in the overuse group than in the control group? There are several possible, often interrelated, explanations for these results that need to be further explored. First, the answer could simply be that repetitive loading does not generate substantial remodeling activity unless strains are above a certain magnitude and that magnitude was not reached in the present experiment. This explanation seems unlikely given past results from wild samples (Lad et al., 2016; Lad et al., 2019) and given the fact that bone cells are capable of responding to low strain loads in regards to uncoupled bone formation and resorption in this model system (Franks et al., 2017; Menegaz et al., 2009; Scott et al., 2014a) and others (Gilsanz et al., 2006; Judex et al., 2007; Rubin et al., 2001; Xie et al., 2006). Second, we have assumed that if deformation occurs then microdamage will accumulate, but this could be faulty. Perhaps microdamage does not occur in the bone examined here, or at least not in the manner assumed, and thus remodeling is not required to repair it. There is some evidence that diffuse damage, microdamage occurring as very small clusters (submicron sized) of cracks as opposed to typical linear microcracks (up to 100 μm in length) does not activate remodeling (Seref-Ferlengez et al., 2014). As noted above, current studies intend to quantify microdamage in rabbit jaws to assess whether the stimulus for remodeling is present.

Third, at least some microdamage forms but the ongoing modeling activity, age, and quality of the bone tissue negate the need for reparative remodeling. Modeling tends to cease or dramatically slow in adulthood (Frost, 1986), while remodeling continues throughout life. The rabbits were raised from weaning to early adulthood, and changes in bony measurements throughout the duration of the experiment (Menegaz et al., 2009; Scott et al., 2014b) evince that modeling occurred throughout the experimental time frame, including in the latter weeks. McFarlin et al., (2008) demonstrated that very few osteons form in newly laid bone, probably in part because fatigue microdamage has not yet occurred, but also because newly formed bone tends to be less mineralized than older bone, which makes it more compliant and able to resist crack propagation (Carter & Hayes, 1976, 1977b; Reilly & Burstein, 1974; Reilly et al., 1974). The microCT results presented here demonstrate that the lateral alveolar portion of the maxilla in the overuse group had greater cortical thickness than the control group at some sites. Although the picture is less clear in this region than in other parts of the rabbit skull, we have reason to believe modeling was still occurring here as well. Additionally, biomineralization is significantly lower in the hard palate compared to other areas of the skull (Franks et al., 2017). Following the same line of thought, the lack of differences in remodeling between overuse and control group palates could be due to the more compliant nature of bone in that region.

### TABLE 3

Results from Mann–Whitney U tests comparing osteon population density (OPD), percent Haversian bone (%HAV), and osteon cross-sectional area (On.Ar) between control and overuse dietary groups

<table>
<thead>
<tr>
<th>Measurement</th>
<th>Alveolar maxilla p-value</th>
<th>Hard palate p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>OPD</td>
<td>.481</td>
<td>1.000</td>
</tr>
<tr>
<td>%HAV</td>
<td>.009</td>
<td>.852</td>
</tr>
<tr>
<td>On.Ar</td>
<td>&lt;.001*</td>
<td>.211</td>
</tr>
</tbody>
</table>

*Note: On.Ar is significantly different between treatments in the alveolar portion of the maxilla.

*Statistically significant (α < .0083).
Furthermore, the possibility that continued modeling precludes the need for remodeling, the age of the rabbits used in this study may also be relevant considering that in humans the complete remodeling cycle can take up to 6 months (Martin et al., 2015a). If the remodeling process is similarly prolonged in rabbits, then the individuals used here may not have been old enough for many remodeling cycles to occur.

Finally, osteogenic responses are known to vary throughout the skull and throughout the skeleton. In some cases, response variation may coincide with differences in loading regimes across skeletal elements, but may also be related to bone quality, and developmental timing. Across the masticatory apparatus, presence of a plastic response varies among the hard palate, condyle, corpus, symphysis (Franks et al., 2017; Scott et al., 2014b), and maxillary alveolus (as demonstrated here). Even when the direction of the response is the same, the magnitude can differ (Scott et al., 2014b). Osteogenic responses to the same stimulus can also occur at different levels of bone architecture (i.e., macrostructural, microarchitectural, or microstructural) (Franks et al., 2016). For example, bone quality differences can be present in the absence of bone quantity differences, and vice versa. Additionally, bony responses can vary by strain mode, causing differences within a single skeletal element, as demonstrated in mammalian limbs (Hsieh et al., 2001; Lieberman et al., 2003; Mason et al., 1995; Skedros et al., 2003). Even here, mean maxillary On.Ar (0.021 mm²) is markedly larger than what is reported in the femur (0.008 mm²) (Martiniaková et al., 2006) (although see caveat about area measurements above) suggesting remodeling differences between the skull and postcrania. Bone material properties can also vary across a single skeletal element, as evidenced in primate mandibles (Daegling et al., 2011; Daegling et al., 2009; Le et al., 2017), potentially affecting bone performance. Thus, the collective osteogenic response to cyclical loading in the rabbit maxilla might not include remodeling despite manifesting bone formation and/or in other levels of bony architecture. Moreover, the absence of remodeling differences in the maxilla does not preclude other aspects of the bony masticatory apparatus from displaying a remodeling response. For example, the load case, especially regarding strain mode, for the rabbit maxilla is not as well-understood as it is for the mandible because the maxilla is involved in other functions of facial structure aside from mastication. Accordingly, the mechanical signal in the maxilla may be more complicated compared to the mandible, for which function is more closely tied to mastication. If osteonal orientation is a result of strain distribution patterns, then the variable orientation of osteons in the maxilla compared to the mandibular corpus or femoral shaft, where osteons are parallel to the long axis of the bone, may support this supposition.

The findings of this study contribute to a larger body of work regarding the bony response to cyclical loading in the rabbit skull. Prolonged periods of cyclical loading do engender greater bone growth in the mandibular symphysis, corpus, and condyle and also in the hard palate (Menegaz et al., 2009; Scott et al., 2014a) but measures of biomineralization do not necessarily follow the same pattern (Franks et al., 2017). The present analyses demonstrate the maxilla does not exhibit a remodeling response and the alveolar process does not have bone formation response to elevated load cycles. Collectively these works demonstrate that the bony response to prolonged cyclical loading is not the uniform throughout the masticatory apparatus and that bone adaptation may occur at different levels of bony architecture throughout the skull.

Overall, the results presented here regarding the rabbit maxilla do not support the hypothesis that cyclical loading incites remodeling in the absence of high strain. However, there are many aspects of bone quality and performance that remain unknown about connective tissues in the mammalian masticatory system. Several ongoing lines of research collectively aim to provide a better understanding of the bony response to cyclical loading across the rabbit skull. Most immediately relevant to the issues at hand is quantification of micromodification across various regions of the rabbit skull to ascertain whether the remodeling stimulus is present, as assumed. Osteon densities and area also need to be measured in other areas of the masticatory system, specifically the mandible, for which loading regimes are far better documented than for the maxilla. Additionally, to fully address the question of whether cyclical loading at low strain magnitudes can cause substantial remodeling, older individuals in which modeling has mostly ceased should also be examined.

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AUTHOR CONTRIBUTIONS

Susan Lad: Conceptualization; formal analysis; investigation; methodology; writing-original draft; writing-review and editing. Rebecca Anderson: Data curation;
methodology. Stephen Cortese: Data curation; formal analysis. Carmen Alvarez: Data curation. Andrew Danson: Data curation. Hannah Morris: Data curation. Matthew Ravosa: Conceptualization; funding acquisition; investigation; writing-original draft; writing-review and editing.

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