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RESEARCH ARTICLE

Prolonged cyclical loading induces Haversian remodeling in mandibles of growing rabbits

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ABSTRACT

Bone adaptation to mechanical loading happens predominantly via modeling and remodeling, but the latter is poorly understood. Haversian remodeling (cortical bone replacement resulting in secondary osteons) is thought to occur in regions of low strain as part of bone maintenance or high strain in response to microdamage. However, analyses of remodeling in primates have revealed an unappreciated association with the number of daily load cycles. We tested this relationship by raising 30 male domestic rabbits (*Oryctolagus cuniculus*) on disparate diets from weaning to adulthood (48 weeks), facilitating a naturalistic perspective on mandibular bone adaptation. A control group consumed only rabbit pellets and an 'overuse' group ate hay in addition to pellets. To process hay, which is tougher and stiffer, rabbits increase chewing investment and duration without increasing bite force (i.e. corpus mean peak strain is similar for the two foods). Corpus remodeling in overuse rabbits was ~1.5 times that of controls, measured as osteon population density and percentage Haversian bone. In the same subjects, there was a significant increase in overuse corpus bone formation (ratio of cortical area to cranial length), consistent with previous reports on the same dietary manipulation and bone formation in rabbits. This is the first evidence that both modeling and remodeling are simultaneously driven by the number of load cycles, independent of strain magnitude. This novel finding provides unique data on the feeding apparatus, challenges traditional thought on Haversian remodeling, and highlights the need for experimental studies of skeletal adaptation that examine mechanical factors beyond strain magnitude.

KEY WORDS: Osteon, Strain, Stress, Microdamage, Mastication, Bone, Mandible

INTRODUCTION

It is well established that bone adapts to its load environment with the overall effect of maintaining the health and structural integrity of the skeleton, *sensu* Wolff (1892). However, bone can adapt in myriad ways as a result of its hierarchical composition. Stresses and strains – the stimuli – can vary in frequency, magnitude and type (i.e. tension, compression, shear), with bony responses across the skeleton not necessarily uniform (Biewener, 1993; Ravosa et al., 2010; Rawlinson et al., 1995; Rubin and Lanyon, 1984). The

feasibility of interpreting load history, and ultimately behavior, from skeletal growth and form requires a precise grasp of how bone responds to mechanical stimuli. Although there is experimental evidence on diet-induced modeling in diverse mammals, few have attempted to address masticatory plasticity and Haversian remodeling (see Bouvier and Hylander, 1981; He and Kiliaridis, 2003; Lad et al., 2021; Lieberman et al., 2004; Ravosa et al., 2016; Yamada and Kimmel, 1991). To this end, we aimed to contribute to understanding this multifactorial process by exploring the relationship between cyclical (prolonged, repeated) loading and Haversian remodeling in mammalian jaws during routine oral processing.

Haversian remodeling (henceforth, 'remodeling') is the replacement of existing cortical bone by new bone via the coordinated activity of osteoclasts (resorption) and osteoblasts (formation), which operate as a basic multicellular unit (BMU) at a single site (Frost, 1969). This differs from bone modeling which refers to bone formation and resorption that result in changes in bone shape and size (Martin et al., 2015). Remodeling also results in secondary osteon formation, whereas modeling does not. Haversian bone is laid down as concentric lamellae that form roughly cylindrical or conical structures (Doube, 2022) encompassing vasculature. Secondary osteons are visible in histological cross-sections and identifiable from the surrounding matrix because of their encircling cement lines (Skedros et al., 2005). The density and area of secondary osteons can be used to quantify how much remodeling has occurred in a bone.

In humans and most mammals, some 'baseline' remodeling occurs more-or-less stochastically, typically where strain is low or where resorption will not compromise mechanical integrity (Burr, 2002; Frost, 1986, 1990b), as it aids in mineral homeostasis (Enlow, 1962). However, remodeling also targets sites of fatigue damage, which accumulates under strain, and serves to repair and strengthen the damaged bone (Bouvier and Hylander, 1996; Burr, 1993, 2002; Burr et al., 1997, 1985; Burr and Martin, 1993; Frost, 1990b; Mori and Burr, 1993; Parfitt, 2002; Verbogt et al., 2000). It has long been thought that remodeling induced by microdamage occurs predominantly in regions of high strain whereas remodeling in low strain environments occurs owing to disuse, as predicted by the Mechanostat (Frost, 1990b). Yet, there is reason to suspect that remodeling might occur outside of these two conditions, i.e. in regions where strain is not particularly high yet other load parameters (e.g. strain frequency, rate, mode) might lead to a sort of 'overuse' condition that causes microdamage. Efforts have been made to correlate secondary osteon distributions with known or presumed load cases in artiodactyls (Skedros et al., 2003; Su et al., 1999), canids (Mori and Burr, 1993), primates (Lad et al., 2016; Lad et al., 2019a,b; Paine and Godfrey, 1997) and rabbits (Lad et al., 2021). In particular, the studies in primates suggest that mechanically mediated remodeling can occur outside of regions of very high strain, and that cyclical loading may drive remodeling

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activity, even when strain is not particularly high. The mandibular symphyses of cercopithecid monkeys experience very high mean peak strains, which presumably incite microdamage, yet there is little to no remodeling in this region (Lad et al., 2016). Macaque ribs have greater secondary osteon densities than do the femora or tibiae despite having lower strains, likely because they endure more daily load cycles (Lad et al., 2019a). Collectively, these findings suggest that (1) there is a close link between remodeling and cyclical loading, (2) high strains do not necessarily incite remodeling, and (3) high strain is not a requirement for remodeling to occur.

The rabbit masticatory apparatus is an ideal model for testing the relationship between remodeling and cyclical loading through experimental methods for several reasons. First, rabbits share anatomical characteristics of the jaws and teeth with many mammals, and they exhibit similar chewing behaviors to other mammals (Weijs et al., 1989; Weijs and Dantuma, 1981; Weijs and de Jongh, 1977). Second, rabbit bone remodels and forms secondary osteons, unlike that of other common animal models such as mice and rats. Third, much is known about relationships between food material properties, chewing behavior and bone formation in rabbits. Rabbits, like many mammals, are known to alter their feeding behavior when consuming mechanically challenging food items. In a key study utilizing the same experimental foods as herein, Ravosa et al. (2015) demonstrated that rabbits increase both chewing investment (i.e. the number of chews required to process a gram of food) and chewing duration (measured in seconds) when eating hay cubes – a stiff and tough food item – compared with rabbit pellets, which are easier to process. Hay has a stiffness of 3335.6 MPa and toughness of 2759.8 J m^{-2} (Ravosa et al., 2007), requiring 474.9 chews (i.e. masticatory cycles) and a chewing duration of 568 s per gram (Ravosa et al., 2015). The stiffness and toughness of pellets are 29.2 MPa and 1030.6 J m^{-2}, respectively, requiring only 161.0 chews and 173 s per gram. Despite the increase in chewing effort, mandibular corpus mean peak compressive strain is not particularly high (∼−300 με) during mastication of either food item (versus typical strains in the limbs, ∼−3000 με), nor does it differ between the two experimental foods (Ravosa et al., 2010; Weijs and de Jongh, 1977). Increased chewing investment and duration (i.e. more chewing) equate to more load cycles, and this 'overloading' of the masticatory apparatus is substantial enough to generate a greater bony response in terms of bone formation in the hard palate as well as the mandibular symphysis, corpus and condyle (Franks et al., 2017; Menegaz et al., 2009; Scott et al., 2014). Rabbits raised on a diet of hay and pellets have greater bone formation in the masticatory apparatus compared with those raised on only pellets because of prolonged repetitive loading.

While the relationship between cyclical loading of rabbit jaws and bone formation is well documented, Haversian remodeling is understudied in relation to cyclical loading. The present study tested the hypothesis that, like bone formation (i.e. modeling), remodeling is also chiefly driven by the number of daily load cycles, rather than high strain, using a feeding experiment designed to modulate feeding behaviors in New Zealand white rabbits (*Oryctolagus cuniculus*). We use microcomputed tomography (microCT) and histological methods to compare both modeling (measured as a ratio of cortical bone area to cranial length) and remodeling (as osteon population density and percentage of Haversian bone) of the mandibular corpus in rabbits raised on disparate diets. Rabbits that ate an 'overuse' diet (i.e. stiff and tough hay) were compared with rabbits raised on a less challenging 'control' diet (i.e. pellets) in an experimental design based on observations of increased chewing

investment and duration in rabbits when eating hay cubes. Because more chews equate to more protracted cyclical loading, the overuse group was predicted to have greater bone formation and more Haversian bone if both bony responses are driven primarily by the number of load cycles. This is the first study, to our knowledge, to use experimental methods to assess both modeling and remodeling responses to overloading in the skull.

The immediate aims of this experiment were to characterize the relationship between cyclical loading and Haversian remodeling in the rabbit mandible and to better understand patterns of load-induced osteogenic responses across the skeleton. The ultimate purpose is to contribute to a broader understanding of this multifarious and hierarchical form–function relationship so that we may refine our ability to use skeletal morphology to make behavioral inferences in paleontological and archeological contexts, while also acknowledging any limitations in morphological inference from skeletal materials.

MATERIALS AND METHODS
Animal model and experimental design
Thirty male New Zealand White rabbits, *Oryctolagus cuniculus* (Linnaeus 1758), were obtained from Harlan Laboratories (www.envigo.com), housed in Freimann Life Science Center, the animal care facility at the University of Notre Dame, and raised in compliance with the Institutional Animal Care and Use (IACUC) guidelines. The rabbits arrived just after the onset of weaning, which marks the transition to eating solid foods and using adult chewing behaviors (Herring, 1985; Weijs et al., 1989).

An experiment was designed (Fig. 1) to induce prolonged cyclical loading in some rabbits but not others. The rabbits were divided into two treatment groups (*n*=15, each): a 'control' group and an 'overuse' group. [Note, the 30 rabbits in this study are a subset of a larger sample of 60 rabbits, which were raised for a related study (not yet published). The 15 rabbits per treatment group used here were randomly selected from the larger treatment group. We reduced the sample size for the present study because it required destructive sampling and preserving some mandibles was a priority for collecting other types of data, not presented here.] Each rabbit, regardless of treatment group was provided with 175 g of rabbit pellets each day for the duration of the experiment. Rabbit pellets provide complete nutrition for captive rabbits. The overuse group's diet was supplemented with 80 g of hay cubes. Any remaining food items were weighed at the end of the day, allowing us to confirm all animals were healthy and to ensure that each rabbit was eating enough to engender the chewing behaviors necessary for the experiment (i.e. grams of food eaten is a rough proxy for number of chews). The experimental duration was 48 weeks, beginning at 5 weeks of age and ending at 53 weeks. Rabbits become skeletally mature around 28 weeks (Masoud et al., 1986); thus, the experimental duration surpassed the entire juvenile growth period.

The material properties of hay and pellets and the consequent rabbit feeding behaviors are discussed in the Introduction and summarized in Fig. 1. Hay is a stiffer and tougher food than pellets, and rabbits substantially increase both chewing investment and chewing duration to process hay compared with an equal serving of pellets. Thus, the overuse group employed more chewing cycles to consume hay versus the control group. Importantly, mean peak strain in the working side mandibular corpus does not differ during the mastication of pellets versus hay (Weijs and de Jongh, 1977). Consequently, despite chewing more to consume hay, the overuse rabbits did not chew more forcefully. It is important to note that mean peak corpus strains in mammals are also low compared with

Treatment groups	Control	Overuse
Diet	Pellets	Pellets + Hay
Food material properties	Less stiff and tough	**Stiffer and tougher**
Elastic modulus (E)[a]	29.2 MPa	**333.6 MPa**
Toughness (R)[b]	1030.6 J m^{-2}	**2759.8 J m^{-2}**
Chewing effects	Chew less	**Chew more**
Chewing investment[c]	161.0 chews g^{-1}	**474.9 chews g^{-1}**
Chewing duration[c]	173 s	**568.0 s**
Working-side corpus mean peak strain[d]	270/−320 με	286/−318 με

Fig. 1. Experimental design. Thirty male New Zealand white rabbits were divided into two treatment groups: control and overuse. The control group was raised on rabbit pellets while the overuse group was raised on pellets with the addition of hay cubes – a tougher and stiffer food. Hay requires more chewing to process yet does not cause higher corpus mean peak strains (tension and compression are shown). Thus, the overuse group had more daily load cycles while strain magnitude was equivalent between groups. [a]Ravosa et al. (2007); [b]Williams et al. (2005); [c]Ravosa et al. (2015); [d]Weijs and de Jongh (1977).

peak functional strains in mammal limbs (Ravosa et al., 2010; Rubin and Lanyon, 1984) and *in vitro* mean peak strains in the macaque lingual symphysis (Hylander, 1984). Therefore, the overuse group had more daily load cycles than the control group, but strain magnitude did not significantly differ and was relatively low compared with what is typical in other regions of the skeleton.

MicroCT scanning
At the end of the experiment the rabbits were humanely euthanized via barbiturate overdose administered by university veterinary staff. The heads were fixed in formalin and stored in ethanol. Each head was scanned using microCT (SCANCO Medical vivaCT 80, Brüttisellen, Switzerland; settings: 70 kVp, 47 µA, 70 µm isotropic voxel size). The reconstructed scans were opened in Amira software (Thermo Fisher Scientific) and placed in proper orientation such that the image stacks would depict sections in the transverse plane.

Histological sectioning
After scanning, the mandibles were dissected out from the heads, washed with Dawn soap and water, and allowed to air dry. A 10 mm section was cut from each right mandibular corpus spanning the second and third molars using an Isomet low speed saw (Buehler) and diamond wafering blade (4×0.012×0.5 inches, or 101.6×0.305×12.7 mm). The sections of bone were again cleaned with Dawn soap and water, dried and then embedded in epoxy resin (Buehler EpoThin 2). One 100 µm transverse thin section was cut from each. The thin sections were polished to remove cut marks using a Buehler Phoenix BETA grinder/polisher and microcloth polishing pad, and then stained with Toluidine Blue O solution following Osborne and Curtis (2005). The stained thin sections were placed under static pressure to prevent warping as they dried and then mounted on microscope slides with Cytoseal 60 (ThermoFisher Scientific), and a slide cover dipped in xylene. The mounted sections were photographed (Fig. 2) at ×100 magnification (×10 occulars, ×10 objective) with a QIClick CCD Camera (QImaging) mounted onto a Nikon Eclipse E600 brightfield microscope (Nikon Instruments Inc.), resulting in approximately 20 overlapping images per thin section. The images were stitched together using PTGui photo stitching software to create a single composite image of each thin section (one per rabbit).

Data collection and analysis
ImageJ image processing and analysis software (Schneider et al., 2012) was used to open each microCT image stack. The cortical area of the left mandibular corpus surrounding the center of the first molar was measured in the transverse plane (Fig. 3). Maximum

cranial length (CL), the distance between the posterior-most point on the skull and the anterior-most point on the premaxilla between the central incisors, was measured in the sagittal plane. Cortical area (Ct.Ar) ratio was calculated as:

$$\frac{\sqrt{\text{Ct.Ar}}}{\text{CL}} \times 100, \tag{1}$$

to assess the modeling response in the corpus. Mann–Whitney U-tests were performed to test for differences in Ct.Ar ratio between groups. Although the primary emphasis of this study is the remodeling response, we used this Ct.Ar ratio as a proxy to indicate whether our experiment generated the desired behavioral differences known to induce a modeling response in rabbits. As cyclical loading is known to generate a greater bony response in terms of bone formation, a significant difference, with higher Ct.Ar ratios in the overuse group, would be consistent with what has been found in similar studies of bone formation in rabbits.

ImageJ was also used to assess two measures of Haversian remodeling from the composite thin section images: osteon population density (OPD) and percentage Haversian bone (%HAV). OPD is defined as the number of secondary osteons (both intact and fragmentary) per mm^2 of cortical bone. %HAV is the percentage of cortical bone area that is osteonal (i.e. contained within an osteon cement line). Secondary osteons were distinguished from primary osteons by the presence of a cement line. Mann–Whitney U-tests were performed for both OPD and %HAV to test for differences between control and overuse groups ($P<0.05$). Ct.Ar ratio, OPD and %HAV data are available in Table S1.

RESULTS
The modeling response, Ct.Ar ratio, was significantly greater ($P<0.005$) in the overuse group than in the control group (Fig. 4). This result is consistent with the findings of previous studies on the modeling responses in rabbits raised on the same diets described herein, indicating that the experiment successfully altered feeding behaviors between the groups. The two measurements of Haversian remodeling, OPD ($P=0.030$) and %HAV ($P<0.005$), were significantly different between the control and overuse rabbits, with higher values found in the overuse group (Figs 2 and 4). Mean OPD was 1.84 in the control group and 2.59 in the overuse group. Mean %HAV was 3.45% in the control group and 5.90% in the overuse group. Secondary osteons were predominantly oriented transversely to the sectioning plane, i.e. running anteroposteriorly within the corpus. Summary statistics for all measured variables can be found in Table 1.

The overuse group ate more food per day, on average, than the control group. Mean daily amount of pellets consumed was
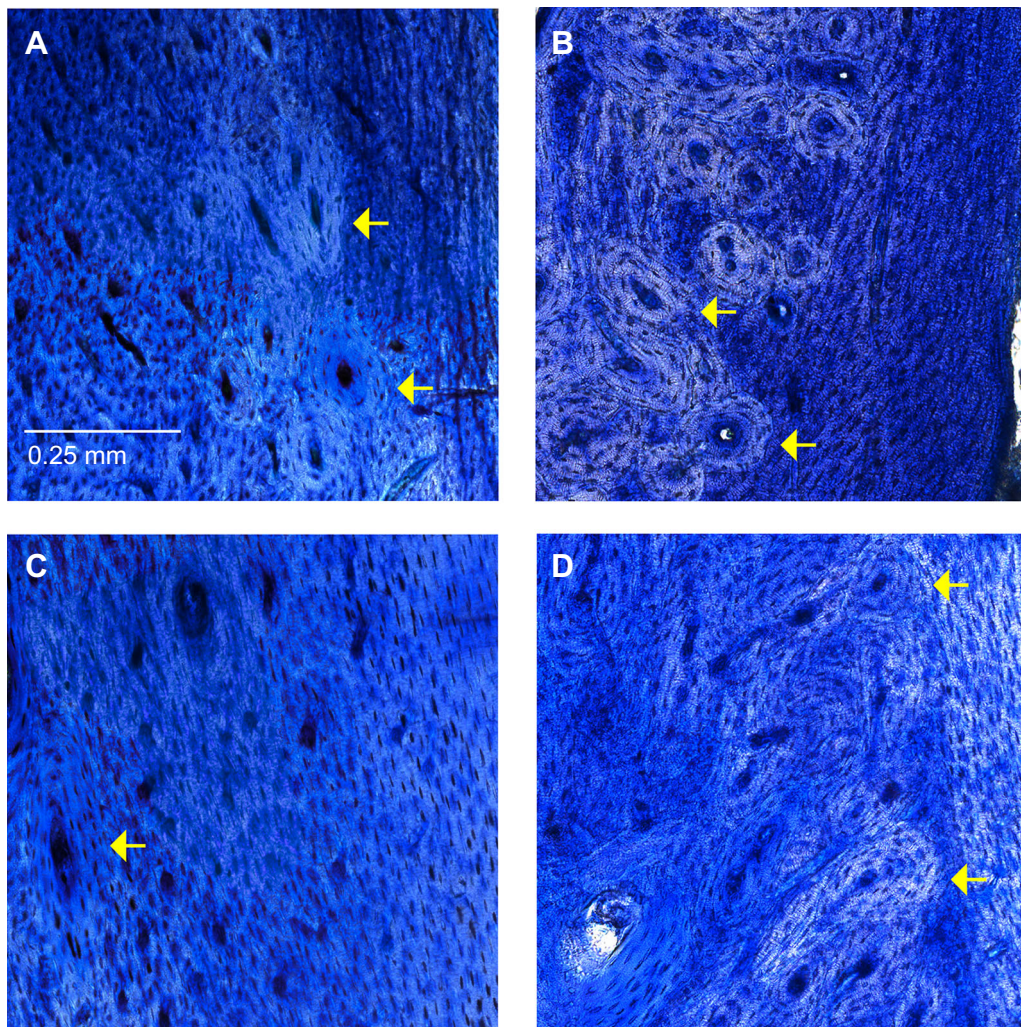
Fig. 2. Histological images of rabbit mandibular corpus show secondary osteons, products of Haversian remodeling. (A,B) Basal and alveolar regions, respectively, from a rabbit raised on the control diet. (C,D) Basal and alveolar regions from a rabbit raised on the overuse diet. Yellow arrows point to some individual secondary osteons, which can be distinguished from the surrounding matrix or from primary osteons by their encompassing cement line, concentric lamellae and central Haversian canal. Although secondary osteons are visible in all images, there was greater osteon population density and percentage Haversian bone in the overuse rabbits.

122.85 g day^{-1} for the control group and 124.65 g day^{-1} for the overuse group. The overuse rabbits also consumed an average of 12.65 g day^{-1} of hay. The consumption of hay cubes plus slightly more pellets per day equates to substantially more mastication cycles in the overuse group, as demonstrated by Ravosa et al. (2015) (Fig. 1).

DISCUSSION
Cyclical loading and Haversian remodeling
The results support the prediction that cyclical loading of rabbit jaws induces elevated remodeling in the mandibular corpus compared with the jaws of rabbits with fewer daily load cycles. This phenomenon occurs presumably because repetitive loading causes microdamage accumulation, which stimulates BMU activity. Importantly, this result occurred in the absence of corpus mean peak strain differences and with relatively low strain in both treatment groups (compared with the postcranial skeleton) (Ravosa

et al., 2010; Weijs and de Jongh, 1977). This novel observation for mammalian jaws conclusively demonstrates that bone remodeling is tied to the number of daily load cycles and that high strain is not requisite for mechanically induced remodeling.

This finding warrants revisiting Frost's (1983, 1987, 1990a,b) Mechanostat hypothesis. The Mechanostat is a model that predicts how strain magnitude modulates bone mass by identifying thresholds of activation for modeling and remodeling. At the time of its proposal, the hypothesis had not been rigorously tested, and even still the predictions about bone remodeling, in particular, are in need of investigation, although some work has been done (e.g. Skedros et al., 2001). Yet, the Mechanostat is widely and often uncritically employed in applied contexts (e.g. anthropological, clinical) as it was originally proposed.

The Mechanostat predicts that remodeling will occur at higher rates when typical peak strain is very low – below a 300 µε threshold – whereas higher strains result in more bone formation and decreased

Table 1. Summary statistics

Group	Cortical area ratio		Osteon population density		Percentage Haversian bone	
	Mean±s.d.	Range	Mean±s.d.	Range	Mean±s.d.	Range
Control	5.47±0.30	5.04–6.06	1.84±0.99	0.30–4.34	3.45±2.23	0.44–8.04
Overuse	5.95±0.38	5.17–6.77	2.59±1.12	0.56–4.77	5.90±2.07	1.96–8.82

Fig. 3. Micro-computed tomography image of the mandibular corpus under the first molar in the transverse plane. The measured cortical area is outlined in red.

remodeling. Remodeling that occurs under that low threshold is termed 'disuse-mode remodeling' (Frost, 2003). The exception is when typical peak strain is very high, above 3000 με, and microdamage

occurs and activates the BMU. Under the Mechanostat, the rabbit mandibular corpus, where mean peak strains are ∼300 με (Weijs and de Jongh, 1977), should experience disuse-mode remodeling and there should be no difference in remodeling activity between treatment groups. The present finding refutes that prediction and demonstrates the importance of recognizing that 'overuse' can take the form of high strain and/or of protracted periods of highly repetitive loading.

It is unclear how much of the total remodeling across the skeleton is targeted to regions of microdamage versus occurring stochastically as a means of balancing mineral content (Burr, 2002). The results presented here provide evidence that remodeling has a significant mechanobiological function (i.e. much of it is targeted). For example, ribs are often used for estimations of age at death because they are presumably freer from the effects of mechanical loading than limb bones (Streeter, 2012), and presumably reveal a systemic baseline rate of remodeling. However, Lad et al. (2019a) found greater OPD in the macaque rib compared with the femur and tibia and concluded this was due to the cyclical loading of the ribs caused by constant expansion and contraction of the ribcage for inhalation and exhalation. The connection between cyclical loading and remodeling found here lends support to that conclusion and suggests that ribs may actually be in an 'overuse' loading environment, despite their non-weight-bearing status.

Modeling and remodeling

The simultaneously greater modeling and Haversian remodeling observed in the overuse group indicates that the detrimental effects of fatigue damage caused by cyclical loading were mitigated in two ways: repair of damaged bone by Haversian remodeling and reduction in corpus strain by modeling (i.e. bone formation). To our knowledge, this is the first experimental demonstration of bony responses to an overloading stimulus in terms of both modeling and remodeling in the skull. Other studies have looked at one or the other osteogenic mechanism, with the majority having focused on modeling. While the relationship between bone formation and cyclical loading has been previously demonstrated, the observation of the two adaptive processes being simultaneously modulated by cyclical loading during development is novel and highlights the need to examine mechanical factors other than strain
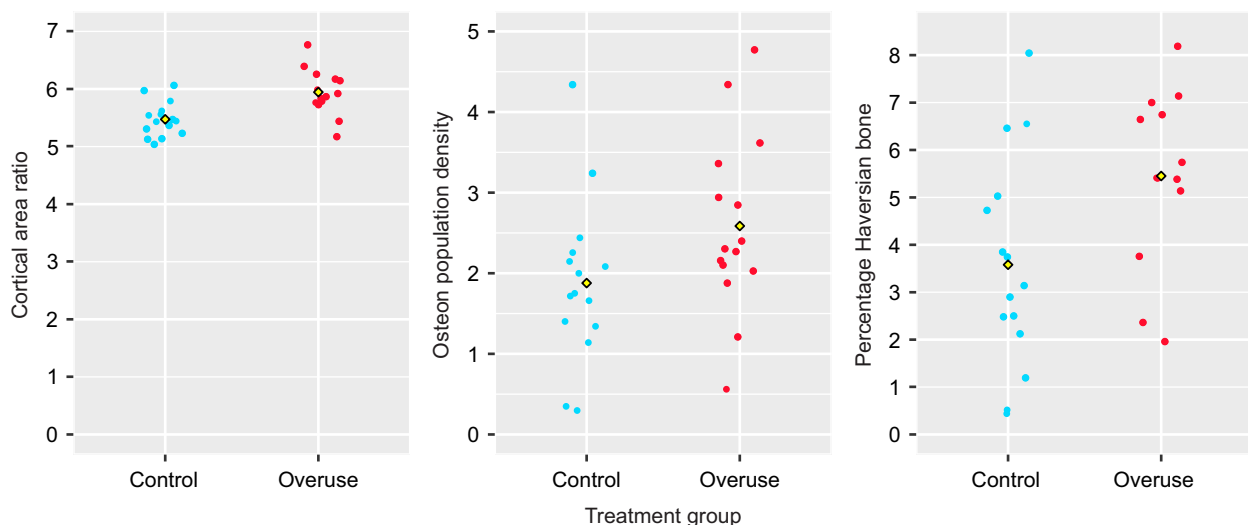

Fig. 4. Modeling and remodeling response to cyclical loading. Jitter plots depict cortical area ratio, osteon population density and percentage Haversian bone, which were greater in the overuse group (n=15) than in the control group (n=15) (Mann–Whitney U-test P<0.005, P=0.030 and P<0.005, respectively), indicating that prolonged cyclical loading results in more Haversian remodeling and more cortical bone formation. The yellow diamonds represent means.

magnitude to understand bone functional morphology. Only long-term *in vivo* studies can provide the means for testing such hypotheses.

Additional considerations

It is important to recognize that not all skeletal elements respond the same way to mechanical stimuli. The material properties of bone tissue may determine how likely it is for repeated strain to cause microdamage (e.g. tougher bone may be more resistant to crack expansion and thus less remodeling occurs) (Martin et al., 2015). Additionally, other load parameters may affect bone turnover, including strain mode (i.e. whether bone is loaded in compression, tension or shear strain) (e.g. Mason et al., 1995; Skedros et al., 1994) and strain rate (i.e. the amount of deformation with respect to time) (O'Connor et al., 1982). Indeed, the negative allometry of both locomotor and chewing rate suggests that determinants of adaptive modeling and remodeling likely vary with body size (Biewener and Taylor, 1986; Druzinsky, 1993; Gerstner and Gerstein, 2008; Heglund and Taylor, 1988; Heglund et al., 1974; Ravosa et al., 2010). Likewise, there is emerging evidence that safety factors, osteogenic potentials and mechanosensitivity differ across the skeleton (Biewener, 1993; Hamrick et al., 2006; Hsieh et al., 2001; Judex et al., 2004; Ravosa et al., 2016, 2010; Rawlinson et al., 1995). The rabbit maxillary alveolus, for example, does not remodel after prolonged cyclical loading (Lad et al., 2021) to the extent that the mandible does, possibly due to it having different material properties or strain distributions and, consequently, less microdamage accrual (Franks et al., 2017). Further complications arise from interrelatedness of some loading parameters (e.g. strain rate and magnitude; Aiello et al., 2015) and difficulty in parsing apart the effects of each.

Implications for interpreting load from skeletal material

We recommend caution in using OPD and %HAV in isolation to infer loading history from skeletal material, as it may be tempting to do in anthropological or paleontological settings. While there is a clear relationship between remodeling and cyclical loading, BMU-activating microdamage can occur from high strain, cyclical loading and could be mediated by other load parameters. An interpretation of 'severe' loading history from high OPD could mean any number of things. The ascription of certain load regimes, and even further extrapolating to behaviors, to animals or people on the basis of secondary osteon densities or distributions is currently dubious. This is of particular concern given the presence of regional variation in mechanobiological factors across the mammalian skeleton (Biewener, 1993; Hamrick et al., 2006; Hsieh et al., 2001; Judex et al., 2004; Ravosa et al., 2016, 2010; Rawlinson et al., 1995). Further investigation of the parameters under which mechanically mediated remodeling occurs may elucidate whether loading histories can be reliably interpreted from bone microstructure. Experimental methods or, at the very least, correlations between bone microstructure and well-documented behaviors of extant animals are necessary.

Conclusion

Haversian remodeling has long been thought to occur in bony regions where strain is either low, as part of bone maintenance processes (baseline remodeling), or very high, as a repair process stimulated by microdamage formation. The novel study presented here is the first experimental assessment of remodeling in relation to cyclical loading in the mammalian skull and provides conclusive evidence that remodeling of mandibular bone occurs in response

to prolonged cyclical loading. The Mechanostat hypothesis, as originally proposed, provides a limited understanding of bone adaptation, as factors other than strain magnitude influence bony morphology. This finding emphasizes the need for long-term multifactorial experimental studies of bone adaptation across the skeleton, rather than relying purely on comparative methods or *in silica* models, which are limited in the types of load parameters that can be incorporated therein.

Data availability
All relevant data can be found within the article and its supplementary information.

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