J Musculoskelet Neuronal Interact 2010; 10(1):46-55

Original Article

Hylonome

Masticatory biomechanics and masseter fiber-type plasticity

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Abstract

Compared to force-resisting elements of the mammalian feeding apparatus, data on jaw-muscle plasticity are less common. This hinders our understanding of the role of force-producing structures in craniofacial development and integration. Thus, we investigated fiber-type abundance and cross-sectional area in the masseter muscle of growing rabbits subjected to diet-induced variation in masticatory stresses. Three loading cohorts were obtained as weanlings and raised until adult on different diets. Immediately following euthanasia, left-sided masseters were dissected away, weighed, and then divided into anterior, intermediate and posterior sections for fiber-type immunohistochemistry. These data were compared to mandibular proportions and biomineralization from the same subjects. Results indicate that growing mammals fed a tougher, fracture-resistant diet develop: absolutely and relatively lower numbers of Type I jaw-muscle fibers; absolutely larger fiber cross-sectional areas; and relative increases in the amount of Type II fibers. These analyses indicate that an early postweaning dietary shift can induce significant variation in muscle fiber types. Such norms of reaction are comparable to those observed in bony elements. Functionally, the processing of fracture-resistant foods results in jaw adductors potentially characterized by faster contraction times and higher force production capabilities, which may influence the frequency and amplitude of forces experienced by oral tissues.

Keywords: Masseter Muscle, Fiber Types, Fiber Cross-Sectional Area, Dietary Properties, Bone, MicroCT, Adaptive Plasticity, Weaning, Postnatal Development, Rabbits, Mastication

Introduction

Weaning in mammals is characterized by pronounced transitions in feeding behaviors, neuromotor activity and loading patterns of the mammalian feeding apparatus[1-7]. Indeed, early post-weaning ontogeny is when particularly marked plasticity responses can be induced[5-7]. With respect to the topic of adaptive plasticity of craniofacial connective tissues, considerable insights have been gleaned from the analysis of alert organisms subjected to diet-induced variation in masticatory stresses[8-11]. For instance, post-weaning responses of the feeding complex following increased loading results in elevated cortical bone remodeling, greater mandibular dimensions and cortical bone thickness, a higher density of temporomandibular joint (TMJ) connective tissue and subchondral bone, thicker TMJ cartilage, and larger craniofacial proportions[5-10,12-18].

This naturalistic modeling of masticatory plasticity mirrors comparative experimental analyses of mammalian feeding behaviors, where it has been shown that stresses due to jaw-adductor muscle, bite and reaction forces are elevated during the biting and chewing of resistant and/or stiff items[19-29]. For instance, mammals that routinely process such diets tend to exhibit relatively larger bony elements related to greater load resistance as well as relatively longer jaw-adductor in-levers and/or relatively shorter bite point out-levers for the more effective production of muscle forces (see reviews[4,30-33]). In many such taxa, some of the adaptive tissue responses noted above appear designed to maintain a sufficient safety factor for a given skeletal element or joint system vis-à-vis routine masticatory loads[2,11,34-37]. Although not documented as extensively, attachment sites for the jaw-adductor muscles appear to exhibit postnatal plasticity in response to muscle hyperplasia[38-40], a finding that remains to be linked more directly to altered loading.

This brief overview of experimental analyses of the mammalian feeding apparatus also serves to highlight the differen-

The authors have no conflict of interest.

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Edited by: M. Hamrick
Accepted 23 January 2010

Food Items (Sample Size)	**Young's Modulus: E, MPa** (Mean, Range)	**Toughness: R, Jm^{-2}** (Mean, Range)	**Hardness: H, MPa** (Mean, Range)
Pellets (N=10)	29.2 (17.0-41.0)	–	11.8 (6.3-19.9)
Wet hay (N=15)	277.8 (124.9-451.0)	1759.2 (643.6-3251.9)	–
Dry hay (N=15)	3335.6 (1476.8-6711.4)	2759.8 (434.0-6625.5)	–

Table 1. Dietary mechanical properties of rabbit experimental foods measured with a portable food tester. Ground pellets require minimal oral preparation, which reduces the amount of cyclical loading during unilateral mastication (Under-Use Diet). The Control diet consisted of intact pellets. Hay requires greater forces to process which, in addition to the greater processing required for intact pellets, results in increased peak loads as well as increased cyclical loading during molar biting and chewing (Over-Use Diet). The mechanical properties of wet hay model the exposure of hay to saliva. Note that 'N' refers to the number of food items tested.

tially 'osteocentric' nature of research on masticatory plasticity. Although the responses of load-resisting elements to elevated and/or altered stresses are well documented, concomitant changes in the force-generating masticatory components underlying such bony and cartilaginous responses are less completely understood. One particular example is the plasticity of jaw-adductor muscles vis-à-vis long-term alterations in dietary properties. Arguably, the absence of such basic data hinders attempts to develop an integrative perspective on the ontogeny and mechanobiology of the mammalian masticatory apparatus, information of great importance for ecomorphological analyses of evolution in the wild as well as translational analyses seeking to mimic biological activities and associated tissues responses[41-45].

Due to its central and multifarious role in the mammalian masticatory system, the development and function of the masseter muscle has drawn considerable attention. This has ranged from work on ontogenetic increases in its internal compartmentalization and differentiation to research on the postnatal ontogeny of stereotypical adult jaw-adductor activity patterns[1,24,25,46-49]. Earlier studies have demonstrated that the architecture[50-52] and fiber-type composition[52-58] of mammalian jaw-adductor muscles varies postnatally. Likewise, long-term dietary modification has been shown to influence masseter physiologic cross-sectional area[59]. There is additional evidence that the organizational units of neuromotor control of mastication are necessarily different than those for locomotion, allowing for finer control of muscle force and associated mandibular movements[60]. Similar comparisons have been performed with regard to the fiber-type composition of jaw versus limb muscles[61].

In terms of jaw-adductor muscle plasticity, it is possible that variation in diet might act upon underlying ontogenetic patterns of change in the masticatory complex[62]. Thus, given documented postnatal increases in the relative abundance and cross-sectional area of Type II fibers of the masseter and temporalis muscles[52-58], one might find alterations in Type II fibers corresponding to shifts in masticatory function during development. However, one of the few studies of masticatory plasticity observed that increased masseter duty time, which tracks daily jaw-muscle activity, appears correlated with larger and greater numbers of Type I fibers[63]. Unfortunately, it is unclear how such an analysis would facilitate the integration of corresponding data on norms of reaction for connective tissues of the feeding complex. Moreover, apart from a recent research on masseter architecture in growing rabbits[59], a long-term investigation of the adaptive plasticity of the masseter muscle in growing mammals is largely lacking[61,64].

To address this gap in the mechanobiology of the developing mammalian masticatory apparatus, the current study investigated the biochemistry and cross-sectional proportions of masseter muscle fiber types in growing rabbits subjected to long-term diet-induced variation in masticatory stresses. As compared to Type I fibers, Type II fibers fatigue more readily but have faster contraction times and can produce larger tensile forces[53,65]. Rabbits exhibit a feeding complex and loading responses broadly similar to primates and other omnivorous mammals[5-7,17,18,23], which facilitates the extrapolation of findings from this study to questions of an evolutionary and clinical nature. With this in mind, the primary objective of this analysis is to evaluate the effects of dietary mechanical properties on the growth and plasticity of the masseter muscle in terms of early postweaning shifts and long-term postnatal variation in jaw-muscle anatomy. To develop an integrative perspective for understanding patterns of masticatory plasticity in musculoskeletal elements, we also evaluated reaction norms in mandibular tissues from the same experimental subjects.

Materials and methods

Sample

New Zealand domestic white rabbits (*Oryctolagus cuniculus*) were obtained as weanlings (4 weeks old) and raised for 26 weeks until attaining young adult status at 30 weeks old[47,66,67]. In accord with an ACUC-approved protocol, three dietary cohorts of 10 male rabbits each were established to induce postweaning variation in jaw-adductor muscle forces and masticatory stresses[5,17,18,68]. For this study, we employed four rabbits from each of the three dietary cohorts for the analysis of jaw-muscle fiber types and three subjects for the mandibular analyses. All individuals were male so as to eliminate the confounding influence in rabbits of sexual variation in jaw-adduc-

tor fiber types[69-71]. Weaning was selected as the starting point for dietary manipulation because (1) plasticity decreases with age[7,15,65,72-74], (2) this approximates shifts in masticatory function in rabbits and other mammals to an adult-like pattern[1-7,49] and (3) we sought to minimize the confounding influence of diets other than those used in our protocol.

Loading cohorts were separated into: an 'under-use' cohort fed only powdered pellets, a 'control' cohort fed intact Harlan TekLad rabbit pellets, and an 'over-use' cohort fed whole rabbit pellets supplemented daily with two 2.5-cm cube hay blocks. A summary of the dietary mechanical properties of the experimental foods is in Table 1. In rabbits and other mammals, fracture-resistant foods such as pellets and especially hay require absolutely larger jaw-adductor muscle activity (i.e., greater amplitude) and perhaps greater cyclical loading during food processing, which in turn increases masticatory peak stresses and corresponding plasticity responses[5-8,17,18,21,23-29]. The inclusion of similar daily amounts of pellets in the diet of all experimental subjects provided adequate nutrition for normal postweaning growth.

Using a portable food tester, the material properties of whole rabbit pellets and hay were assessed (Table 1: samples, means, ranges) and were routinely monitored to assure consistency[5]. The elastic, or Young's, modulus (**E**) represents the stress/strain ratio at small deformations, characterizing the stiffness or resistance to elastic deformation. Toughness (**R**) is an energetic property describing the work performed propagating a crack through an item. Hardness (**H**) is employed to quantify indentation. While the properties of crushed pellets differ little from intact pellets, the latter entail greater repetitive loading due to a longer processing time. Therefore, the sequence from crushed pellets to whole pellets (only) to pellets with hay tracks diets with longer preparation time and progressively greater elastic moduli, hardness and toughness (well known to result in increasingly elevated masticatory stresses[19-29]).

Tissue harvest

At the end of the period of dietary manipulation, all rabbits were euthanized by IV pentobarbital overdose with death assured via cervical dislocation. Immediately thereafter, superficial masseter muscles from the left side were dissected away and weighed. After the muscle was detached, it was carefully split into anterior (AM), intermediate (IM) and posterior (PM) regions parallel to fiber orientation. These regions, and subsequent histological sections, correspond to more inferior superficial masseter compartments examined previously by electrical stimulation[75] and immunostaining[52,76]. Each section was embedded in OCT tissue-freezing medium, frozen slowly in chilled 2-methylbuterol, quick-frozen in liquid nitrogen, and then stored in a -80ºC freezer until immunohistochemical analysis.

Immunohistochemistry

For examination, the embedded masseter muscles frozen in OCT were thawed to -22ºC, oriented vertically and sectioned roughly orthogonal to central tendon orientation in a microtome at 10 µm. Sections were collected onto charged slides. All masseter muscles were fixed in a mixture of methanol : acetone : acetic acid : water (35:35:5:25) overnight. The sections were treated with hydrogen peroxide (3% v/v in PBS) for 30 minutes to reduce endogenous peroxidase activity, followed by pre-incubation in Teng-T buffer (10mM Tris, 5mM EDTA, 150mM NaCl, 0.25% gelatin, 0.05% Tween-20, pH 8.0) for 30 minutes to reduce non-specific binding. To identify Type II muscle fibers, the pre-treated sections were incubated with primary antibody: myosin heavy chain fast (Vector) at a dilution of 1:20 and incubated overnight at 4ºC. On the second day, following a PBS wash 3 times, the sections were incubated with Biotinylated Universal Antibody (Vector) for 30 minutes. Sections were then reacted with VECTASTAIN *elite* ABC reagent (Vector) for 30 minutes. The color reaction was developed in DAB with chromogen (Biogenex) solution for 2-10 minutes until the appropriate darkness of Type II fibers was achieved. Sections were rinsed with distilled water, tap water, and distilled water, and run through alcohols to xylene. Finally, the sections were mounted with Permount.

It is important to note that the above protocol does not directly stain for Type I fibers. Rather, fibers not staining positively as Type II fibers were designated as representing Type I fibers. Therefore, Type I and Type IM fibers are classified as similar although the latter muscle fibers exhibit some functional characteristics intermediate between Type I and Type II fibers[76]. However, given the small percentage of Type IM fibers present in the rabbit masseter muscle, particularly in the more inferior compartments[76] sampled herein, this is unlikely to have posed a significant problem for this investigation.

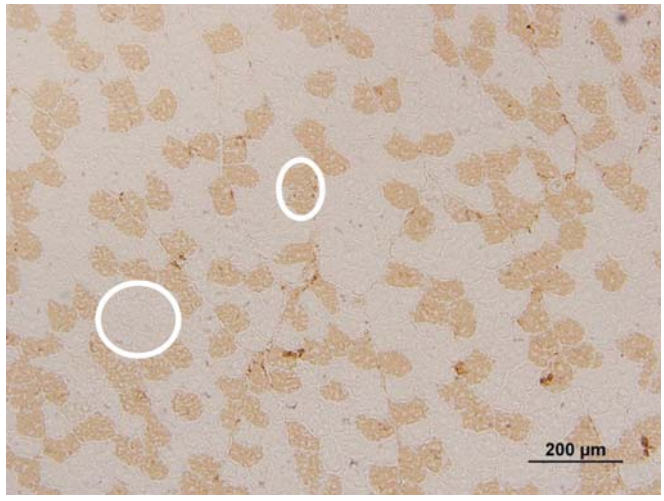

Figure 1. Immunohistochemical staining of Type I (large oval, unstained) and Type II (small oval, brown stain) fibers in the intermediate region of an adult rabbit masseter muscle. Type I fibers have slower contraction times, are more fatigue resistant and generate lower forces. In contrast, Type II fibers exhibit faster contraction times, fatigue more quickly and facilitate the production of larger jaw-adductor muscle forces.

Masseter Region	Under-Use Mean (N, SD)	Control Mean (N, SD)	Over-Use Mean (N, SD)	OU vs. UU % DIFF	OU vs. C % DIFF	C vs. UU % DIFF
AM type I fiber count***	111.82 (44, 75.20)	76.94 (32, 20.91)	62.75 (48, 31.50)	-43.9	-18.4	-31.2
AM type II fiber count**	270.59 (44, 28.46)	266.38 (32, 54.77)	249.46 (48, 42.81)	-7.8	-6.3	-1.6
AM total fiber count***	382.41 (44, 77.60)	343.31 (32, 64.02)	312.21 (48, 63.51)	-18.4	-9.1	-10.2
AM type I fiber abundance***	0.27 (44, 0.11)	0.23 (32, 0.05)	0.19 (48, 0.07)	-29.6	-17.4	-14.8
AM type II fiber abundance***	0.73 (44, 0.11)	0.77 (32, 0.05)	0.81 (48, 0.07)	11.0	5.2	5.5
IM type I fiber count***	88.57 (150, 74.22)	44.63 (131, 18.64)	33.67 (188, 14.05)	-62.0	-24.6	-49.6
IM type II fiber count***	199.15 (150, 55.24)	230.92 (131, 47.71)	199.47 (188, 41.06)	0.2	-13.6	16.0
IM total fiber count***	287.72 (150, 79.18)	275.55 (131, 58.49)	233.13 (188, 48.55)	-19.0	-15.4	-4.2
IM type I fiber abundance***	0.28 (150, 0.18)	0.16 (131, 0.05)	0.14 (188, 0.05)	-50.0	-12.5	-42.9
IM type II fiber abundance***	0.72 (150, 0.18)	0.84 (131, 0.05)	0.86 (188, 0.05)	19.4	2.4	16.7
PM type I fiber count***	55.94 (47, 32.11)	27.02 (44, 13.32)	18.88 (48, 16.41)	-66.2	-30.1	-51.7
PM type II fiber count***	222.43 (47, 43.24)	192.64 (44, 27.43)	183.58 (48, 48.36)	-17.5	-4.7	-13.4
PM total fiber count***	278.36 (47, 44.24)	219.66 (44, 26.88)	202.46 (48, 59.75)	-27.3	-7.8	-21.1
PM type I fiber abundance***	0.20 (47, 0.11)	0.12 (44, 0.06)	0.09 (48, 0.06)	-55.0	-25.0	-40.0
PM type II fiber abundance***	0.80 (47, 0.11)	0.88 (44, 0.06)	0.91 (48, 0.06)	13.8	3.4	10.0
Masseter Mass*	11.39 (10, 1.19)	12.45 (10, 1.25)	13.01 (10, 0.93)	14.2	4.5	9.3

Table 2. Basic statistics (mean, SD) and ANOVAs of superficial masseter fiber-type counts and relative abundance in all three muscle regions (AM, IM, PM) as well as muscle mass (g) for the dietary/loading cohorts ('N' refers to number of histological sections [fiber types] or subjects [masseter mass]; Kruskal-Wallis: ***=$p<0.001$, **= $p<0.01$, *=$p\leq0.05$). The three columns on the right present the % mean difference based on pairwise group comparisons. Consistency in the plasticity response across all three dietary groups suggested by a significant ANOVA will be reflected by the similarity in the direction and magnitude of the % value ('+' or '-') for a variable.

Muscle analyses

The anterior, intermediate and posterior regions were analyzed separately. For each treatment group, a total of four individuals were evaluated. For each subject, a minimum of 20 sections was examined for fiber cell counts in a standardized field area (10x) in each of the three regions. At least four sections per subject were examined for computer-based determination of Type I and Type II fiber areas based on digital images of all cross-sections obtained at a fixed magnification setting (10x). Image J software (NIH) was used to derive the quantity of Type I versus Type II muscle fibers and muscle fiber type cross-sectional area. Muscle fiber-type counts and cross-sectional areas (Figure 1) were performed using a WACOM tablet and associated tracing stylus (WACOM Co., Ltd.). Fiber counts entailed a consideration of both absolute and relative amounts (i.e., % of one fiber type count vs. combined Type I and Type II fiber count).

Morphometry and microCT analysis of skeletal biomineralization

To facilitate comparisons between norms of reaction between force-generating (masseter muscle) and load-resisting structures in the same animal model, a series of mandibular measures were collected. After euthanasia, external measures of the TMJ condyle, symphysis and corpus as well as a masseter attachment site (ramus

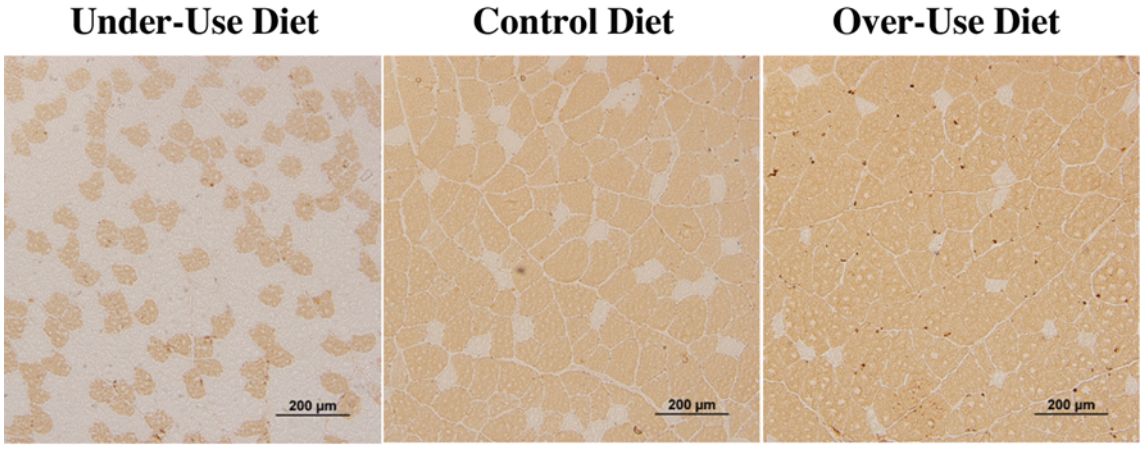

Figure 2. Plasticity in fiber-type biochemistry and cross-sectional area for the intermediate region of the masseter muscle in adult rabbits. In comparing under-use to control to over-use loading cohorts, note the trade-off in the abundance and proportions of Type I (unstained) versus Type II (brown) muscle fibers. Versus under-use rabbits, over-use rabbits exhibit less, smaller Type I fibers and more, larger Type II fibers. This increase in Type II fibers appears related to a masticatory apparatus that has the potential to generate more rapid, elevated forces during the biting and chewing of a more fracture-resistant diet.

height) were obtained using digital calipers (0.1 mm)[2,4-7,30-33]. Subsequently, microCT was performed on fixed jaw joints to assess variation in tissue mineral density of the articular surface, subchondral bone and cortical bone as a function of masticatory stress, with increased biomineralization tracking greater resistance to compressive loads[5-7,40]. Using a Scanco Medical MicroCT 40 (PA, USA), the microfocus x-ray tube was operated at 70 kV and 57 µA with an effective energy of 30 keV, and the beam passed through a 0.13 mm thick beryllium window on the x-ray tube and through a 0.50 mm thick aluminum filter before encountering a sample. With this beam system, data were collected with the longest integration time (0.30 seconds per view) and the highest sensitivity mode (1000 projections over 180°, 2048 samples per projection). Reconstruction was with 8µm voxels (volume elements). The linear attenuation coefficient (µ) was measured in reconstructed slices parallel to the coronal plane: five equidistant sites per symphysis (labial, anterior, middle, posterior, lingual) and three equidistant sites per TMJ (anterior, middle, posterior)[5-7,40]. At each joint site, five contiguous slices covering 0.31 mm were imaged, with one slice chosen to represent a site. Values of 'µ' were pooled for each jaw joint and used to characterize among-group variation in tissue mineral density for regions of the TMJ and symphysis[5-7,40]. Thus, the TMJ was divided into three regions (joint surface - five points; subchondral - four points; condylar neck - six points), whereas the symphysis was considered as two regions (joint surface - five points; cortex - four points)[5-7,40].

Statistical analyses

Non-parametric ANOVA was used to investigate variation in masseter muscle fiber-type abundance, cross-sectional area, and mass among treatment cohorts (Kruskal-Wallis, $p \leq 0.05$). The application of parametric tests provided similar results, so only the former are presented.

Results

Masseter analyses

Analyses of freshly dissected, intact whole muscles indicate a significant size gradient for the masseter with over-use diet rabbits exhibiting the largest muscles, control diet rabbits having intermediate-sized masseters, and under-use diet rabbits possessing the smallest muscles (Table 2). Subsequent analyses focused on diet-related variation in fiber-type cross-sectional area and abundance in the anterior, intermediate and posterior regions of the rabbit superficial masseter.

In all three masseter regions, there were absolutely and relatively lower numbers of Type I fibers, relative increases in the number of Type II fibers, and lower numbers of total fibers in over-use diet rabbits (Table 2, Figure 2). Under-use and over-use rabbits are typically at opposite ends of the spectrum, with mean values for control-diet rabbits in between those for the other two treatment groups. Diet-induced variation in the number of Type II fibers characterized the anterior and posterior regions; in the intermediate region of the masseter, the primary difference is between under-use rabbits vs. control and over-use rabbits. In the intermediate and posterior masseter regions, there were increases in the cross-sectional area of Type II fibers coupled with decreases in the area of Type I fibers in over-use rabbits (Table 3, Figure 2). In the anterior masseter region, the main difference is between the control group and the other groups.

Qualitative comparisons across muscle regions provide further information regarding intramuscular and among-cohort variation in fiber-type abundance and cross-sectional area. In each of the three treatment cohorts, there is an anteroposterior decrease in the number of Type I fibers and total fiber counts (Table 2). Similar anteroposterior decreases in Type II fiber

Masseter Region	Under-Use Mean (N, SD)	Control Mean (N, SD)	Over-Use Mean (N, SD)	OU vs. UU % DIFF	OU vs. C % DIFF	C vs. UU % DIFF
AM type I fiber Average Area***	1902.40 (44, 823.98)	1355.31 (32, 295.70)	1801.16 (48, 569.98)	-5.3	32.9	-28.8
AM type II fiber Average Area*	4682.51 (44, 1188.95)	4310.90 (32, 766.23)	4635.69 (48, 1728.63)	-0.1	7.5	-7.9
IM type I fiber Average Area*	2624.78 (18, 948.38)	2135.73 (19, 467.70)	1948.09 (19, 380.71)	-25.8	-8.8	-18.6
IM type II fiber Average Area*	5473.19 (18, 915.12)	6020.20 (19, 945.99)	6112.31 (19, 938.51)	11.7	1.5	10.0
PM type I fiber Average Area***	1971.18 (47, 494.89)	1704.79 (44, 499.624)	2471.07 (48, 961.34)	25.4	45.0	-13.5
PM type II fiber Average Area***	4882.42 (47, 967.64)	6394.34 (44, 1283.34)	8379.09 (48, 2216.72)	71.6	31.0	31.0

Table 3. Basic statistics (mean, SD) and ANOVAs of superficial masseter fiber-type cross-sectional areas (μm^2) in all three muscle regions (AM, IM, PM) for the dietary/loading cohorts ('N' refers to the number of histological sections; Kruskal-Wallis: ***=$p<0.001$, **=$p<0.01$, *=$p\leq0.05$). The three columns on the right present the % mean difference based on pairwise group comparisons. Consistency in the plasticity response across all three dietary groups suggested by a significant ANOVA will be reflected by the similarity in the direction and magnitude of the % value ('+' or '-') for a given variable.

counts occur in the control and over-use cohorts (Table 2). In control and over-use diet rabbits, there is an anteroposterior increase in mean cross-sectional area of Type II fibers (Table 3). In the under-use cohort, Type I and Type II mean fiber cross-sections were largest in the intermediate region and roughly equivalent between the anterior and posterior regions (Table 3). In the control group, Type I mean fiber cross-sectional area was largest in the intermediate region, followed by the posterior and then anterior regions. In over-use rabbits, Type I mean fiber size exhibited a posteroanterior gradient with the largest cross-sections in the posterior region.

Mandibular analyses

External measures of the mandibular corpus, symphysis and ramus indicate a significant size gradient with over-use diet rabbits possessing the largest bony structures, control diet rabbits having intermediate-sized elements, and under-use diet rabbits exhibiting the lowest mean values (Table 4). TMJ AP condyle length also shows such a pattern but it is non-significant. TMJ ML condyle breadth is more variable and there is no significant diet-related variation across cohorts.

MicroCT analyses of the articular surface, subarticular bone and cortical bone along the TMJ condyle as well as articular and cortical bone along the symphysis indicate that significant variation develops in tissue mineral density across dietary groups. In particular, over-use diet rabbits exhibit the highest levels of jaw-joint biomineralization, followed next by control diet subjects and then lastly by under-use diet individuals (Table 4). Considerably more broadly, the magnitude of the plasticity response among dietary groups is similar for the masseter and the mandible although there is considerable variability across specific parameters (Tables 2-4).

Discussion and conclusions

Research on muscle plasticity has direct implications for understanding the nature of adaptive or compensatory responses in other tissues, structures and ultimately organisms[77,78]. By identifying the physiological changes inherent to the plasticity response, one can also gain insight into the potential genetic bases of phenotypic variation in jaw muscles[79,80]. Despite the considerable importance of information on mammalian jaw-adductor plasticity for understanding determinants of craniofacial integration, such studies had lagged behind similar research on limb muscles[61]. To complement recent work that has largely rectified this discrepancy[57,63,79,80], our experimental analyses focused on the influence of long-term variation in dietary properties on the developmental plasticity of masseter fiber-type composition and cross-sectional area. While by no means an exhaustive study of all masseter compartments and fiber-type isoforms, our analyses of long-term masticatory plasticity initiated herein at weaning and followed until adulthood provide a more naturalistic perspective on norms of reaction for jaw-adductor muscles that can be considered vis-à-vis patterns of plasticity in masticatory connective tissues.

As diet-induced plasticity influences the development of postnatal form via the modulation of relative growth patterns[62], it is noteworthy that previous analyses of monkeys, mice, dogs and rabbits have documented postnatal increases in Type II fibers of the masseter and temporalis muscles[53-58]. In rabbits, the masseter also exhibits postnatal differential increases in the cross-sectional area of Type II fibers[52]. This suggests that as growing mammals ingest increasingly adult-like diets, they may benefit from the elevated force-generating capabilities conferred by developing greater numbers of relatively larger

Mandibular Variables	Under-Use Mean (N, SD)	Control Mean (N, SD)	Over-Use Mean (N, SD)	OU vs. UU % DIFF	OU vs. C % DIFF	C vs. UU % DIFF
TMJ Joint Surface***	1.743 (9, 0.067)	1.873 (9, 0.159)	2.138 (9, 0.091)	22.7	14.2	7.5
TMJ Subchondral***	1.954 (9, 0.102)	2.122 (9, 0.135)	2.381 (9, 0.136)	21.9	12.2	8.6
TMJ Neck***	1.901 (9, 0.096)	1.947 (9, 0.106)	2.181 (9, 0.119)	14.7	12.0	2.4
TMJ AP Length^{NS}	11.800 (3, 0.669)	12.037 (3, 0.488)	12.473 (3, 0.587)	5.7	3.6	2.0
TMJ ML Breadth^{NS}	5.080 (3, 0.106)	5.517 (3, 0.987)	5.200 (3, 0.241)	2.4	-5.7	8.6
Symphysis Surface***	1.851 (15, 0.058)	1.927 (15, 0.126)	2.073 (15, 0.074)	12.0	7.6	4.1
Symphysis Cortex***	2.220 (10, 0.073)	2.418 (10, 0.141)	2.640 (11, 0.142)	18.9	9.2	8.9
Symphysis Length*	22.283 (3, 1.128)	24.857 (3, 2.819)	27.073 (3, 2.382)	21.5	8.9	11.6
Symphysis Breadth*	5.547 (3, 0.450)	6.783 (3, 0.596)	8.060 (3, 0.098)	45.3	18.8	22.3
Corpus Height*	15.720 (3, 1.038)	16.763 (3, 0.787)	18.500 (3, 1.438)	17.7	10.4	6.6
Corpus Width*	5.440 (3, 0.090)	7.283 (3, 1.994)	8.290 (3, 1.235)	52.4	13.8	33.9
Ramus Height*	46.380 (3, 2.927)	49.807 (3, 1.776)	53.297 (3, 3.150)	14.9	7.0	7.4

Table 4. Basic statistics (mean, SD) and ANOVAs of mandibular external proportions (mm) and biomineralization values (μ) for the dietary/loading cohorts ('N' refers to the number of microCT-based sections or jaw specimens; Kruskal-Wallis: ***=$p<0.001$, **= $p<0.01$, *=$p\leq0.05$, ^{NS}=$p>0.05$). The three columns on the right present the % mean difference based on pairwise group comparisons. Consistency in the plasticity response across all three dietary groups suggested by a significant ANOVA will be reflected by the similarity in the direction and magnitude of the % value ('+' or '-') for a given variable.

Type II muscle fibers[81]. Alternatively, such findings may simply document a postnatal pattern of fiber-type differentiation in mammalian jaw adductors still in need of explication. As processing a more fracture-resistant diet requires greater muscle and bite forces[26,44,45,82], given the unique role of large Type II fibers in the production of increased jaw-adductor forces, growing rabbits raised on a more fracture-resistant diet may further emphasize or accentuate underlying postnatal increases in the proliferation of such muscle fibers.

Indeed, our experimental analyses indicate that a postweaning shift to a more fracture-resistant diet induces physiological variation in the abundance of jaw-muscle fiber types as well as fiber-type cross-sectional areas. In particular, reliance on a fracture-resistant diet appears to necessitate a masseter muscle comprised differentially of larger Type II fibers that facilitate the potential generation of larger forces and faster contraction times, factors that in turn positively influence the ability and speed with which such foods are processed along the postcanine toothrow. Such plasticity responses result in especially high concentrations of Type II fibers in the posterior regions of the masseter, a region likewise characterized by the largest Type I and Type II fibers. In conjunction with findings that higher masseter duty time, indicative of more extensive daily muscle activity, results in larger and increased numbers of Type I fibers[63], this demonstrates that the masseter muscle has considerable adaptive potential in growing mammals to accommodate alterations in dietary material properties. Thus, our observations and those of prior studies are not contradictory. Instead, they suggest that variation in dietary properties may, via its influence on the peak forces and/or duration of chewing bouts required of jaw-adductor muscles, have significant and disparate effects on the development of fiber-type characteristics.

If the processing of hard, resistant food items differentially results in jaw adductors characterized by faster contraction times and higher force production capabilities, this is likely to affect the loading rate and amplitude of reaction forces experienced by connective tissues of the oral cavity such as bone, cartilage and ligaments. In fact, osteogenic and chondrogenic activities are well known to be modulated by dynamic variation in loading frequency and magnitude[5-8,11,34-38,74,83-85]. Such a relationship is sup-

ported by our analyses of mandibular parameters, which demonstrate that norms of reaction in the masticatory apparatus are comparable between skeletal and muscular structures but vary depending on the specific parameter. Thus, the processing of more fracture-resistant foods during postweaning development results in a series of changes in force-generating structures (i.e., jaw adductors) with corresponding increases in the overall size and biomineralization of bony elements (e.g., mandible) that serve as muscle attachments and counter greater masticatory stresses.

Given the presence of ontogenetic variation in jaw-adductor muscle fiber types[52-58], architecture[50-52] and mechanical advantage[30,33], the relative contribution of such influences to postnatal variation in masticatory loading parameters remains to be more fully explored. In this regard, further investigation into the mechanotransduction networks underlying the plasticity of muscle fiber types is warranted[79,80]. Arguably, the broader implications of our observations for ecomorphological studies of the feeding apparatus will be more readily understood via recourse to detailed behavioral data on factors that likewise influence craniomandibular loading patterns, such as dietary properties, the duration of daily feeding bouts, bolus size and daily forage intake in the wild[37]. Such an integrative perspective is beneficial for advancing our understanding of fundamental principles of musculoskeletal design and tissue plasticity relevant to biomechanical *and* bioengineering research on the mammalian skull.

Acknowledgements

The authors thank Mark Hamrick for the invitation to contribute to this special issue on muscle-bone interactions. M. Hamrick, F. Rausch and two anonymous reviewers provided helpful comments on an earlier version of this manuscript. D.B. Costley was supported by the MU EXPRESS program. A.N. Daniel was supported by a MU LS UROP Summer 2009 Fellowship. R. Menegaz, S. Neff and W. Phillips are thanked for assistance with tissue harvesting. B. Wright graciously performed the analyses of rabbit food properties. M.A. Rogers and B. Stanley kindly provided lodging to M.J.R. during the collection of microCT data. We also acknowledge use of the Scanco MicroCT-40 of Northwestern's MicroCT facility. Finally, this research was funded by the NSF (BCS-0924592 to M.J. Ravosa) and the Department of Pathology and Anatomical Sciences at the MU School of Medicine.

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