Rossiter, Jeffrey A., The Effect of Dietary Loading on Structural Determinants of Force Production in the Rat Masseter. Master of Science in Medical Sciences - Anatomy, May 2020.

Biomechanical loading associated with feeding is known to direct cranial bone growth, however less is known about its effects on masticatory muscle growth and performance. Peak muscle contractile forces are determined by a combination of factors including total muscle mass, fiber length, and fiber type. Here, we test two hypotheses: that mechanically challenging diets will (1) increase the physiological cross-sectional area (PCSA), an estimate of maximum contractile force at tetanus, and (2) increase the number and proportion of type II (fast-twitch) muscle fibers in the masseter of the rat.

Sprague-Dawley rats were raised on either a hard/tough (overuse) diet or a soft (underuse) diet (n=5/cohort). The superficial masseters were dissected and photographed using a trifocal stereo microscope, and muscle fiber length (6/individual) were measured using ImageJ. Muscle volumes were calculated from in-situ diffusible iodine-based contrast-enhanced μCT scans. Muscles were stained using an IHC protocol for the fast isoform of myosin heavy chain, allowing the number and areas of type II (stained) and type I (unstained) fibers to be quantified in ImageJ.

Results from this study do not support our hypotheses, most likely due to the small sample sizes (n=5/treatment group) available for this study. Paradoxical results were found, with rats raised on a soft diet tending to have longer superficial masseter muscle fibers and more type II muscle fibers with larger cross-sectional areas in the posterior masseter. Rats raised on a hard diet tend to have larger masseter muscle volumes. However, these trends were not statistically significant ($p > 0.05$).

Mechanically challenging diets tend to be associated with greater masticatory muscle volumes and thus increased PCSA. The fiber type results from the posterior masseter (with more deep masseter fibers) were the opposite of those previous results from the middle masseter (with more superficial masseter fibers) in the same animals. Future studies with increased sample sizes are needed to better understand the structural determinants of force production in the rat masseter.

THE EFFECT OF DIETARY LOADING ON STRUCTURAL DETERMINANTS OF FORCE PRODUCTION IN THE RAT MASSETER

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THE EFFECT OF DIETARY LOADING ON STRUCTURAL DETERMINANTS OF FORCE PRODUCTION IN THE RAT MASSETER

INTERNSHIP PRACTICUM REPORT

Presented to the Graduate Council of the

Graduate School of Biomedical Sciences

University of North Texas

Health Science Center at Fort Worth

in Partial Fulfillment of the Requirements

For the Degree of

MASTER OF SCIENCE

By

Jeffrey Rossiter, B.S.

Fort Worth, Texas

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CHAPTER I
INTRODUCTION TO THE STUDY

Research has shown that biomechanical loading affects the growth of bone (Lanyon and Rubin 1984). In the skull, mechanically challenging diets have been shown to increase biomechanical loading which in turn affects skull growth and bone mineralization (Ravosa et al. 2006). While the skeletal responses to masticatory loading has been well studied, less is known about the changes occurring within the masticatory muscles. Use increases mass, but mass alone is a poor predictor of function/strength. Factors such as fiber length, mass, and pennation angle all contribute to both the strength and efficiency of the muscle. Experimental studies have shown that skeletal muscle may respond to increased biomechanical loading through a number of structural mechanisms, including increased number of stronger type II muscle fibers, increased fiber length, increased PCSA, and increased mass (Barber et al. 2011, Kiliardis and Engstrom 1988, Ravosa et al. 2010, Taylor and Vinyard 2004, Taylor et al. 2006).

This project aims to expand our current knowledge on the changes in muscle architecture that occur in response to variation in dietary material properties. Specifically, this study investigates the relationships among diet, muscle fiber type, and physiological cross-sectional area (PCSA, an estimate of maximum contractile force at tetanus) in the rat masseter. Sprague-Dawley rats were raised on two dietary treatments of either high loading (overuse) or low loading (underuse). We predict that more mechanically challenging diets, as seen in the overuse cohort, will be associated with changes in muscle fiber type (e.g. increased number and area of fast-twitch type II muscle fibers). We also predict that more mechanically challenging diets, as seen in the overuse cohort, will be associated with changes in muscle architecture (e.g. increased PCSA) related to stronger, more efficient muscle contractions. This research will elucidate the architectural and physiological elements that contribute to muscle strength.

CHAPTER II
BACKGROUND AND LITERATURE REVIEW

Musculoskeletal Plasticity

The effects of diet on craniofacial bone are well studied, in part due to the ease of preserving bone and due to methodological advances such as CT scanning that facilitate the visualization and quantification of bone. Dietary loading has been demonstrated to affect bone mineralization (Ravosa et al. 2006) and morphology (Kiliaridis et al. 1996, Menegaz and Ravosa 2017) of the skull. Harder and/or tougher diets increase the peak and cyclical bite forces used during food processing (Kiliardis and Shyu 1988), increasing the strain experienced by the facial skeleton and ultimately stimulating increased bone growth and remodeling (Menegaz and Ravosa 2017, Ravosa et al. 2006).

The function of masticatory muscles during feeding is well studied (Hiimae, 1971a,b,c), as is plasticity related to interindividual variation in biomechanical loading (Kiliaridis et al. 1988, Kiliaridis and Shyu 1988, Ravosa et al. 2010). However, very few studies examine both architectural and physiological adaptations to increased loading within the same muscles. By looking at several levels of tissue organization and adaptation, this study offers a more holistic look at muscular plasticity. This study examines two parameters of muscle force production - fiber type and PCSA - to address this gap in the literature.

Muscle Fiber Types

Muscle fibers fall into one of three categories: Type I, Type IIA, and Type IIB. Type I muscle fibers produce energy via oxidative phosphorylation, which makes them resistant to fatigue. These fibers are primarily used for low effort activities, such as walking or distance running (Lieber 2010). Type IIB muscle fibers produce energy via glycolysis. While significantly stronger, Type IIB muscle fibers fatigue much quicker than their Type I counterparts. These fibers are primarily used for maximal effort activities, such as jumping (Lieber 2010). Type IIA

fibers use a combination of both oxidative and glycolytic metabolism, which renders them with intermediate fatigue resistance and force production (Lieber 2010).

Interindividual variation in biomechanical loading has been demonstrated to result in increased force production in rat masseters (Kiliardis and Shyu 1988). A portion of this increase in force production can be explained by changes in fiber type. Kiliardis et al. (1988) and Ravosa et al. (2010) both detected changes in muscle fiber type depending on dietary consistency. In rats fed soft diets, more IIb (fast glycolytic) than IIa (fast oxidative) fibers were seen in the masseter (Kiliaridis et al. 1988). In a rabbit model, harder and tougher diets were associated with a proportional increase in type II ("fast twitch") muscle fibers which produce greater force but exhaust more quickly (Ravosa et al. 2010).

Physiological Cross-Sectional Area (PCSA)

PCSA is defined as the area of muscle perpendicular to the muscle fibers. This measurement is directly proportional to maximum force produced by the muscle at tetanus (Lieber 2010). Several studies have shown that hard/tough diets are associated with an increase in PCSA (Taylor and Vinyard 2004, Taylor et al. 2006). However, there is no consensus in the literature regarding which structural variables produce the best measurements of PCSA. Some studies suggest that changes in PCSA are related to variation in muscle mass alone (Taylor et al. 2006), whereas others have detected differences in mass, pennation angle, and fiber length (Barber et al. 2011, Taylor and Vinyard 2004). In studies that examine PCSA, muscle massa and fiber length are the most consistently included variables. Furthermore, some PCSA variables such as pennation angle and sarcomere length may be most important when controlling for variation in stretch or distortion that occurs during the muscle fixation process (Anapol and Gray 2003, Felder et al. 2005, Lieber 2010). These variables therefore may be less significant in masticatory muscles where each specimen was fixed with similar temporomandibular joint position (minimal occlusion). In an effort to compare the advantages and drawbacks of two popular formulas, this study will calculate PCSA using two different formulas. One formula includes muscle mass and fiber length (Felder et al. 2005), and one includes mass, fiber length, and pennation angle (Taylor et al 2006) to calculate PCSA.

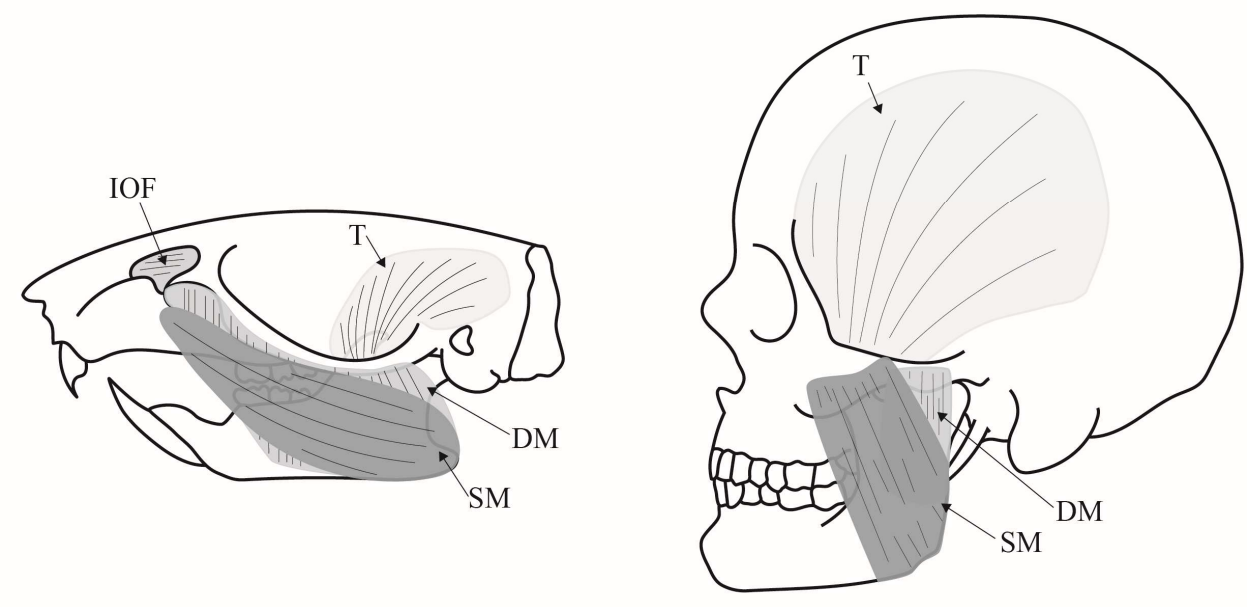
Anatomy of the Masseter

In humans, the masseter is subdivided into superficial and deep parts (or bellies) (van Eijden et al., 1993). In rats, the masseter is similarly divided into superficial and deep parts, with an additional part of the anterior deep masseter arising from within the infraorbital foramen (the infraorbital part of the deep masseter) (Hiimae et al., 1971a).

In humans and rats, the fibers of the superficial masseter are oriented in an oblique anteroposterior direction so that this muscle serves to protract the jaw during contraction (Hiimae 1971c; van Eijden et al., 1993). Unilateral contractions assist with lateral rotations of the mandible during feeding (van Eijden et al., 1993). In rats, protraction of the mandible contributes to anterior movements during the occlusal phase of mastication (Hiimae 1971c). Thus, the superficial masseter contributes to the grinding and reduction of food during mastication.

The deep masseter of both species has fibers aligned in a vertical (superoinferior) direction (Hiimae 1971c; van Eijden et al., 1993). The primary function of this part of the muscle is elevation of the mandible and the production of the vertical bite force during incision, mastication, and other oral behaviors (Hiimae 1971c; van Eijden et al., 1993). Additionally, the infraorbital portion of the anterior deep masseter in the rat is specialized to produce forward movements of the jaw during feeding and incision (gnawing) (Hiimae 1971a,c), however this portion of the muscle was not examined in our analyses.

Figure 1. Muscles of mastication in the rat (left) and human (right). Note that deep muscles (pterygoid and zygomaticomandibularis muscles) are not shown. Key: DM deep masseter; IOF, infraorbital part of the deep masseter; SM, superficial masseter; T, temporalis.



CHAPTER III
SPECIFIC AIMS

Specific Aim 1: Examine the effect of mechanically challenging (hard/tough) diets on muscle fiber type in the rat masseter (overuse vs underuse cohorts).

Hypothesis 1: The number and area of fast-twitch type II will be increased in the overuse cohort compared to the underuse cohort.

Specific Aim 2: Examine the effect of mechanically challenging (hard/tough) diets on muscle architecture and PCSA in the rat masseter (overuse vs underuse cohorts).

Hypothesis 2: Muscle mass, fiber pennation, fiber length, and PCSA will be increased in the overuse cohort compared to the underuse cohort.

CHAPTER IV
SIGNIFICANCE

This study investigates the role of feeding behavior in the development of masticatory musculature. Many structural components may affect the force production of chewing muscles, such as muscle mass, lever arms, fiber type, and PCSA. This project examines the variables affecting force production/muscle strength including PCSA and fiber type, and how these variables are affected by behavior and use. This more complete understanding of muscle plasticity is important for studies involving development, athletic performance, and aging.

CHAPTER V
MATERIALS AND METHODS

Experimental Model

All procedures for this study were conducted in accordance with University of Missouri Institutional Animal Care and Use Committee (IACUC) approved protocol (#6827).

Male Sprague-Dawley rats were raised from weaning (4 weeks) to adulthood (16 weeks), and randomly separated into two dietary treatment groups: the "overuse" group was raised on a hard/tough diet consisting of whole compressed rodent diet pellets; and the "underuse" group was raised on a soft diet consisting of the same pellets ground into a fine powder. Nutritional content was comparable between the diets.

Table 1. Dietary treatment groups

Cohort	**Diet Type**	**Diet**
Overuse	Hard/Tough	Pellets
Underuse	Soft	Powder

Sample Size

The sample size is 5 individuals per treatment group (total n = 10).

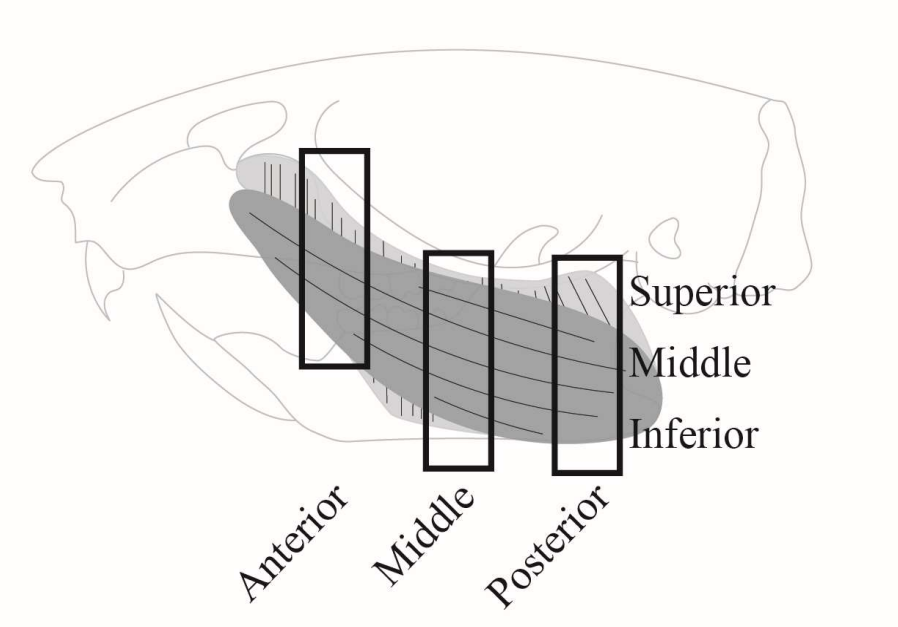
Tissue Collection

Following sacrifice, the right masseter was dissected from the cranium and weighed. Cranial tissues were fixed in 4% paraformaldehyde for 48 hours at 4°C. Tissues were then stored in 70% ethanol at 4°C.

Muscle Measurements: Fiber Type

Muscle histology was processed by the UNTHSC histology core facility. After being embedded in paraffin, the whole masseter (including both muscle bellies) was divided into three regions for coronal sectioning: anterior, middle, and posterior. Muscle sections were stained with a primary antibody for the fast isoforms of myosin heavy chain (MHC) (ab 91506) and a fluorescent secondary antibody (ab 150077). Under this staining protocol, fibers positive for the fast isoform of MHC (type II cells) fluoresce green under 488 nm laser excitation (Figure 2). Photos of the sections were taken at 10x using a fluorescent confocal microscope. Six histological sections per individual per region were photographed. In an effort to capture intramuscular variation, the photos containing regions of interest (ROIs) were taken along a superoinferior axis such that the six photos per region were composed of two superior ROIs, two middle ROIs, and two inferior ROIs. Thus, the resulting histological ROIs represent anteroposterior (anterior/middle/posterior) and superoinferior (superior/middle/inferior) variation within the masseter muscle.These photos were then processed in ZenBlue to create slide tiffs for further analysis.

Figure 2. Histology regions sampled for these analyses. Sections were taken from three blocks distributed along the anteroposterior axis, then ROIs were photographed along the superoinferior axis.

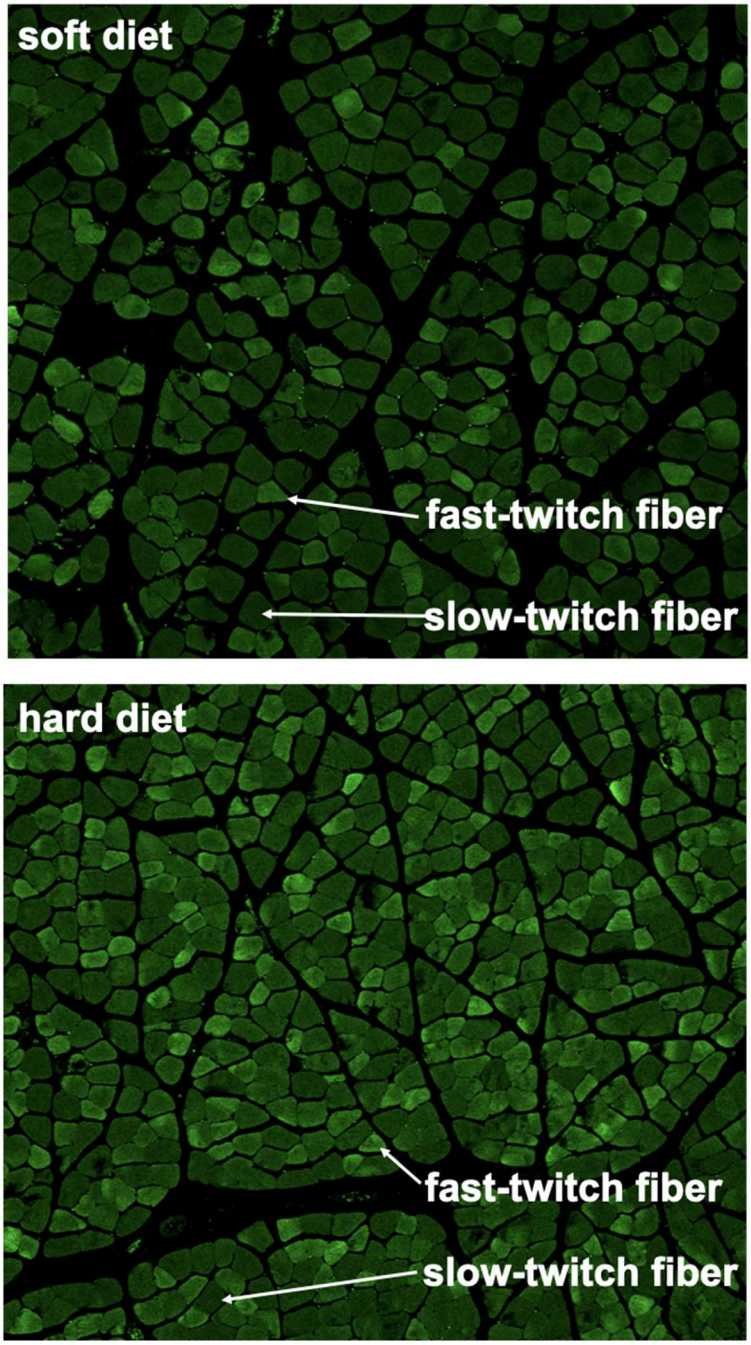


For each individual, six ROIs per muscle region were analyzed in ImageJ. The following variables were measured in each section: total muscle cell area, type I muscle cell area, type II muscle cell area, type I muscle cell number, and type II muscle cell number. From these

measurements, ratios of type I/II muscle cell number to total cell number and type I/II cell area to total cell area were calculated. All variables were compared between treatment groups using Mann-Whitney U-tests ($\alpha = 0.05$). Some slides were excluded due to poor sectioning or staining; the final sample size for muscle fiber histology was overuse n = 4 / underuse n = 3.

A previous student in the Menegaz lab (Holly L. LaRocque) collected fiber type data from the middle masseter. Jeffrey Rossiter collected data from the posterior masseter slides as part of this practicum; the results from the posterior masseter are reported here.

Figure 3. Examples of IHC-stained histological sections (ROIs) of muscle. Top: soft diet; bottom: hard diet.



Muscle Measurements: PCSA

The left-side masseter volumes were previously collected in our lab using diffusible iodine-based contrast-enhanced computed tomography. After fixation, the specimens were soaked in Lugol's solution and imaged using a Skyscan 1171 microCT scanner (18 μm^3 voxels). Volumes were calculated in 3D Slicer software from models created using a semi-automatic segmentation protocol (Menegaz et al. 2020).

Due to the complexity of measuring the deep masseter without destroying the specimen, only the superficial masseter was measured. The left-side superficial masseters were dissected under a Meiji EMZ-5 trifocal dissecting scope and photos were taken using the integrated camera. A total of 6 fiber lengths per individual were then measured in ImageJ.

PCSA for each muscle (average fiber length/muscle/individual) were calculated using the following formula (Felder et al. 2005):

$$PCSA = V_m / L_f$$

Where V_m = muscle volume (cm^3) and L_f = fiber length (cm).

PCSA measurements for the left and masseter muscles were calculated using average fiber length for each individual. Mann-Whitney U-tests ($\alpha = 0.05$) were then used to compare muscle volume, fiber length, and PCSA between the treatment groups.

Limitations

The small sample sizes (5/treatment group) used in this study represent one potential limitation. Additionally, the rat masseter contains bellies that are convergent (superficial masseter) and parallel (deep masseter types), without large central tendons. Without using acid digestion, only the superficial masseter was able to be measured. Since fiber length was measured from tendon to tendon, the disappearance of fibers behind bone or fascia could have introduced additional error. Not all histology slides were usable, further reducing the potential sample size for fiber type analysis. Muscle slides were only stained for myosin heavy chain, which could miss detection of type IIb intermediate fibers.

CHAPTER VI
RESULTS

Masseter Weights

The right masseter was dissected away from the skull at the time of sacrifice. Muscles were blotted dry and weighed on a scale with accuracy to the second significant digit (0.00). No significant difference was found in body mass ($p = 0.170$) or masseter mass ($p = 0.369$) (Figure 3), most likely due to the small size of the muscles and resolution of the scales used to collect this data.

Figure 4. Body mass (g) at the time of sacrifice (16 weeks).

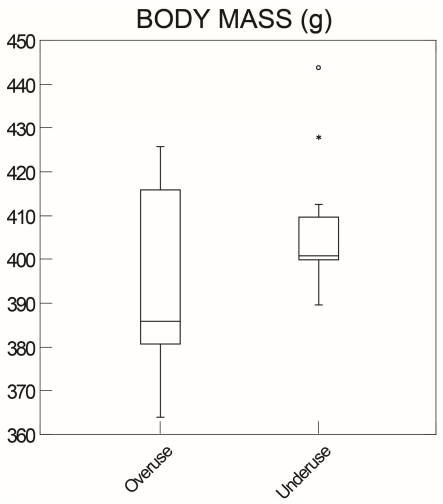
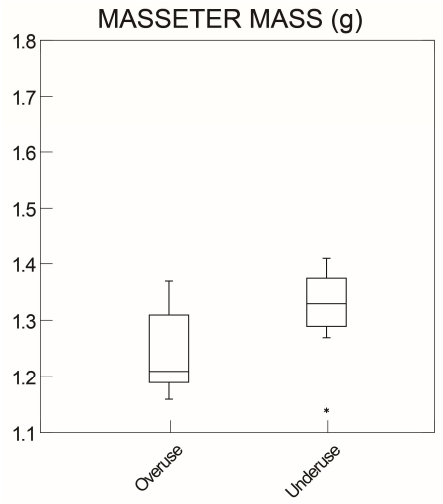


Figure 5. Masseter mass (g) at the time of sacrifice (16 weeks).



A scale with accuracy to the fourth significant digit (0.0000) was used to measure the fixed muscles. It was discovered that fixation and long term storage of the muscles had shrunk an average of ~61%. Due to this shrinkage, muscle volumes calculated from dice-CT cans were used as a measure of muscle size for PCSA calculations.

Table 2. Pre- and Post-Fixation Mass Comparison

Rat	Diet	Pre-Fixation	Post-Fixation	% Change
1	Overuse	1.37	0.7638	55.7518
3	Overuse	1.18	0.6647	56.3305
5	Overuse	1.31	0.7572	57.8015
6	Overuse	1.16	0.6989	60.2500
9	Overuse	1.21	0.7387	61.0496
21	Underuse	1.31	0.7934	60.5649
25	Underuse	1.41	0.8705	61.7376
27	Underuse	1.35	0.8331	61.7111
29	Underuse	1.40	0.9517	67.9786
31	Underuse	1.30	0.8670	66.6923

Posterior Masseter Fiber Type

The overuse cohort (hard diet) was found to have a decreased ratio of type II fiber number to total fiber number of 17.32% compared to 19.20% in the underuse cohort (soft diet). The overuse cohort was also found to have a decreased ratio of type II fibers area to total fiber area of 11.40% compared to 17.01% in the underuse cohort. However, these differences were not statistically significant (Table 3).

Figure 6. Number ratios (fiber type number/total fiber number) for hard diet (left) and soft diet (right).

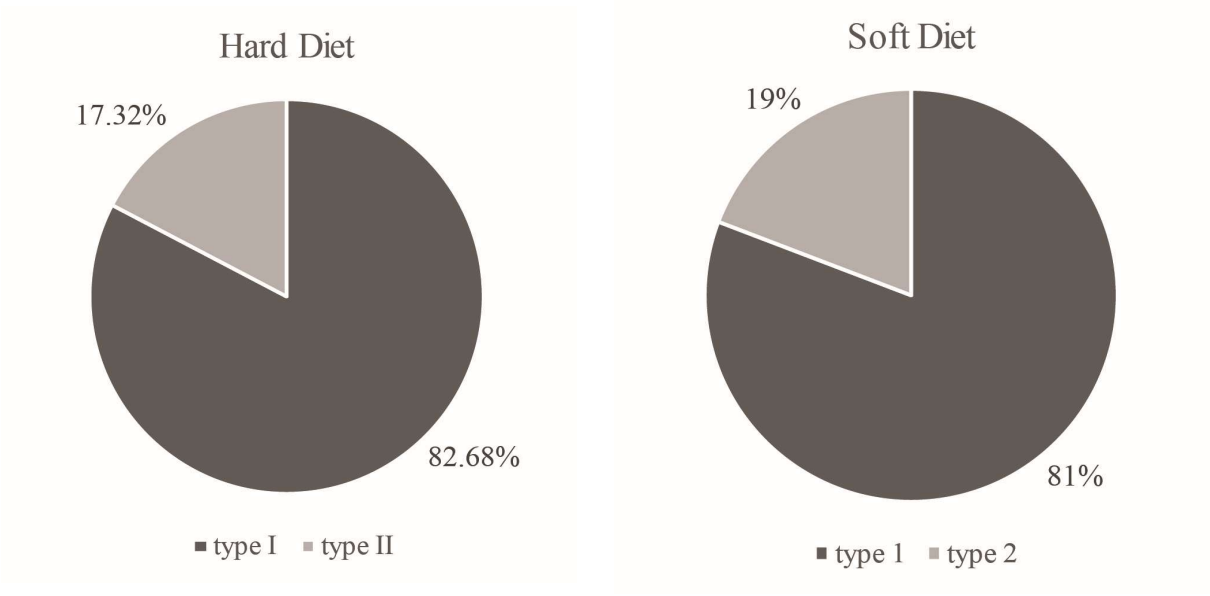


Figure 7. Area ratios (fiber type area/total fiber area) for hard diet (right) and soft diet (left).

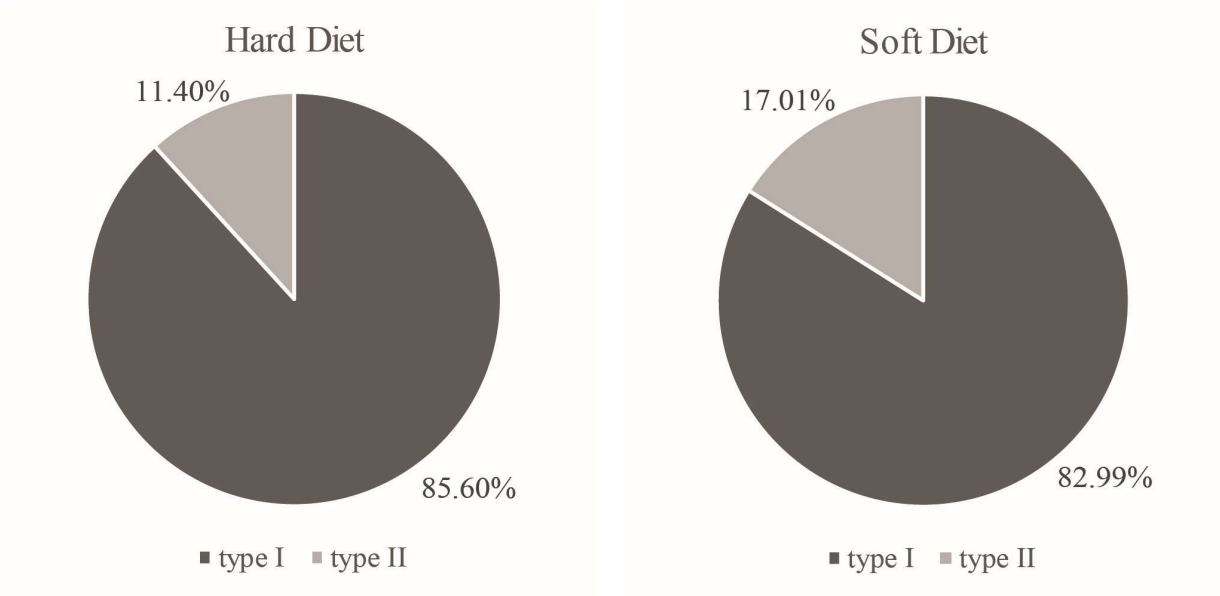


Table 3. Posterior masseter fiber variables measured in this study (mean ± SD) and Mann-Whitney U-test p-values.

	Overuse Diet	**Underuse Diet**	**p-values**
Total fiber area	573,534.09 ± 62937.25	577,832.90 ± 82483.95	1.000
Type I fiber area	506,139.82 ± 27592.62	485,197.19 ± 116450.56	0.480
Type I fiber area / Total fiber area (%)	88.59% ± 4.66%	82.99% ± 7.84%	0.289
Type II fiber area	67,394.27 ± 37242.20	92,635.71 ± 33973.51	0.289
Type II fiber area / Total fiber area (%)	11.40% ± 4.66%	17.01% ± 7.84%	0.289
Total fiber number	314.25 ± 42.62	425.72 ± 106.52	0.229
Type I fiber number	257.83 ± 22.00	338.61 ± 59.11	0.070
Type I fiber number / Total fiber number	82.68% ± 7.54%	80.80% ± 8.16%	0.857
Type II fiber number	56.41 ± 31.12	87.11 ± 49.45	0.289
Type II fiber number / Total fiber number	17.32% ± 7.54%	19.20% ± 8.16%	1.000

Superficial Masseter PCSA

Mean PCSA was found to be greater in the overuse cohort (374.46 ± 58.19 cm^2) compared to the undeuse cohort (399.47 ± 88.37 cm^2) (Table 4). However, the results are not statistically significant (p=0.20) likely due to the small sample size. The increase in PCSA for the overuse cohort appears to have been largely driven by mean muscle volume rather than mean fiber length (Table 4).

Figure 8. Superficial masseter PCSA in the overuse cohort versus underuse cohort.

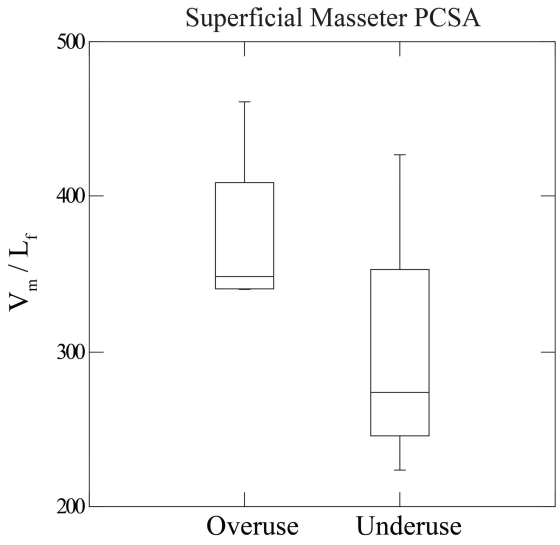


Figure 9. Superficial masseter fiber length (mm) vs superficial masseter volume (cm^3). White circles = overuse, black circles = underuse.

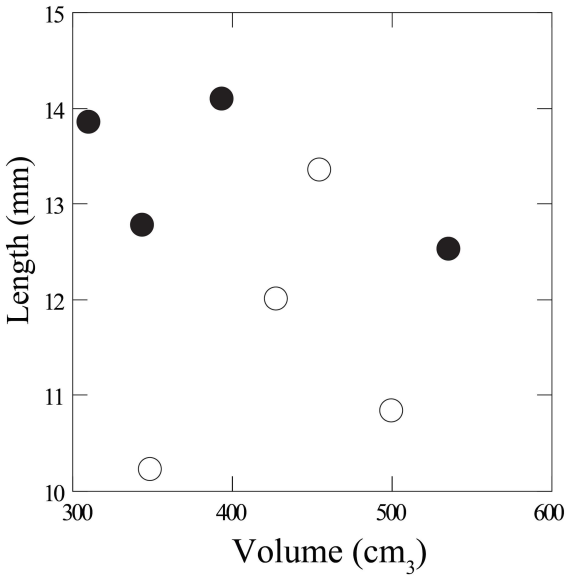


Table 4. Superficial masseter architectural variables measured in this study (mean ± SD) and Mann-Whitney U-test p-values.

	Overuse Diet	**Underuse Diet**	**p-values**
PCSA (cm^2)	374.46 ± 58.19	299.47 ± 88.37	0.200
Superficial masseter mass (cm^3)	432.24 ± 54.93	410.30 ± 92.21	0.690
Superficial masseter fiber length (mm)	11.61 ± 1.38	13.32 ± 0.788	0.110

CHAPTER VII
DISCUSSION AND CONCLUSIONS

We found a seemingly paradoxical decrease in the type II fiber number and area in the posterior masseter of rats raised on hard diets compared to the soft diet cohort. A previous study (LaRocque et al. 2020) found a slight increase in both of these ratios in the middle section of the masseter in the same rats. This finding is less surprising considering that the posterior section of the masseter would contain a higher proportion of the deep masseter than the middle section analyzed by LaRocque. Since the deep masseter is used for non-masticatory behaviors (such as biting and gnawing), it might be less likely to respond to changes in loading related to mastication (grinding) since the anterior-posterior motions involved in this action are largely produced by the superficial masseter (Hiimae et al. 1971a,c.).

We found no significant difference in fiber length between the overuse and underuse diet rats, and our results failed to support our hypothesis that a mechanically challenging diet would result in increased masseter PCSA. However, our findings that changes in PCSA were driven by muscle volume are consistent with those of Taylor et al. (2006) in the masseters of postweaning rabbits fed hard/soft diets, who found that the PCSA change was driven by changes in muscle mass but not fiber length or pennation angle. While we anticipated an increase in fiber length in response to an increase in biomechanical loading, it would most likely be exceedingly difficult to detect within physiological ranges due to the small sample sizes available for this study.

Our current understanding of muscle plasticity suggests that muscles respond to biomechanical loading by increasing the number and proportion of type II fast-twitch muscle fibers, and by increasing PCSA and the underlying factors (mass, fiber length) that determine it (Barber et al. 2011, Kiliardis and Engstrom 1988, Ravosa et al. 2010, Taylor and Vinyard 2004, Taylor et al. 2006). This leads us to hypothesize that similar structural and physiological changes exist in the rat model, but that the methods and sample size we used in this study were unable to capture these differences. This conjecture is supported by the fact that Taylor et al. (2004) and Ravosa et. al (2010) were able to detect significant differences in muscle mass and fiber type proportions in

a similar dietary modification experiment in rabbits. LaRoque et al. (2020) reported fiber type changes (although not statistically significant) in the anticipated direction in the same rat sample described in this practicum, but in a different section of the muscle. Overall, this study emphasizes the importance of statistical power, tissue preservation methods, and data collection protocols for the detection and repeatability of biologically relevant results.

CHAPTER VIII
FUTURE DIRECTIONS

In future studies, the use of larger sample sizes would make it easier to detect differences between PCSA and the factors that determine it. Assuming that there is an appreciable difference between fiber length, a larger sample size would help detect it.

Furthermore, I would like to see a methodological comparison between the two formulas for calculating PCSA that I encountered over the course of my background studies.

$$PCSA = (mass \times \cos\Theta)/(I_f \times 1.0564\ g/cm^3) \text{ vs } PCSA = V_m / L_f$$

Where mass = muscle mass (g), Θ = pennation angle, I_f = fiber length (cm), and 1.0564 g/cm^3 = the density of skeletal muscle (Murphy and Beardsley 1974). This comparison would help shed light on potential error that can be introduced using this methodology because measuring the variables for the former formula are more difficult in an attempt to capture what is likely a nominal contribution within physiological ranges.

I would also like to see a methodological comparison between the specimen conserving method of measuring fiber length versus an acid digestion protocol for isolating and measuring individual fibers. This would help us understand if current efforts to preserve the specimen come at the expense of introducing unnecessary error into the fiber length measurement.

Finally, muscles should be stained shortly after fixation in order to minimize curling or the specimens prior to histological staining in order to help maximize the number of usable slides. It would also be beneficial to use additional stains in order to pick up transitional fibers that our staining protocol could have missed.

CHAPTER IX
INTERNSHIP EXPERIENCE

This internship practicum was performed at the University of North Texas Health Science Center in Fort Worth, TX under the supervision of Rachel A. Menegaz, PhD the course of the 2019-2020 school year as a partial requirement for the degree of Master of Science in Biomedical Science. I was introduced to this project and recent studies conducted in the Menegaz lab in May of 2019 when I started this program. At this time, we created a rough schedule for the upcoming year that was adjusted by either Dr. Menegaz or myself over the course of the year as needed. Between the months of May and August of 2019, I met with Dr. Menegaz once a week to discuss the background literature relevant to this project. In October of 2019, I presented my practicum background and research proposal as a "Work-in-Progress" (or WIP) seminar for my committee and peers in the Center for Anatomical Sciences.

In August and September 2019, I took a hiatus from my research duties for a few weeks in September and October of 2019 to focus on my Head & Neck Anatomy and Structural Neuroscience electives.

Over the course of the Fall 2019 semester I worked on fiber type analysis and measurement in ImageJ. Dr. Menegaz trained me in our fiber length measurement protocol after the conclusion of my classes and I began data collection. For the remainder of the Fall 2019 semester, I collected fiber length data for the overuse and underuse cohorts before using previously collected muscle volume data in order to calculate physiological cross sectional area. After which, I resumed collection of fiber type data.

During the Spring 2020 semester, I took a Physiology course and the Anatomy Laboratory Practicum (teaching assistantship) course. I also finished collecting fiber type data. Upon the completion of data collection, Dr. Menegaz and I analyzed and discussed the implications of our results. These results have been reported in this practicum report and my accepted abstract for the 2020 UNTHSC Research Appreciation Day (which has since been canceled due to concerns over COVID-19). Data from this practicum also contributed to abstracts accepted by the 2020 annual meetings of the Society for Integrated and Comparative

Biology (Menegaz et al., 2020) and Experimental Biology (LaRocque et al., 2020); I was a co-author on these abstracts.

This research and public speaking experience I gained during this journey presented me with experience in problem solving, which allowed me to refine my critical thinking skills and has improved me as a student. I was given the opportunity to learn from and develop professional relationships with my PI and committee members, which has helped me grow as a person who will be better equipped for an increasingly interdisciplinary industry in healthcare.

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