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14. ABSTRACT <u>Purpose:</u> To develop interventions aimed at preventing Obstructive Sleep Apnea (OSA) incidence or manage OSA risk in asthma patients. <u>Scope:</u> To test the effects of orally administered inhaled Fluticasone Propionate (FP) on tongue muscle function, muscle fiber morphology and Myosin Heavy Chain (MHC) isoform expression. <u>Major Findings:</u> FP-treated rats, compared to placebo, displayed significant weight loss that was not entirely explained by reduced food consumption. On tongue contractile force measurements, FP-treated rats generated greater retrusive and protrusive force (expressed as percentage of maximum tetanic force generated during testing) at 40 Hz stimulation frequency, suggesting more engagement of slower-twitch fibers. Additionally, protrusive muscle endurance was elevated in FP-treated animals compared to placebo. On muscle fiber morphometry, FP-treated animals had greater Cross-Sectional Area (CSA) of MHC-2A expressing muscle fibers in the genioglossus – a major protrusive muscle of the tongue. The size increase of these intermediate-twitch, fatigue-resistant fibers is in line with the physiological findings of higher protrusive endurance and greater force at low stimulation frequencies. <u>Significance:</u> Our findings provide empirical evidence that long-term oral administration of FP alters tongue muscle structure and function, and may predispose patients on FP therapy to obstructive events during sleep. Additionally, our findings have implications in other functions of the tongue, such as speech and swallowing.					
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INTRODUCTION

Obstructive Sleep Apnea (OSA) is a sleep disorder characterized by repeated airway obstruction during sleep, leading to inability to breathe, drops in blood oxygen and frequent interruption of sleep. Asthma patients are at increased risk for OSA, but it is not known why. There is increasing evidence from clinical studies that inhaled corticosteroids (ICS), the most widely used treatment for asthma, affect muscle properties of the tongue and upper airway, and may lead to upper airway collapse during sleep, but mechanisms remain unknown. To investigate the mechanisms whereby ICS affect tongue muscle physiology and contractile properties, we treated rats with 4 weeks of orally administered ICS (Fluticasone Propionate) or placebo (propellant gas only). After the drug or placebo treatment, tongue forces, and tongue muscle fiber characteristics were assessed.

KEYWORDS: Obstructive Sleep Apnea; Inhaled Corticosteroids; Fluticasone Propionate; Tongue Physiology; Genioglossus; Styloglossus; Myosin Heavy Chain isoforms; Rats.

ACCOMPLISHMENTS:

What were the major goals of the project?

1. To test the effects of Fluticasone Propionate (FP) on tongue protrusive and retrusive function in response to hypoglossal nerve stimulation in rats.
2. To test the effects of FP on Myosin Heavy Chain isoform expression and fiber cross-sectional area (CSA) in rat genioglossus (GG) muscle.
3. To test the effects of FP on protein degradation and glucose metabolism in GG muscle homogenates.

What was accomplished under these goals?

Methodology:

Experimental Setup: Male, 9 month-old F344 rats (Envigo) were housed one animal to a cage with ad libitum access to drinking water and fed standard rodent chow. Animal weight and food consumption for the last 24-hour period were monitored daily for each rat. Animals were subdivided into the following 3 groups: 1) *Fluticasone Propionate (FP)* rats received oral administration of Fluticasone

Propionate 220 mcg/puff, 1 puff twice-daily, from a metered dose inhaler. 2) *Food Restricted (FR)* rats received twice-daily oral placebo administration. Placebo treatment consisted of a puff from a canister containing propellant gas only (generously provided by H&T Presspart). Each FR animal was weight-matched to a rat from the FP group at the beginning of the study and received only as much food as the FP-treated rat consumed during the previous 24-hour period. This was done to control for steroid-induced weight loss observed in published literature (1-3). 3) *Placebo (P)* animals received twice-daily oral placebo administration and *ad libitum* access to food.

Fluticasone and placebo treatment continued for 28 days. On Day 29, tongue contractile properties were measured and animals were euthanized for tissue sample collection.

Tongue Contractile Property Recordings were conducted as described in (4, 5). Briefly, animals were anesthetized via intraperitoneal injection of sodium pentobarbital and placed in a dorsal recumbent position. Hypoglossal nerves were exposed bilaterally and instrumented with stimulation electrodes. The tongue was connected with a loop of suture to a force transducer. To measure retrusive force, hypoglossal nerves were stimulated with a single 100 μ s pulse to elicit a twitch contraction, or a 200ms – long train of pulses (at frequencies of 20, 40, 60, 80 and 100 Hz) to elicit a tetanic contraction. To measure endurance, hypoglossal nerves were stimulated repeatedly with 100Hz pulses for 2 minutes. The endurance index was calculated as the ratio of the tetanic force at the end of 2 minutes of stimulation relative to the initial tetanic force.

The lateral branch of the hypoglossal nerve was transected bilaterally, and force measurements were repeated as above after a 45-minute wait, to measure protrusive twitch and tetanic forces, as well as the endurance index. Following these measurements, the animal was euthanized with pentobarbital/phenytoin mixture. Genioglossus and styloglossus muscles were excised and preserved at -80°C for analysis.

Myosin Heavy Chain Immunofluorescence and Fiber Morphometry: Muscles were cross-sectioned on a cryostat and immunostained with MHC isoform-specific antibodies as described in (6). MHC isoform expression, cross-sectional area (CSA) and minimum Feret diameter for individual muscle fibers in cross-section were calculated with SMASH analysis software (7).

Results:

Animal Weight and Food Consumption: Placebo-treated animals maintained their initial weight over the course of the study (Figure 1A). In contrast, FR and FP animals displayed progressive and significant weight loss beginning on Day 4 of the treatment, losing 8 – 10 % of their initial body weight by the Day 28. At Day 18, the weight of FR animals stabilized, whereas FP-treated animals continued to lose weight, with the amount of weight loss becoming significantly different from FR after Day 24 (Fig. 1A).

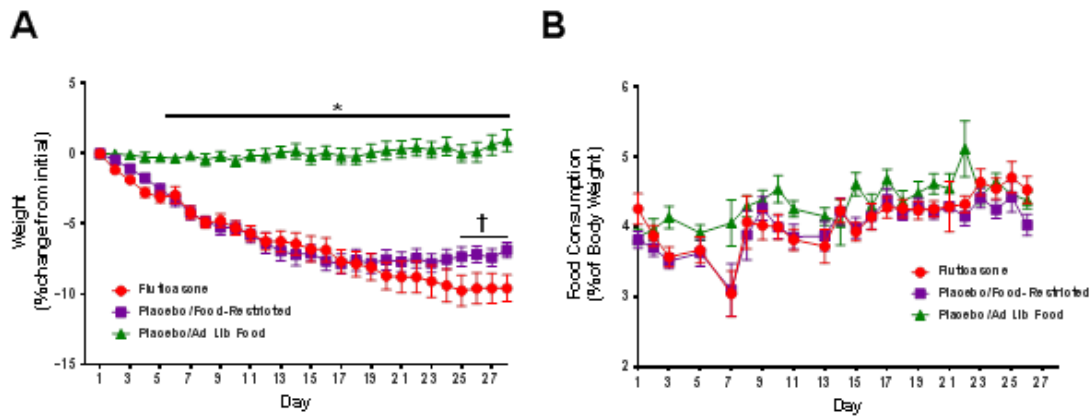


Figure 1. Weight Loss as percent of initial body weight **(A)** and Daily Food Consumption **(B)** over the course of the experimental protocol. Significant differences: *FP vs. P; †: FP vs. FR, $p < 0.05$, 2-way RM ANOVA with Holm-Sidak post-hoc tests.

FP and FR-treated animals consumed significantly less food than Placebo-treated rats (treatment effect $p = 0.008$, 2-way RM ANOVA, Figure 1B). At no point was the food consumption significantly different between FP and FR animals, suggesting that the continued weight loss observed in fluticasone-treated animals after Day 18 was not due to steroid-induced anorexia.

Twitch Force contraction time (time between stimulus and half-maximum force) and decay time (time between stimulus and decay from maximum force halfway down to baseline) did not change with experimental treatment (Figure 2 C - F). FP-treated animals tended to have specific retrusive twitch force (maximum twitch force divided by the mean fiber cross-sectional area (CSA) of the styloglossus) higher than placebo-treated animals ($p = 0.063$, Figure 2A). Specific protrusive twitch force (normalized to genioglossus mean fiber CSA) did not significantly change with treatment (Figure 2B).

Tetanic Force: Specific maximum tetanic force did not significantly change with experimental treatment (Figure 3 A,B). However, when tetanic force at different stimulation frequencies was expressed as percentage of maximum tetanic force attained, FP animals produced significantly higher retrusive (Figure 3C) and protrusive (Figure 3D) force at 40 Hz stimulation frequency, compared to placebo treated animals (retrusive and protrusive) and compared to FR animals (protrusive). The difference was much more pronounced for protrusive force (8%, Figure 3C) than for retrusive force (3%, Figure 3D).

Furthermore, the protrusive endurance index was significantly higher in FP and in FR animals compared to placebo (Figure 3F). These results suggest more involvement of slow-twitch, fatigue-resistant muscle fibers during protrusive muscle contractions in FP-treated animals.

Genioglossus Fiber Morphometry: We immunostained sections of the genioglossus muscle with antibodies specific to four MHC isoforms: MHC-I (slow-twitch, fatigue-resistant), MHC-IIa (intermediate),

MHC-IIb and MHC-2x (fast twitch, fatigue-prone) (representative image in Figure 4A). No differences in

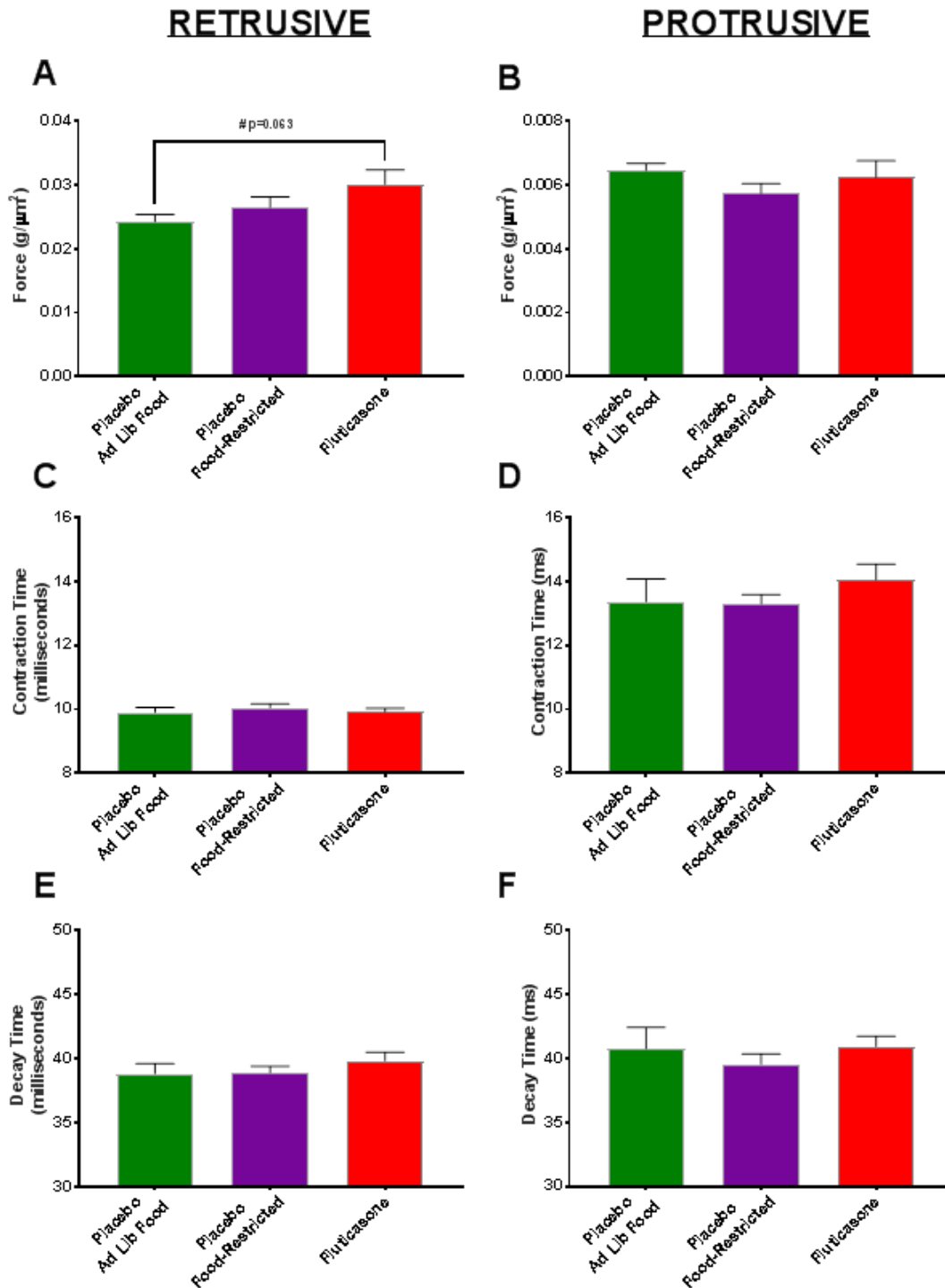


Figure 2: Twitch Force. **A.** Specific retractive twitch force (grams/average styloglossus (SG) fiber CSA), n=6-7/group. **B.** Specific protrusive twitch force (grams/average genioglossus (GG) fiber CSA), n=6-9/group. Contraction (**C,D**) and Decay (**E,F**) time for retractive twitch (**C,E**) and protrusive twitch (**D,F**) contractions, n= 14-16.

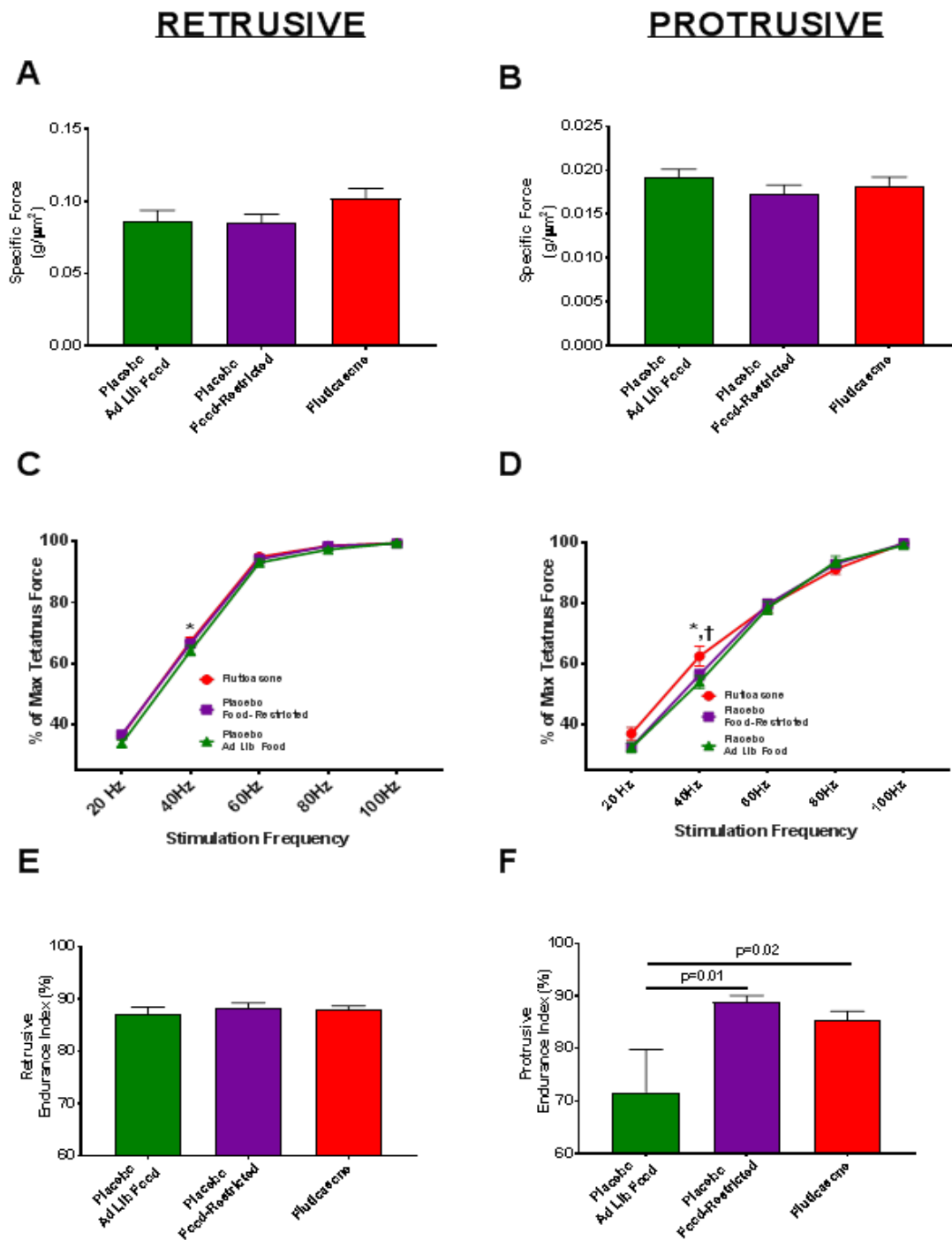


Figure 3: Tetanic Force and Endurance Indices. (A,B): Specific retrusive (A) and protrusive (B) maximum tetanic force. N=6-10/group **(C,D)** Percentage of maximum tetanic force attained at indicated stimulation frequencies. N=13-16/group. **(E,F)** Endurance index. N=13-15/group. Significant differences: *FP vs. P; †: FP vs. FR, p<0.05; ANOVA with Holm-Sidak post-hoc tests.

the fraction of fibers expressing any particular isoform were found between the treatment

groups. Average fiber CSA tended to be elevated in FP-treated animals compared to placebo (Figure 4B), but the difference did not reach significance. MHC-IIa fibers were significantly larger (bigger CSA; $p=0.048$) in FP-treated animals compared to placebo (Figure 4C), whereas MHC-2b fibers tended to be larger in FP-treated animals ($p=0.09$, Figure 4D).

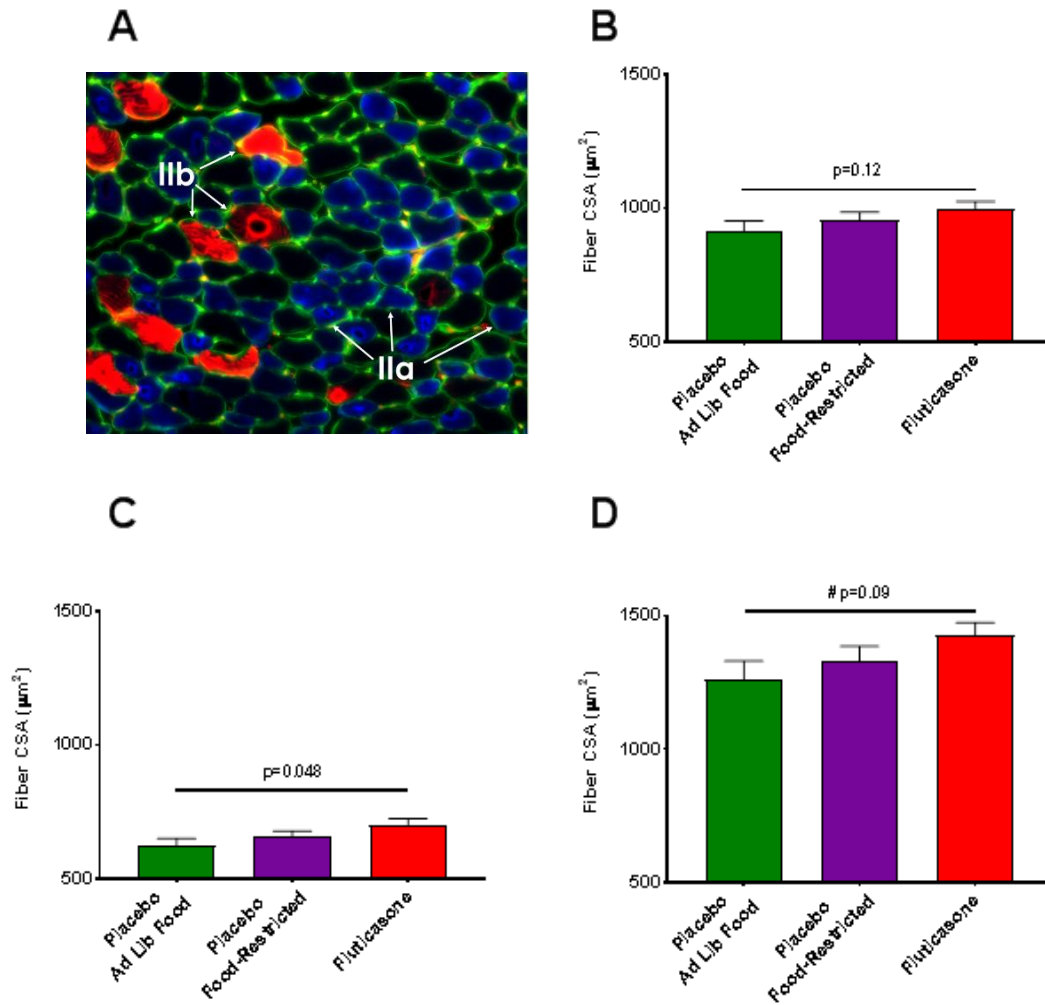


Figure 4: Genioglossus fiber morphometry. (A): Representative image of rat genioglossus muscle, 20x magnification. Red: MHC-2B; Blue: MHC-2A; Green: Laminin. **B-D**: Mean Fiber CSA for all fibers (B), MHC-2A fibers (C) and MHC-2B fibers (D). N=6-10/group. P-values: Holm-Sidak post-hoc tests.

Styloglossus Fiber Morphometry: In the styloglossus (SG), the average fiber CSA tended to be smaller in FP-treated animals compared to FR or Placebo ($p=0.0996$ for both comparisons, N=6-7/group; Figure 5).

No significant differences were found in the CSA of specific fiber types, or in MHC isoform expression within the SG muscle.

Summary and Interpretation: Our data thus far shows that in response to orally inhaled FP administration: 1) the animals lost 8-10% of body weight; 2) twitch contraction characteristics were largely unaffected, except for a small increase in the maximum retrusive twitch force; 3) slow-twitch muscle fibers were involved in tetanic contractions to a significantly higher degree, especially in protrusive muscles; 4) protrusive muscle endurance increased, while retrusive muscle endurance remained unchanged; 5) slower-twitch, fatigue resistant MHC-2A fibers in the genioglossus muscle increased in cross-sectional area, while styloglossus muscle fibers tended to decrease in cross-sectional area, regardless of fiber type.

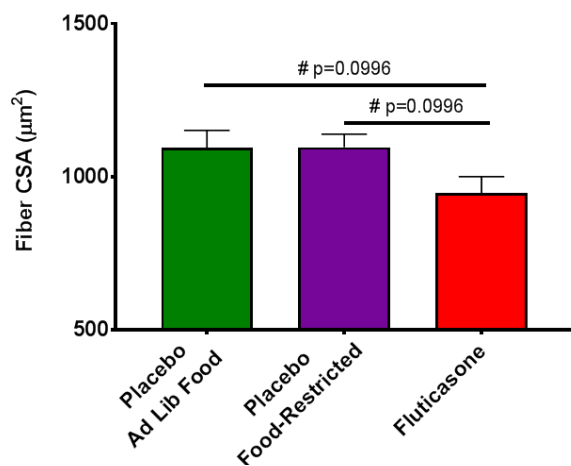


Figure 5: Styloglossus fiber CSA. N=6-7/group. P-value: Holm-Sidak post-hoc tests.

Studies in anesthetized, tracheotomized rats have shown that protrusive and retrusive tongue muscles activated together in response to tracheal occlusion, and that the net response was a retraction of the tongue (8). In a later study, the same researchers reported that co-activation of these muscles in the rat improved pharyngeal stability compared with isolated stimulation of protrusive muscles(9). Moreover, combined stimulation of protruder and retractor muscle during sleep in humans led to greater reductions in pharyngeal collapsibility than stimulating the genioglossus alone (10). Thus, animal and human studies both show that a synergistic action of protrudors and retractors is needed to stiffen the tongue and provide optimal

protection against posterior displacement during inspiration(8), thus protecting airway patency. Our results thus far suggest that chronic FP oral administration may disrupt this synergy, by creating an imbalance in the fiber cross-sectional areas and overall endurance of protrusive and retrusive muscle groups. The discord in muscle force production as retractor muscles fatigue faster may lead to a greater risk of airway obstruction. The only way to further test this hypothesis is to measure critical airway pressure (Pcrit) in anesthetized animals, as described in Polotsky et al. (11). We are in the process of setting up a collaboration with that research group led by Dr. Alan Schwartz, to measure Pcrit in anesthetized FP-treated rats in our future studies.

What opportunities for training and professional development did the project provide? Nothing to report.

How were the results disseminated to communities of interest? Results were disseminated by oral presentations in meetings with collaborating scientists, and intramural seminars in the University of Wisconsin School of Medicine and Public Health. Our work to develop a modified mouthpiece for the

Fluticasone Propionate MDI to adapt it for rats, and Fluticasone delivery efficiency testing of various mouthpiece prototypes was presented at the American Thoracic Society conference in May 2018(12). An abstract of the findings described in this report has been accepted for poster presentation at the 2018 Military Health Systems Research Symposium in August 2018, and a manuscript of these findings is currently in preparation.

What do you plan to do during the next reporting period to accomplish the goals? During the next reporting period we plan to complete MHC isoform-specific histology and fiber morphometry on the hyoglossus (another retrusive muscle of the tongue), as well as soleus and extensor digitorum longus (EDL) muscles, in order to generate a more complete picture of oral corticosteroid administration upon skeletal muscle. In parallel, we will complete the third goal of the project by assaying glutamine synthase and glycogen synthase activity in GG muscle tissue. We also plan on developing our collaboration with Dr. Alan Schwartz, to expand on our current findings, as part of a near future grant submission to this office.

IMPACT

What was the impact on the development of the principal discipline of the project? Our study generated very important empirical evidence that oral administration of corticosteroids, the standard therapy for asthma and COPD, changes the histological properties and force generation capacity of tongue muscles leading to protrusive vs. retrusive function imbalance. This may result in upper airway dysfunction during sleep that could increase the risk of OSA. These findings have critical clinical relevance, since ICS are the standard therapy and many patients with asthma are using them. Thus, further studies are paramount to clarify the relevance of our findings during sleep and wakefulness.

What was the impact on other disciplines? By testing several prototypes of a modified Fluticasone MDI for ease of use and drug delivery efficiency(12), we advanced the methodology of future studies involving oral powder delivery to laboratory animals. The disproportional histological and force generation capacity changes between retrusors and protrusor muscles have the potential to cause speech and swallowing deficits, during wakefulness and sleep, opening a new set of clinically very significant questions, since vocal cord and swallowing pathology are very common in patients with asthma. As well, our data has relevance to patients with chronic obstructive pulmonary disease, who also are increasingly prescribed these medications.

What was the impact on technology transfer? Nothing to report.

What was the impact on society beyond science and technology? Nothing to report.

CHANGES/PROBLEMS

Changes in approach and reasons for change.

Chronic administration of systemic corticosteroids (such as dexamethasone) leads to increased insulin resistance(13-16). The effect of chronic oral corticosteroid use on insulin resistance and glucose metabolism has not been studied previously, nor is it known whether changes in glucose uptake by tongue muscles could contribute to FP-induced changes in the function of those muscles. To investigate

the effect of chronic FP administration on insulin resistance and insulin sensitivity, we added an overnight fast and pedal vein blood draw to our experimental protocol. After the evening treatment on Day 28, all food was removed from the animal cage. On the morning of Day 29, blood was collected from the pedal vein. Following the blood collection, food was returned to the animal. Glucose and insulin concentration in rat sera were measured using commercially available reagents. Insulin resistance and insulin sensitivity were calculated as described in (17).

On the advice of our collaborators, Dr. John Russell and Dr. Miranda Cullins, we used SMASH software, instead of Metamorph, for muscle fiber morphometry. By making this change, we reduced project expenditures (see below) and were able to take advantage of Dr. Cullins' expertise with the software and her pre-optimized methods for rat tongue muscle morphometry.

Actual or anticipated problems or delays and actions or plans to resolve them.

- Four animals (2 in the FR group, and 2 in the placebo group) died of unknown causes during the 28-day period of experimental treatment. Necropsies of these animals showed evidence of acute lung toxicity, and veterinary examination found evidence of distress immediately after administering a puff from one particular placebo canister. The canister was eliminated from the study and submitted for gas chromatography analysis (results pending). From that point on, all new placebo canisters were tested by administering puffs to rats that were not included in the study, and observing the rats for adverse effects. The problem did not recur and no more adverse events occurred during the study.
- One animal went into cardiac and respiratory arrest during pentobarbital anesthesia and could not be resuscitated. This is an anticipated risk of anesthesia and was built into estimating the animal numbers needed for the study. Tongue contractile force data could not be obtained from this animal, but muscle tissue samples were still collected for analysis.
- Protrusive tongue data could not be obtained for 3 animals from the Placebo/Ad-Lib Fed treatment group because of complications in transecting the lateral branch of the hypoglossal nerve or malfunctions of the force transducer equipment. The force transducer was successfully repaired.

Changes that had a significant impact on expenditures

The loss of 4 rats in the FR and Placebo groups, as well as incomplete data from 4 other animals, forced us to add additional animals to the study. Eighteen rats (6 per treatment group) were purchased from Envigo in addition to the 30 originally planned for the study. The addition of these 18 animals to the study did not require an amendment to our IACUC protocol because we did not exceed the maximum approved number of animals under the IACUC protocol.

- SMASH software for muscle fiber morphometry is publicly available at no cost. Using this software obviated the need to purchase Metamorph software, enabling us to conduct experiments at less cost.

Significant changes in use or care of human subjects: Not applicable to this project.

Significant changes in use or care of vertebrate animals. As a follow-up to our fasting metabolic studies, we intended to perform a weekly overnight fast followed by morning blood draw. These changes were approved by the School of Medicine and Public Health Institutional Animal Care and Use Committee (IACUC) on 3/5/2018 and reported to the Animal Care and Use Review Office (ACURO) on 3/17/2018. However, before implementing, we were concerned the repeat fasting may impact the outcomes in the muscles; therefore, this plan was not implemented. Currently, our plan is that after completion of enzymatic assays, guided by those data, we will conduct additional experiments in similarly treated animals to better understand the associated metabolic disturbances on the ICS.

Significant changes in use of biohazards and/or select agents: Nothing to report (Not applicable to this project).

PRODUCTS:

Publications, conference papers and presentations:

- **Journal publications:** Nothing to report
- **Books or other non-periodical, one-time publications:** Nothing to report.
- **Other publications, conference papers or presentations:**
 1. Morel N, Broytman O, Martinez J, Teodorescu M. Development of an Automated Metered Dose Inhaler to Deliver Fluticasone to Rats and Examine the Effects of Corticosteroids on Upper Airway Function and Structure. Poster presentation, American Thoracic Society; San Diego, CA 2018.
 2. Setzke C, Broytman O, Russell JA, Connor N, Teodorescu M. Effect of Fluticasone Propionate on Tongue Muscles Physiology. Poster Presentation, Military Health Systems Research Symposium (MHSRS), Kissimmee FL 2018.

Website(s) or other Internet site(s) Nothing to report

Technologies or techniques Nothing to report

Inventions, patent applications, and/or licenses Nothing to report

Other Products Nothing to report

PARTICIPANTS AND OTHER COLLABORATING ORGANIZATIONS:

What individuals have worked on the project?

Name	Mihaela Teodorescu
Project Role	Principal Investigator
Researcher Identifier	ORCID ID: 0000-0002-4490-6926
Nearest Person Month Worked	1.2
Contribution to Project	Dr. Teodorescu planned and directed the scientific studies on this project, provided scientific guidance and administrative oversight. Dr. Teodorescu is also the official registrant for DEA controlled substances used on this project.
Funding Support	US Veterans Administrations MERIT funding, this PRMRP Award, University of Wisconsin School of Medicine and Public Health intramural funds.

Name	Oleg Broytman
Project Role	Associate Scientist
Researcher Identifier	N/A
Nearest Person Month Worked	2.1
Contribution to Project	Dr. Broytman planned the scientific studies, performed rat weighing and health checks, drug dosing and pedal vein blood draws. Dr. Broytman also assisted with tissue collection, histology, data analysis and interpretation.
Funding Support	US Veterans Administrations MERIT funding and this award.

Name	Christopher Setzke
Project Role	Research Intern
Researcher Identifier	N/A
Nearest Person Month Worked	10
Contribution to Project	Mr. Setzke performed rat weighing, health checks, husbandry and study drug administration. Mr. Setzke did the microscope slide preparation, immunostaining, image acquisition and muscle fiber morphometry.
Funding Support	This award only.

Name	John A. Russell
Project Role	Associate Scientist
Researcher Identifier	ORCID ID: 0000-0003-1755-3448
Nearest Person Month Worked	2.4

Contribution to Project	Dr. Russell performed the hypoglossal nerve electrode placement surgery and the contractile force recording. Dr. Russell also participated in tissue sample collection and data analysis.
Funding Support	National Institute of Deafness and Other Communication Disorders (NIDCD); National Science Foundation

Name	Nadine Connor
Project Role	Co-Investigator
Researcher Identifier	
Nearest Person Month Worked	0.5
Contribution to Project	Dr. Connor provided scientific guidance for the project, analysis and interpretation of results, and will participate in dissemination and further extension of study findings.
Funding Support	National Institute of Deafness and Other Communication Disorders (NIDCD); National Science Foundation

Name	Natalie Morel
Project Role	Undergraduate Research Assistant
Researcher Identifier	N/A
Nearest Person Month Worked	2
Contribution to Project	Ms. Morel designed the modified Metered Dose Inhaler mouthpiece for use in rats and conducted drug delivery efficiency testing.
Funding Support	University of Wisconsin School of Medicine and Public Health intramural funds.

Has there been a change in the active other support of the PD/PI or senior/key personnel since the last reporting period? Nothing to report.

What other organizations were involved as partners? Nothing to report.

SPECIAL REPORTING REQUIREMENTS

None.

APPENDIX 1: CONFERENCE ABSTRACTS

Development of an Automated MDI to Deliver Fluticasone to Rats and Examine the Effects of Corticosteroids on Upper Airway Function and Structure

Natalie Morel; Oleg Broymtman, PhD; Juan Martinez BS; Mihaela Teodorescu, MD, MS

Introduction: Obstructive Sleep Apnea (OSA) is more common among patients with asthma and inhaled corticosteroid usage may be a reason. Corticosteroids have been shown to cause myopathy of skeletal muscles. Herein, we are developing a rat model to test this effect on upper-airway muscles' function and structure. We are developing an MDI device to deliver Fluticasone automatically when a rat bites the MDI mouthpiece.

Methods: After priming a Fluticasone Metered Dose Inhaler (MDI) 220 mcg/puff, a 10G oral gavage needle was attached to its opening, to deliver the medication in a smaller radius, more appropriate for a rat mouth. A device is currently being built to automatically actuate the MDI when the rat bites on this needle. A separate, unmodified MDI was used as the comparator for the unmodified inhaler trials. As a first step, we tested the efficiency of this model by weighing the amount of medication dispensed in four trials, and comparing it to that dispensed from an unmodified inhaler. In each trial, powder from five consecutive actuations was captured on an adhesive surface and weighed with an electronic scale. During the trials, we observed deposition of drug: in the unmodified inhaler, leftover powder could be seen around the inside of the mouthpiece; in the modified inhaler, drug was deposited on the external needle surface at the point of attachment to the MDI, as well as inside the needle. Data were analyzed in Excel. A two-tailed heteroscedastic t-test was used to determine differences between the two delivery methods. Efficiency of the modified inhaler was calculated by dividing its mean by the mean delivery from the unmodified inhaler.

Results: The Table presents means of all actuations for each trial, followed by the overall mean, standard deviation and standard error of the mean for each method. Compared to the unmodified MDI version, our modified rat inhaler's efficiency rate was calculated at 46.2%.

	Unmodified Inhaler	Modified (Rat) Inhaler
Trial 1	125 mcg	120 mcg
Trial 2	220 mcg	120 mcg
Trial 3	220 mcg	60 mcg
Trial 4	300 mcg	100 mcg
Mean	216.25 mcg	100 mcg
Standard Deviation	71.6	28.3
Standard Error of the Mean	35.8	14.1
Efficiency		46.2%
T-Test, p-value	0.02	

Discussion: The current version of our system delivers inhaled medication to the rat at approximately half efficiency to the unmodified MDI. Steps to further optimize it, including sealing around the needle connection to the MDI, assessment of electrostatic properties and changing to a needle of a plastic material or lubricating the current one's inner surface are currently undertaken.

Effect of Fluticasone Propionate on Tongue Muscles Physiology

Christopher Setzke BS^{1,3}; Oleg Broymtman PhD^{1,3}; John A. Russell, PhD²; Nadine Connor, PhD²; Mihaela Teodorescu MD, MS^{1,3}

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Introduction: Obstructive Sleep Apnea (OSA) is more common in patients with asthma and inhaled corticosteroid treatment may contribute. The patency of the human upper airway depends on a fine balance of tongue protrusive and retrusive forces, that dilate and collapse, respectively, the airway. This study aims to test effects of inhaled fluticasone protrusive and retrusive tongue muscle physiology.

Methods: Male Fischer 344 inbred rats (9-months old) were dosed twice daily with a modified human inhaler containing either Fluticasone Propionate (220µg/puff) or a propellant gas (placebo), for 28 days. The rats were grouped in threes by weight and assigned to treatment groups (n=6-10/group): 1. Fluticasone, *ad libitum* fed (FP); 2. Placebo, food restricted (FR) to match FP rat food consumption; and 3. placebo, *ad libitum* fed (P). On day 29, animals were anesthetized with pentobarbital. The hypoglossal nerve was stimulated bilaterally, first intact (for retrusive tongue measurements), then with the lateral branch severed (for protrusive tongue assessments). Recordings of twitch, tetanus and fatigue index (end force/start force*100%) were acquired at each location. Twitch and tetanus data were normalized for body weight. Data were analyzed using either a 1-way or 2-way ANOVA.

Results: On protrusive testing, no significant changes occurred for max twitch force. There was a significant interaction between dose and stimulation frequency for tetanus force data (% of max tetanus force) ($p=0.0473$). No significant effect of treatment was noted in the fatigue index. On retrusive testing, FP and FR vs. the P group showed a significant increase in twitch force ($p = 0.013$ and 0.01 , respectively). There was a higher response for FP vs. P at stimulation frequencies of 20 and 40 Hz during tetanus recordings (% of max tetanus force) ($p = 0.02$ and 0.008 , respectively). Both FP and FR were significantly more fatigue resistant than P group ($p= 0.01$ and 0.004 , respectively).

Conclusions: We found that FP and FR, compared to P, led to greater twitch force and increased resistance to fatigue in the retrusive tongue muscles, whereas no changes were noted in the protrusive muscles. These data suggest that corticosteroids' muscle effects may be caused by metabolic changes related to decreased food intake; these changes are leading to stronger, more fatigue resistant retrusive compared to protrusive tongue muscles. Such physiologic alterations could deleteriously impact the balance of factors maintaining upper airway patency, setting the stage for collapse during sleep. Moreover, findings have implications for swallowing, an important process for patients with respiratory diseases.

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