**Distribution Agreement**

In presenting this thesis as a partial fulfillment of the requirements for a degree from Emory University, I hereby grant to Emory University and its agents the non-exclusive license to archive, make accessible, and display my thesis in whole or in part in all forms of media, now or hereafter now, including display on the World Wide Web. I understand that I may select some access restrictions as part of the online submission of this thesis. I retain all ownership rights to the copyright of the thesis. I also retain the right to use in future works (such as articles or books) all or part of this thesis.

Carol Zhu April 5, 2021

# APPKO mice exhibit dysphagia symptoms separate from whole body sarcopenia

By

Carol Zhu

Hyojung Choo, Ph.D

Adviser

Department of Biology

Hyojung Choo, Ph.D

Adviser

Rachelle Spell, Ph.D

Committee Member

Astrid Prinz, Ph.D

Committee Member

2021

# APPKO mice exhibit dysphagia symptoms separate from whole body sarcopenia

By

Carol Zhu

Hyojung Choo, Ph.D

Adviser

An abstract of
a thesis submitted to the Faculty of Emory College of Arts and Sciences
of Emory University in partial fulfillment
of the requirements of the degree of
Bachelor of Sciences with Honors

Department of Biology

2021

Abstract

APPKO mice exhibit dysphagia symptoms separate from whole body sarcopenia

By Carol Zhu

As the global population of elderly individuals continues to increase, economic and healthcare systems are strained by their need for long term care. One prevalent disease that impacts this demographic is Alzheimer's Disease, making up more than half of dementia case, defined as general symptoms of cognitive decline. In addition to the accumulation of ß-amyloid plaques and worsening cognitive decline, other common early symptoms of Alzheimer's include body weight loss, sarcopenia (loss of muscle mass), and dysphagia (difficulty swallowing), used as early diagnostic tools. This paper utilizes an APPKO mouse model with an amyloid precursor protein double knockout to study the relationship between sarcopenia and dysphagia, measured through lick rate. We hypothesized that APPKO mice exhibit dysphagia which leads to sarcopenia and body weight loss. This would be determined by phenotypic analysis, measurement of muscle fiber cross sectional area, and behavioral analysis. Our findings indicate that APPKO mice exhibit decreased body weight, slower rate of growth, and decreased lick speeds, although food and water consumption remain within normal range. Additionally, APPKO mice exhibit decreased endurance, locomotor activity, and TA muscle fiber size. Our findings suggest that behavioral and muscular deficiencies are independent of nutrient intake despite reduced pharynx muscle size.

# APPKO mice exhibit dysphagia symptoms separate from whole body sarcopenia

By

Carol Zhu

Hyojung Choo, Ph.D

Adviser

A thesis submitted to the Faculty of Emory College of Arts and Sciences
of Emory University in partial fulfillment
of the requirements of the degree of
Bachelor of Sciences with Honors

Department of Biology

2021

Acknowledgements:

I would like to first acknowledge my mentor and advisor, Dr. Hyojung Choo, for her unwavering support and guidance. I would also like to thank Fang Wu, Danbi Lim and Chris Zeuthen from the Choo lab for their help. Lastly, I would like to thank Dr. Rachelle Spell and Dr. Astrid Prinz for serving on my committee.

## Table of Contents

# Index of Figures

# Introduction:

## Elderly populations suffer from high rates of dementia:

The elderly population, commonly defined as individuals 65 years or older, has been rapidly growing throughout the past century at twice the rate as younger populations [1]. Despite advancements in medical technology, mortality rates for the elderly continue to rise, especially those related to cognitive decline[2]. The disproportionate amount of resources that elderly patients consume places a growing strain on healthcare systems [3–6]. A prevalent long-term illness that many elderly patients face is dementia, used as a general term to describe various forms of cognitive decline. Dementia is not characterized as a singular disease but is often a marker of underlying brain disorders. While treatment may help symptoms, there is currently no known cure for dementia, making early diagnosis critical for ameliorating or delaying severe symptoms. Both the proportion and population of dementia patients are increasing, and the total number of affected individuals worldwide is projected to exceed 110 million by 2050 [3,7], emphasizing the importance of improving early diagnostic criteria.

While dementia can be a marker for a number of underlying causes such as vascular blockages and strokes, the most common cause is due to Alzheimer's disease. Specifically, Alzheimer's disease (AD) makes up more than half of dementia cases [8,9]and is the 6th most common cause of death in the US [10]. Despite the prevalence of Alzheimer's disease and the rapidly increasing at-risk population, there is still much we do not know about the connections between the neurological, physiological, and behavioral symptoms.

## Alzheimer's Disease presents in a range of neurodegenerative, cognitive, and physiological symptoms*:*

Alzheimer's disease in a neurodegenerative disorder that presents itself through either major or minor cognitive decline. Major cognitive decline is characterized by significant interference with the patient's daily functioning such as periods of significant confusion, difficulty thinking, forgetfulness, or inability to recognize common objects or people [11]. Other diagnostic tools include analysis of cerebral spinal fluid and brain imaging using MRIs or CT scans, but the high cost of these tests creates an accessibility barrier. It is important to note that, while Alzheimer's disease cannot be definitively diagnosed until post-mortem autopsy confirms the presence of amyloid plaques in brain tissues, advancements in imaging techniques and non-neurological early diagnostic criteria have allowed for higher accuracy of diagnosis.

Examination of a patient's physiological symptoms may provide clarity when differentiating a diagnosis of Alzheimer's disease from dementia. On average, 75% of AD patients exhibit dysphagia symptoms such as difficulty eating and drinking or choking [12]. The number of dysphagic patients ranges as high as 89% in late stage individuals, but, even in the early stages, around half of patients are dysphagic, indicating that dysphagia is a marker for AD throughout all stages, even when there is little cognitive decline [12]. Dysphagia increases the risk for aspiration pneumonia even in early stages and also poses the risk of nutrient deficiencies that may lead to weight loss [13].

Physiologically, patients with Alzheimer's disease experience accelerated body weight loss that is also associated with loss of total brain volume, white matter, and cognitive performance [14]. Conversely, weight gain seemed to be an ameliorating factor for the progression of Alzheimer's disease while subjects who lost weight tended to progress faster and exhibit more

severe symptoms [15]. However, it is unclear if these symptoms are a direct or indirect consequence of Alzheimer's disease or age-related physiological muscle loss, known as sarcopenia [14].

Cognitive and neurological trends tend to be noticed in mid to late-stage patients. In early stages, when there is little to no cognitive decline, markers such as sarcopenia and dysphagia are used as diagnostic criteria. Weight loss has long been considered as one of the criteria for the clinical diagnosis of dementia [16], which affects many patients prior to development and diagnosis of Alzheimer's. Long term studies have now shown that these changes occur prior to cognitive decline in Alzheimer's patients, indicating that they are not just due to pre-Alzheimer's dementia, and that weight loss may be used as an early diagnosis specifically for Alzheimer's [17]. Specifically, sarcopenia (loss of muscle mass), decreased muscle strength, and slow gait are also considered early non-cognitive features of AD[18]. The loss of muscle mass, as opposed to fat or bone density, has been linked to cognitive defects[19] and is considered to be a risk factor that precedes cognitive decline[20]. However, sarcopenia is more difficult to diagnose due to a lack of consensus regarding diagnostic cutoffs in clinical settings so general weight loss is more commonly used as a marker for elderly patients [19]. Individuals exhibit doubled weight loss at around 1.2 pounds per year compared to normal, age-related weight loss starting around 1 year before diagnosis of Alzheimer's [21]. Understanding the mechanisms of sarcopenia may help us create clearer diagnostic criteria, so that treatment may begin even prior to cognitive defects.

## Understanding age related muscular weakness (sarcopenia):

Skeletal muscle comprises nearly half of total human body mass and is capable of an incredible range of fine and gross motor skills. However, with age, muscle mass, strength, and endurance decrease due to decreases in synthesis rates of muscle proteins and mitochondrial

proteins necessary for ATP production [22]. These factors are also impacted by lifestyle factors such as nutrient intake, growth hormone levels, and testosterone levels.

In elderly individuals, sarcopenia has been proposed as a marker of biological age, separate from chronological age [23], but the quantity and quality of nutrition are important factors that may ameliorate the pathogenesis of frailty and sarcopenia [24]. Ingesting a high protein diet is crucial to protecting against sarcopenia, and it is recommended that elderly patients consume 1.67 times as much protein per kilogram of body weight as compared to health adults [25]. However, protein deficiencies are a notable characteristic in sarcopenic elderly patients [26], perhaps due to difficulties with mastication. In conjunction with poor nutrition, other lifestyle changes such as decreased exercise, especially outdoors, may lead to Vitamin D deficiencies that are also implicated in sarcopenia severity [27,28]. It is also important to note that muscle denervation and changes in neuromuscular junction (NMJ) remodeling are markers of aging, as well as sarcopenia and muscular atrophy [29]. Ageing is a complicated process involving a myriad of factors. Thus, it may be difficult to ascertain the impacts of Alzheimer's pathology on these factors separately from normal aging. Mouse models such as the APPKO strain, which show early stage defects, allows us to study the muscular effects of Alzheimer's mutations prior to neuropathological changes and separate them from the aging processes.

## Amyloid protein precursor mutations in mice provide an applicable modelling scheme.

From the neurological side, AD is characterized by β amyloid plaques, created from an overproduction and aggregation of the Aβ protein. Aβ proteins range from 39-42 amino acids long and are made from cleaving β amyloid precursor proteins (APP) in the Aβ region using β-secretase followed by γ-secretase [30]. The most common products are 40 and 42 amino acid peptides, known respectively as Aβ 40 and Aβ 42. Cleavage failure leads to aggregation of Aβ,

which injures neurons, leads to neuroinflammation and eventually neuronal loss that are also signs of Alzheimer's disease [31]. Mutations in the APP gene and within the APP family result in the production of longer Aβ peptides that aggregate more easily [31]. Many of these lead to higher extracellular concentrations of Aβ42, which are deposited early in senile plaques in all forms of Alzheimer's disease [32]. Decreased levels of Aβ 42 and 40 in cerebral spinal fluid and changes in the Aβ 42:40 ratio have been considered as an early biomarker of Alzheimer's, indicating accumulation within the brain, although the direction of associations for each component is inconclusive [33]. These findings are reflected in mouse models as well. In transgenic APP695SWE mice, a precursor model to the one used in this study, there is a dramatic increase in Aβ 42 levels between 6 and 7 months [34].

APP mutations clearly play an important role in Alzheimer's progression and were used in some of the first mouse models to study AD. Many lines of APP mice develop age-dependent amyloid pathology representative of Alzheimer's disease, even when crossed with other AD mutation models such as one that overexpresses the PSEN1 protein [35]. However, few lines seek to emulate the early onset symptoms of dysphagia and sarcopenia with the notable exception of the APP knockout (null) model. These mice have no detectable transcriptional or translational APP gene product, nor do they exhibit behavioral or physical abnormalities at birth [36]. With age, they exhibit the impaired learning and memory performance characteristics of Alzheimer's mouse models, as well as consistently ~15% lower body weight [36]. However, there is no significant loss of hippocampal neurons or synaptic boutons (axon terminals), indicating that these deficiencies occur prior to noticeable cognitive decline [37].

A more popular mouse model utilizes the APP695SWE mutation which exhibits elevated Aβ 42 levels combined with a L166P mutation in the PSEN1 gene. PSEN1, also known as

Presenilin-1, functions as the catalytic subunit of the γ-secretase enzyme involved in cleavage[38], and its mutations are the most common source of familial Alzheimer's disease (FAD), which makes up 15-25% of all AD cases [39]. While these APP/PS1 mice do not exhibit the physiological defects that APP knockout mice do, they exhibit early age amyloid accumulation beginning from 6 weeks of age in the neocortex, which spreads through the rest of the brain through 4-5 months [40]. The accumulation of amyloid plaques, cognitive impairment and neuronal loss [41] makes this mouse model an excellent representation of Alzheimer's disease that is commonly used in current research.

## Experimental objective and hypothesis:

This paper aims to identify the relationship between dysphagia and sarcopenia in an APP knockout mouse model, known to display reduced body weight and muscular deficiencies. We will measure food and water consumption to determine if dysphagia occurs prior to and induces body weight and composition deficiencies. Additionally, we will investigate differences in muscle histology between craniofacial swallowing and limbic muscles. Phenotypic analysis will also be conducted on APP/PS1 mice to determine if findings can have a broader application, as these mice are the more commonly studied model. We expected APPKO mice to exhibit impaired food and water consumption due to dysphagia, as well as atrophy of swallowing muscles that cause sarcopenia. However, the similarities in consumption data and early onset of body weight loss indicates that dysphagia is not a cause of sarcopenia despite atrophy in pharynx muscles. Understanding the relationships between early diagnostic markers allows for increased accuracy of detection and may enhance the accuracy of early diagnosis procedures.

# Methodology

## Experimental Mice

Two mouse models, C57BL/6J mice (wild type, Jax000664), *App*$^{tm1Dbor}$/J mice (APP null, Jax004133) were purchased from Jackson Laboratories (Bar Harbor, ME; www.jax.org). APP null (APPKO) male mice (n=3) and wild type (WT) male mice (n= 4) were utilized in these experiments. Eight to 18 weeks old mice were used as noted in figure legends. Additionally, an APP PS1 mouse model (B6.Cg-Tg(APPswe,PSEN1dE9)85Dbo/Mmjax) was used to determine if findings were applicable to other AD mouse models. Experiments were performed in accordance with approved guidelines and ethical approval from Emory University's Institutional Animal Care and Use Committee and in compliance with the National Institutes of Health.

## Food and water consumption.

At the start (day 1) of weeks 8, 9, 10, and 16, mice were weighed and placed in individual cages with 25mL water pipettes with graduated 0.2mL markings and a predetermined amount of food pellets. Body weight, water level, and remaining food weight were recorded from day 1 through day 4 every 24 hours. If leakage occurred in the water pipettes, data from all three categories from that day was excluded from analysis as water consumption positively correlates with food consumption and body weight.

## Dysphagia assays

At the end of day 4 of food and water consumption experiments, water pipettes are removed for 16 hours overnight to motivate drinking for lick speed analysis on next day. Pipettes are replaced one at a time and mice are recorded for a total of approximately 30 seconds of drinking. Videos are exported to an editing app such as iMovie and slowed down 10x in order to

determine the number of licks per second. Week 7 was used as an acclimation period for water pipette usage before dysphagia experiments.

## Rotarod experiments

Mice (17 weeks old) were introduced to the rotarod machine one at a time using a modified version of the Standard Operating Procedure from Dr. Stacey J Sukoff Rizzo (Jackson Laboratory Mouse Neurobehavioral Phenotyping Facility). Mice were introduced one at a time at a constant speed of 20rpm for five trials, with a maximum time of 300 seconds per trial. Prior to placing each mouse and in between subjects, the rod and trip plate were sanitized with 70% EtOH solution and urine and feces were removed. Experiments were conducted over four days at increasing speeds of 20, 25, and 30rpm with one day of training.

## Muscle collection

Mice were euthanized at 18 weeks following rotarod experiments using 5% isoflurane soaked on a cotton ball in a closed container, followed by spinal dislocation to ensure death. Three craniofacial muscles (pharynx, masseter, and tongue) and four limb muscles (left and right TA, left and right GA) were collected and frozen in OCT and cooled using methylbutane suspended above liquid nitrogen. TA and GA muscle weight and total body weight were measured prior to freezing.

## Cryostat sectioning

A representative craniofacial muscle (pharynx) and limb muscle (TA) are dissected from APPKO (n=3) and WT mice (n =4) and sectioned using a cryostat at 10µm thickness. Sections are mounted on Superfrost slides and stored in a -20°C freezer until staining.

## Immunofluorescence staining

Muscle sections are immunostained for laminin and DAPI. Sections are first blocked off with a hydrophobic PAP pen before being immersed in blocking solutions, comprised of 50µL 5% donkey serum (DS) and 100µL 10% bovine serum albumin (BSA) in 1mL PBST for 1 hour at room temperature. Then, primary antibody solution of 1:400 rabbit laminin in PBST is applied overnight at 4°C. Sections are washed five times for three minutes each with PBST before secondary antibody solution is added, which is comprised of 1:200 donkey anti rabbit FITC in PBST for 1 hour at room temperature. Following another five rounds of three-minute washing with PBST, a PBS wash is added for five minutes. Then, slides are soaked in 1:1000 4′,6-diamidino-2-phenylindole (DAPI) in PBS for 2 minutes. Following one last PBS wash, slides are mounted in Vectashield and sealed with nail polish and a cover slide.

In order to visualize the histology of sections, H&E staining was done using a 2-minute soak in hematoxylin, followed by 2 washes, 1 minute in Scott's Water, 1 wash, and 1 minute each in each of the following solutions: Eosin, 95% EtOH, 95% EtOH, 100% EtOH, 100% EtOH, 1:1 50% EtOH and 50% xylene, 100% xylene, 100% xylene. Lastly, slides are mounted in toluene and sealed with nail polish.

Images are taken using a fluorescent microscope at 10x magnification with embedded size metadata.

## Data interpretation:

Laminin and DAPI stained muscles are imaged using a fluorescence microscope and analyzed using an ImageJ macro (Myosoft)[42] to count total fiber number and average cross sectional area of fibers. This data will be compared between craniofacial and limb groups as well as between experimental and control mice to determine if muscle size is different in an APPKO

mouse model. Furthermore, H&E stained muscles will be used to determine physiological markers to ensure that the areas analyzed are consistent between samples.

## Statistical Analysis:

Amount of food and water consumed and change in body weight were averaged for each group and compiled by week to compare rate of change. Time and number of tongue protrusions for each dysphagia assay video were summed for each mouse and averaged by week for both groups. Muscle weight data was determined as a percentage of muscle weight to total body weight. Immunofluorescence staining was analyzed using Myosoft, an ImageJ macro [42]. Cross sectional areas of muscle fibers are averaged for each group for comparison. All statistical analysis is conducted using Prism using standard thresholds of *$p<0.05$ and **$p<0.01$ to determine significance.

## Results

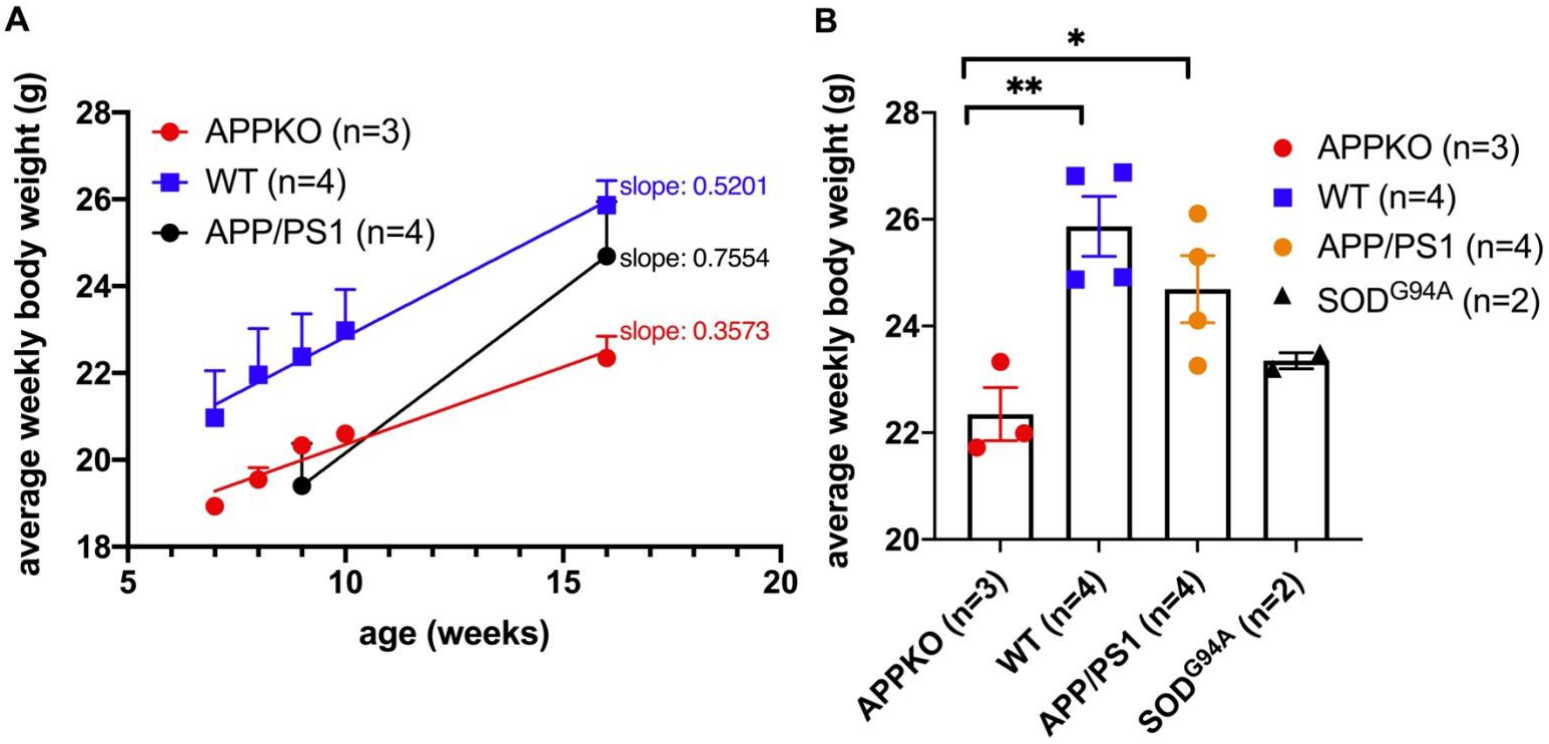



Figure 1: **APPKO mice exhibit lower body weight and rate of growth compared to WT.** (*p<0.05, **p<0.01). a) Body weight and rate of growth over time. b) Comparisons of body weight at week 16. APPKO body weight is comparable to $SOD^{G94A}$ (ALS model) while APP/PS1 (AD model) mice are comparable to WT. Data were measured weekly and averaged from 7-10 weeks and 16 weeks. $SOD^{G94A}$ mice were used as a positive control as they are known to exhibit decreased body weight. Bars represent SEM. Two tailed t tests were conducted between all group pairings for each week.

In order to determine body weight differences between APPKO and WT mice, we tracked body weight starting from adolescence (7 weeks) until early adulthood (16 weeks) (Figure 1). Data revealed around a 9.7% difference between experimental and control groups

week 7, which grew to 13.6% by week 16. Additionally, linear regression revealed that the rate of growth for APPKO mice was slower compared to WT mice. Linear regression indicated a growth rate of 0.3482 g/week for APPKO mice and 0.5347g/week for WT mice (Figure 1). Comparison to a positive control of 16 weeks old $SOD^{G94A}$ mice revealed that APPKO body weights were comparable at 16 weeks. APP/PS1 mice, another commonly used AD mouse model exhibited similarly low body weight as APPKO mice at 9 weeks. However, by 16 weeks, body weight data was consistent with WT mice and significantly higher than APPKO body weight. Comparison of data at week 16 revealed similarities between WT and APP/PS1 groups, while both groups had higher body weight compared to APPKO and SOD mice.

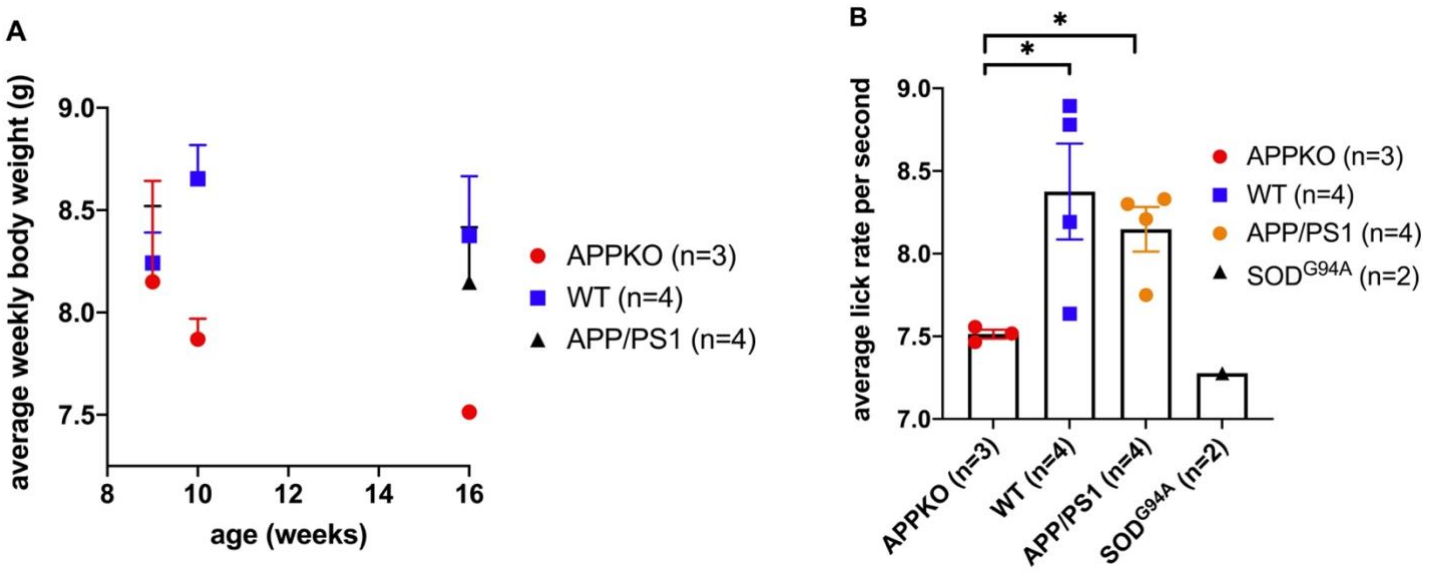



Figure 2: **APPKO mice exhibit slower lick speeds beginning at 10 weeks** a) Lick rate from weeks 9, 10 and 16. b) Comparison of lick rates at week 16. Approximately 30 seconds of drinking were recorded and analyzed for the number of tongue protrusions per second. Male mice were utilized at 9, 10, and 16 weeks of age. $SOD^{G94A}$ mice were used as a positive control as they are known to exhibit dysphagia. Error bars represent SEM. SEM is too small for visualization in 16-week APPKO mice (n=3). Two tailed t tests were conducted between APPKO, APP/PS1 and WT groups at all available weeks (*$p<0.05$)

In conjunction with body weight data, we also tracked lick speeds of male mice as a measure of drinking capability and dysphagia (Figure 2). Lick rate was determined to be similar from at 9 weeks for both groups. However, lick rates begin to diverge at 10 weeks. APPKO mice exhibited significantly lower lick rates, with an average of 7.87 licks per second for APPKO mice and 8.65 licks per second for WT mice. At 16 weeks, difference increases with an average of 7.51 licks per second for APPKO mice and 8.38 licks per second for WT mice. Similar to body weight results, APPKO mice exhibit similarly slow lick rates to the $SOD^{G94A}$ (ALS mouse

model) mice, used as a positive control group. Preliminary comparison to APP/PS1 mouse models at 9 weeks revealed no significant difference compared to WT. Data from the week 16 dysphagia assay indicates that APP/PS1 lick rates are comparable to WT and significantly higher than APPKO lick rate.

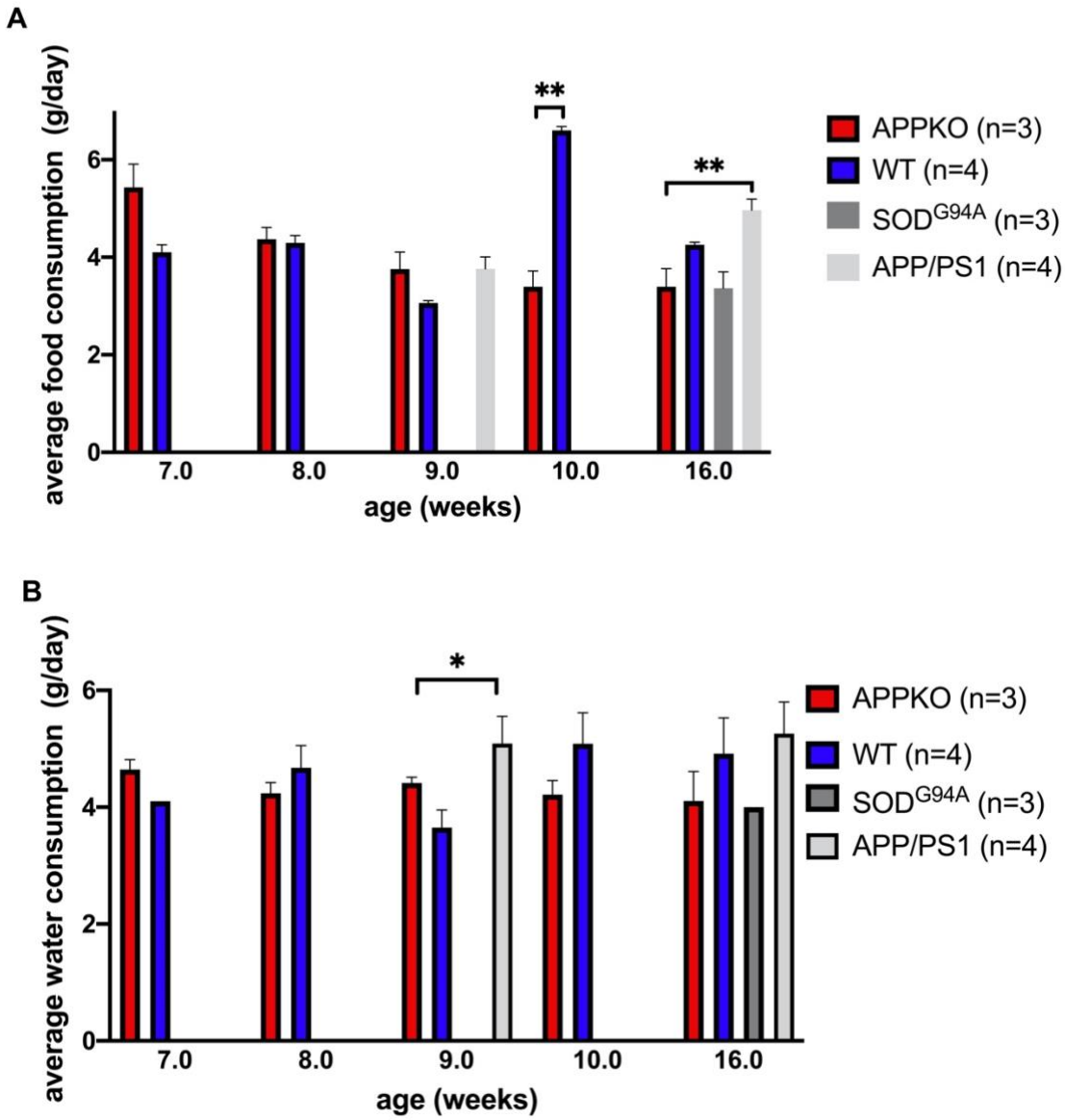



Figure 3. **APPKO mice exhibit few differences in consumption rates compared to WT** (*p<0.05, **p<0.01). a) Food consumption remains within normal range. WT mice exhibit higher food consumption compared to APPKO mice at week 10. APP/PS1 mice exhibit higher food consumption compared to APPKO mice at week 16. b) Water consumption remains within normal range. APP/PS1 mice exhibit higher water consumption compared to APPKO mice at week 9. Bars represent SEM. Two tailed t tests were conducted between all group pairings per week. Male mice aged 7, 8, 9, 10, and 16 weeks were individually studied for four consecutive

days prior to dysphagia assay experiments. Measurements were taken at the same time every day to ensure consistency.

We were interested in food and water consumption levels both as an indicator of swallowing success and a possible contributing factor to body weight differences. Despite differences in body weight, rate of growth and licking speeds, no consistent differences were observed in food and water consumption data for any of the four groups. Food and water consumption trends are poorly described in Alzheimer's mouse models. However, all groups of mice remained within generally accepted ranges of 4.3 +/- 1.1 g per 30 g body weight to 8.8 +/- 0.2 g per 30 g body weight for food consumption, and 3.9 +/- 0.2 ml/mouse to 8.2 +/- 0.3 ml/mouse for water consumption[43]. Comparisons were conducted at 10 weeks consistent with prior studies[43].

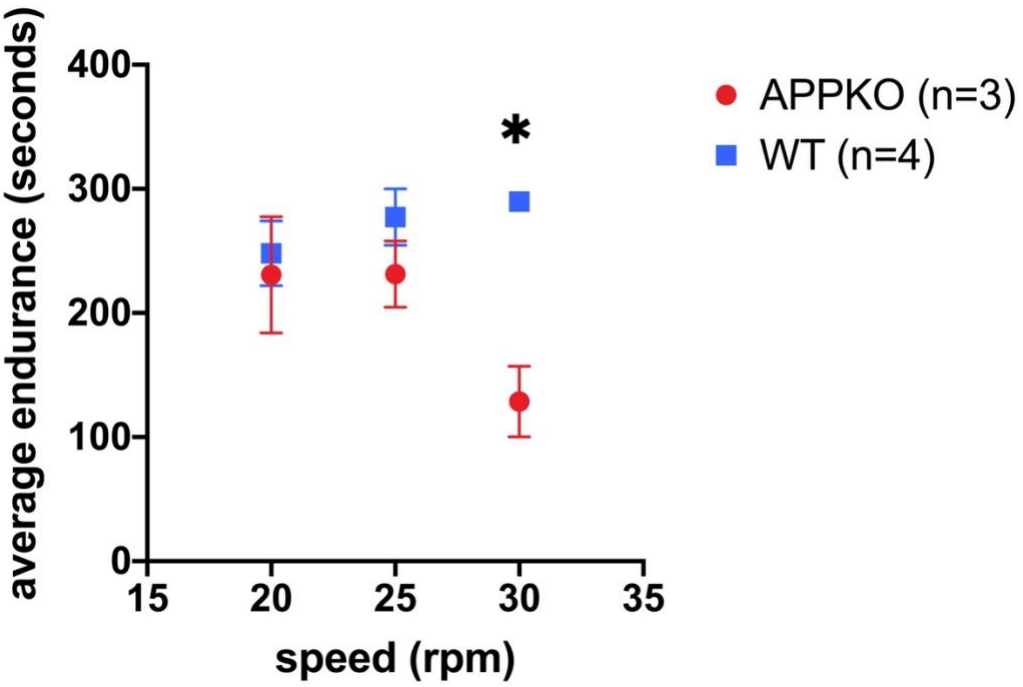



Figure 4a. **APPKO mice exhibit decreased rotarod performance at high speeds** Following one day of training, each male mouse aged 17 weeks underwent a maximum of 5 trials capped at 300 seconds each at speeds of 20, 25 and 30 revolutions per minute (rpm). Trials for each speed were conducted on separate days. Error bars represent SEM. All WT mice were able to reach maximum endurance of 300 seconds at 30 rpm. Two tailed t tests were conducted for APPKO and WT groups for each week (*$p<0.05$).

In order to determine if there were locomotor and grip strength defects, all mice underwent a rotarod endurance test at 17 weeks, following the last week of tracking and dysphagia experiments. Rotarod endurance tests, administered at 20, 25, and 30 rpm revealed differences in overall success, as well as success rates of maximum endurance between groups. Although there were no significant differences between performances at 20 and 25 rpm, APPKO mice exhibit decreased endurance times compared to WT mice at 30rpm.

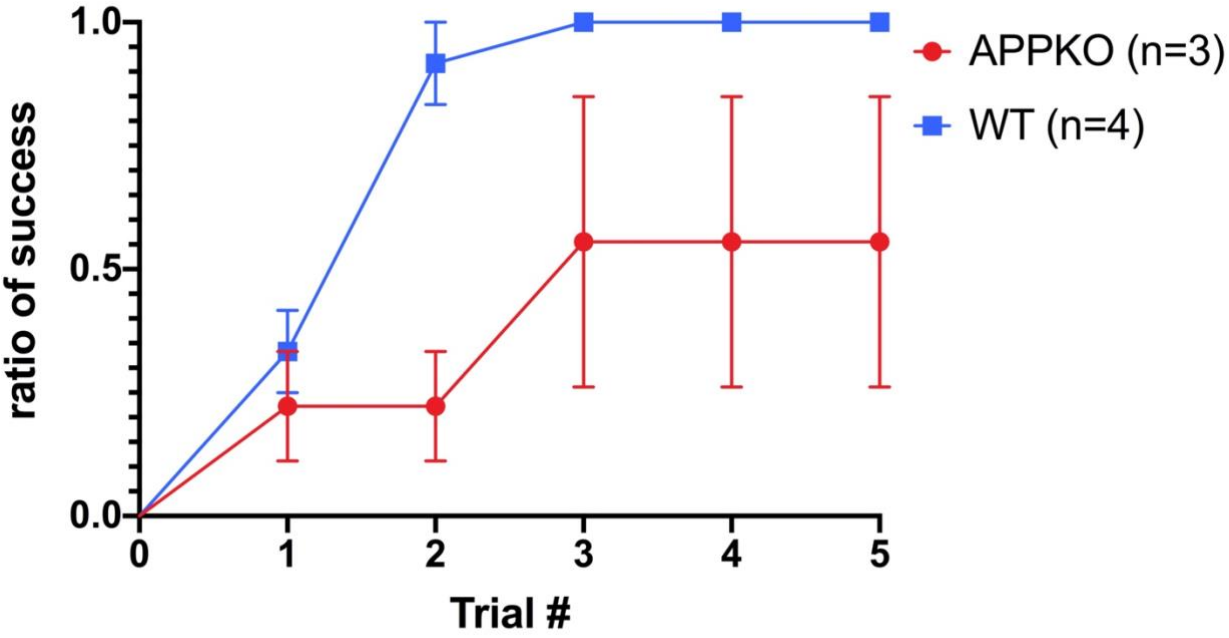



Figure 4b: **APPKO mice consistently exhibit decreased endurance.** Success rate for rotarod performance of 17-week-old male mice was averaged across performances at all three speeds. Success rate was defined as the percentage of mice who were able to reach the maximum endurance time of 300 seconds. Error bars indicate SEM. All WT mice were able to reach complete success by trial #3 so SEM is 0.

As a measure of learning success and endurance, the success rate was determined for each of the 5 trials and averaged between the three speeds. Quantitative data shows that WT were consistently able to reach the maximum endurance time of 300 seconds at all three speeds, while all three APPKO mice failed in all trials at 30 rpm. Additionally, WT mice were consistently able to reach maximum time in fewer trials compared to APPKO mice, as seen through the higher success rate. These findings are consistent on average, and across all three speeds (Supplemental Figure 1).

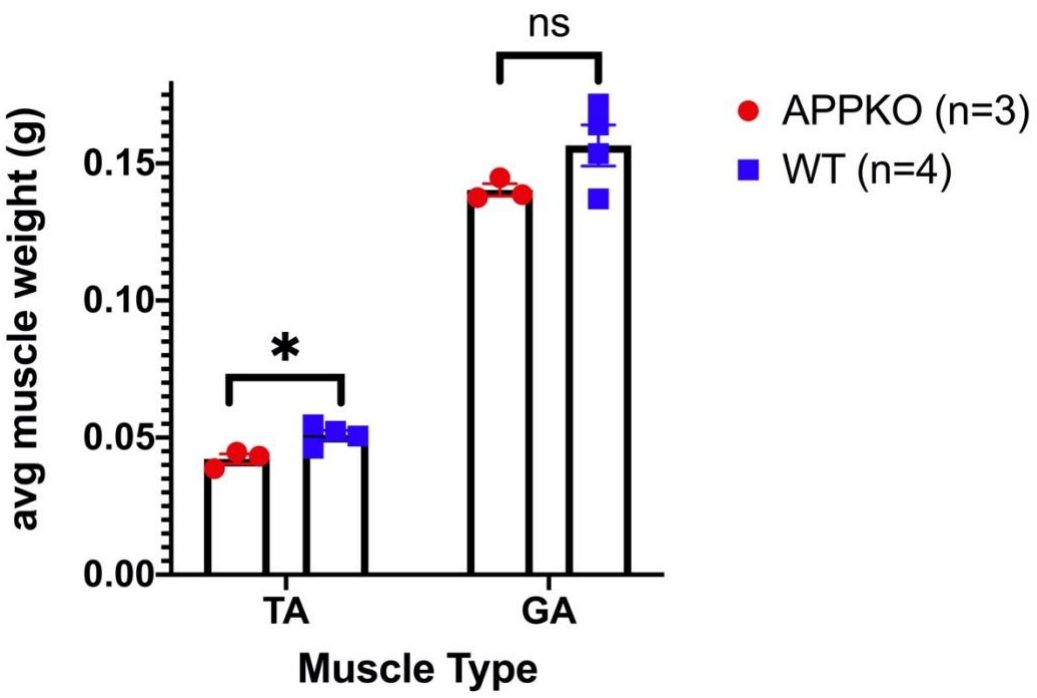



Figure 5: **APPKO mice exhibit decreased TA muscle mass** (*p<0.05). WT mice exhibit higher absolute muscle mass for TA (tibialis anterior) but not GA (gastrocnemius) limb muscles. Samples from both left and right hindlimbs were collected from male mice aged 18 weeks and weighed prior to freezing. Bars indicate SEM. Two tailed t tests were conducted between APPKO and WT groups for each muscle type.

In order to determine limb muscle deficiencies that could explain the poor rotarod performance of APPKO mice, TA and GA muscles were weighed and normalized against total body weight data. Comparison of muscle weight was done only on TA and GA data due to the ease of whole sample collection. The absolute weight of TA but not GA muscles of APPKO mice were decreased compared to WT mice. Larger variation in GA muscles may be due to difficulties with dissection due to its more proximal location to the body.

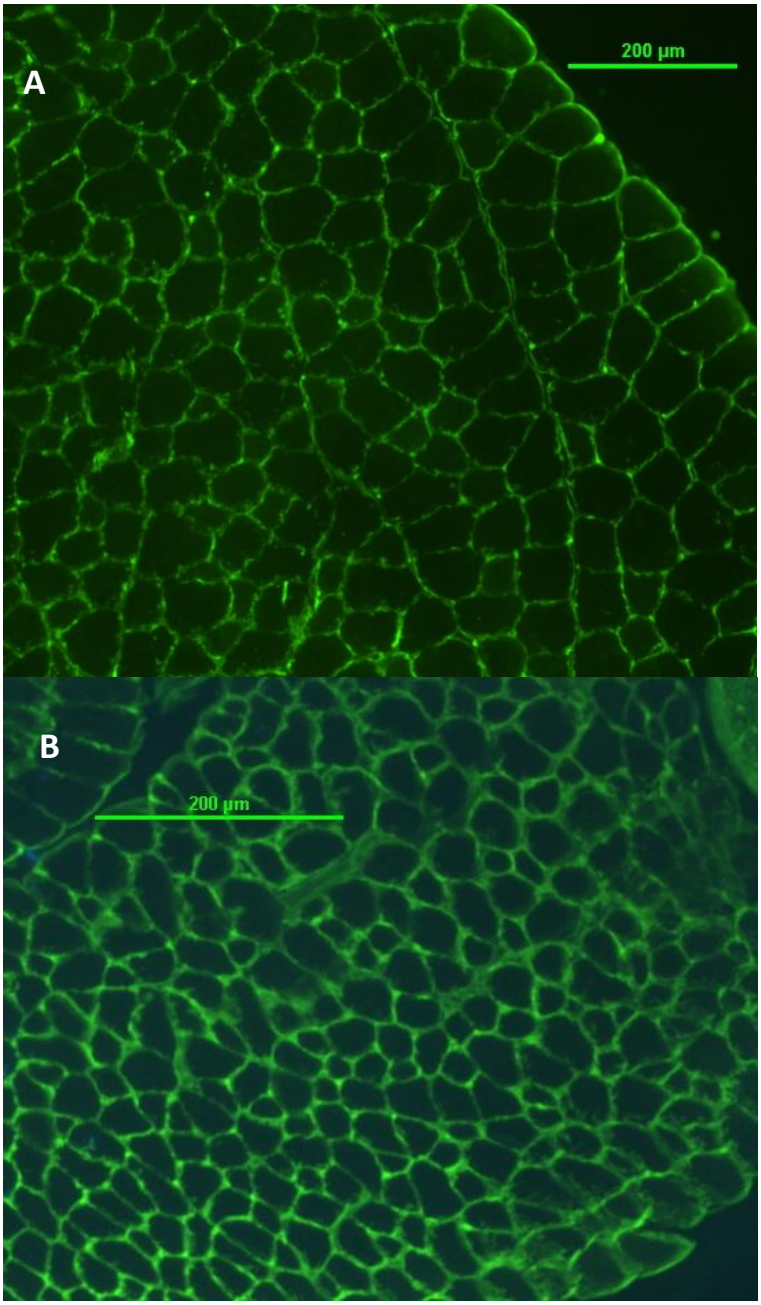



Figure 6: **Representative images of TA and pharynx sections**. a) Close up image of TA muscle fibers. b) Close up image of target CP (cricopharyngeal bar) area. Muscles were cryosectioned at 18 weeks and stained with anti-laminin antibody (green fluorescence).

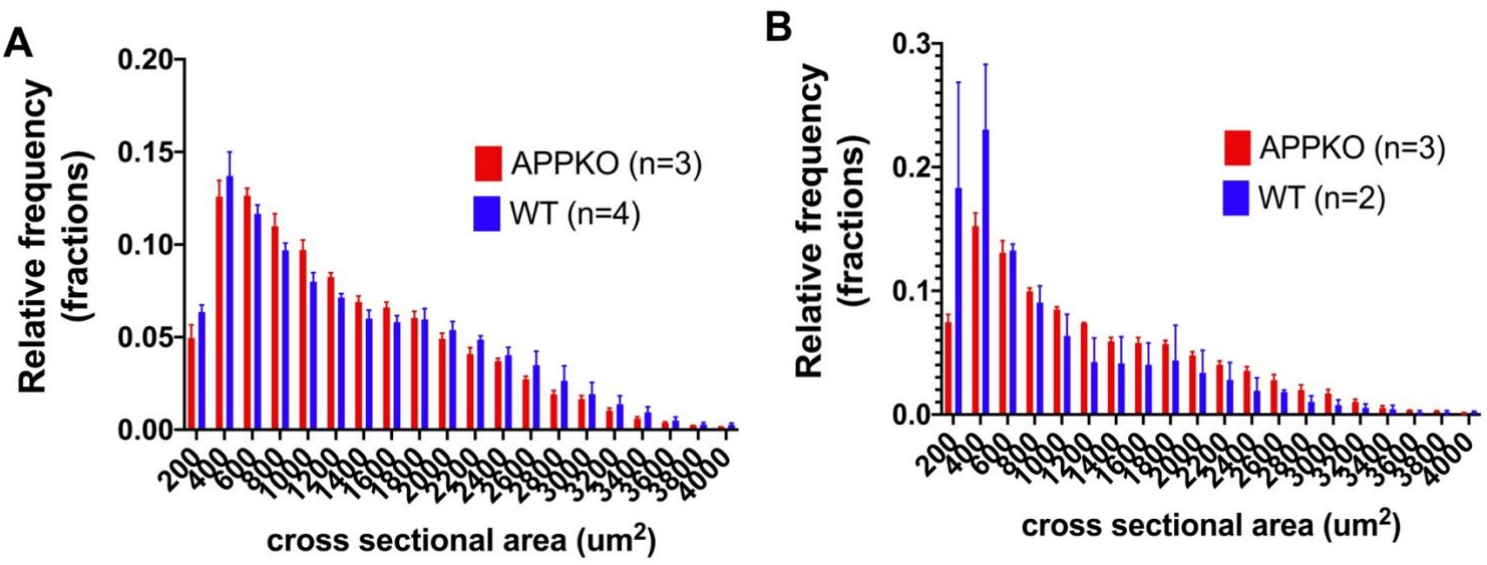



Figure 7. **APPKO mice exhibit similar fiber size in pharynx and TA muscles** a) WT TA muscle have a bimodal distribution. TA fiber size is equivalent between groups. Whole section images were analyzed for TA muscles. Distributions for all mice were averaged b) APPKO pharynx cross sectional area is equivalent between groups. Distributions for all mice were averaged. Landmarks for pharynx analysis were determined using H&E staining. Muscles were cryosectioned at 18 weeks and stained with anti-laminin antibody (green fluorescence). WT pharynx has sample size n=2 due to loss of samples during handling. Two-way ANOVA was conducted between samples, +/- SEM pictured.

In order to determine if there were differences in muscle histology, muscles were membrane stained using laminin to determine cross sectional area. Pharynx, specifically the cricopharyngeal bar, was chosen as a representative craniofacial muscle due to its importance in swallowing function and TA (tibialis anterior) was chosen as the representative limb muscle due to its importance in gross motor function. Average cross-sectional area of muscle fibers was

analyzed to determine if findings were consistent across muscle groups. Comparison of TA muscle size revealed comparable APPKO muscle fiber cross sectional area size compared to WT. Pharynx cross sectional area was smaller for APPKO mice, although the small sample size of WT mice presented challenges for statistical analysis.

# Discussion:

## Overall findings and implications:

We found that APPKO mice exhibited lower body weights and dysphagia symptoms, determined through slower lick rate. However, food and water consumption data revealed no differences and low body weight precedes onset of dysphagia, indicating that dysphagia does not cause sarcopenia. APPKO mice also exhibit decreased locomotor function and endurance in rotarod trials at high speeds, as well as lower rates of success which may be indicative of cognitive defects or defects in fine motor skill such as decreased strength. Despite similarities in compiled CSA data, decreased cross sectional area of pharynx muscles and TA muscles when compiled by mouse indicates defects in both swallowing muscles and limb muscles but to differing degrees. APPKO mice exhibit early-stage physiological defects such as decreased body weight and growth rate, as well as decreased locomotor activity possibly indicative of cognitive defects earlier than expected. Body weight, food, water, and lick rate data from APP/PS1 mice through 16 weeks indicate that APP/PS1 exhibit physical phenotypes comparable to WT. Therefore, the findings in this paper are not applicable to general Alzheimer's disease studies or other mouse models.

## Body weight results:

Both APPKO and WT mice in this study exhibited lower body weights than previously reported, perhaps due to metabolic of activity differences. Increased activity or decreased ability to incorporate nutrients could explain the differences in body weight and growth despite similarities in food intake. At 9 weeks of age, Zheng et al[36] noted an average body weight of 28.7 +/- 1.1g for male wild type mice, significantly higher than the 22.37 average noted in my group. APPKO mice were averaged at 23.0g +/- 0.3, while corresponding mice in this study

averaged around 20.22g (Figure 1). Despite differences in overall body weight, data seems to confirm previous findings that the APP null mouse model exhibits decreased body weight as well as a slower rate of growth, although with a smaller difference. APPKO mice in this study exhibit 9-13% lower body weight compared to the previously reported 15% [36].

### Dysphagia results:

Most current research focuses on neuropathology of AD mouse models as well as behavioral or learning defects. This paper presents evidence that an APP knockout mouse model displays symptoms of dysphagia beginning at 10 weeks as expected, shortly before reaching maturation and adulthood at 3 months. Although the time limitations of this study prevented further analysis, these dysphagia symptoms are shown to persist into adulthood until at least 16 weeks (Figure 2). Together these data confirm that the APP knockout mouse model is an accurate model to study sarcopenia and dysphagia. Although APP/PS1 data is preliminary, the similarity of results suggests that these findings are applicable to other AD mouse models as well.

### Food and water consumption results:

Food and water consumption data revealed overall lower than average food and water consumption in APPKO mice but no statistical difference between groups. Circadian rhythm disruptions have been shown to significantly impact mouse food and water consumption. Average food consumption for 7–10-week-old mice over 4 days ranged from 20.36+/- 0.53 under normal light/dark conditions, to 18.03 +/- 0.36g under constant light conditions. The same group of mice drank on average 30.06 +/- 1.22 mL under normal conditions but only 26.17 +/- 0.63 mL under constant light conditions[44]. When adjusted to daily consumption rates as measured in this study, mice in constant light conditions ingested approximately 4.5g of food and

6.5 mL of water per day. While lights in the experimental room were turned off at the end of each day, ambient light from the external lab could have affected circadian rhythm and therefore food and water consumption. While food and water consumption are independent from each other, constant light and changes in circadian rhythm are known to affect both[45].

Additionally, while dysphagia symptoms were noted in APPKO mice, it is possible that mice compensate by taking longer to eat and drink. A final explanation for these data may be due to the notable engineering difficulties we faced during pipette construction. If leakage was noted overnight, both food and data consumption from that day was excluded during analysis meaning that the sample size was less than ideal. Additionally, air bubble could be trapped within the drinking apparatus, preventing water from moving downwards and thus skew measured data. Pipette construction must be improved for further studies, perhaps through a sealed system that can be weighed to determine changes in water level. However, it is important to note that the pipette tip is a necessary part of the construction to allow mice time to acclimate to the drinking system prior to dysphagia assays. The similarities in consumption data seems to indicate that decreased body weight and growth are not due to nutritional defects stemming from dysphagia symptoms. Rather, muscular and behavioral defects are independent from nutrient intake.

### Analysis of locomotor function findings

Rotarod data experiments further confirmed muscular and behavioral deficiencies in APPKO mice. Due to procedural limitations, the maximum length of time that a mouse could stay on the machine was 300 seconds per trial, thus preventing us from gathering a complete measure of endurance. Additionally, capping the endurance time skews data during analysis. However, comparison of average endurance time and qualitative observations of raw data confirmed observations by Zheng *et. al* of reduced forelimb grip strength, necessary for the

climbing movement required in the rotarod experiments and general decreased locomotor activity [36]. In conjunction with dysphagia and body weight data, this further confirms the muscle defects in APP knockout mice and suggests that it affects both limb and craniofacial muscles.

## Muscle histology analysis:

While many diagnostic criteria such as cognitive decline tend to be noticed after significant amyloid protein accumulation, there is also evidence that neurological impacts such as memory defects, decrease in long term potentiation, and behavioral deficits occur prior to plaque deposition [34]. Muscle mass analysis revealed decreased TA limb muscle mass consistent with lower body weights in APPKO mice. Cross sectional area analysis of TA muscles confirms muscle mass findings; despite similar fiber numbers, WT TA muscle fibers exhibit a bimodal distribution and larger average size compared to APPKO data. Alternatively, these data may be indicative of early neurocognitive deficiencies with fine motor skills such as grasp and balance adjustment that go unnoticed in early stages, prior to cognitive decline. APPKO mice exhibit reduced branching in hippocampal neurons beginning from 2-3 weeks after birth, resulting in reduced synapse formation [46], as well as increased levels of calcium channels which may cause defects in calcium handling, synaptic plasticity and neuronal network properties[47,48]. Further analysis of post-mortem brain sections may clarify the underlying causes of behavioral defects.

## Implications:

Sarcopenia and dysphagia are unrelated early diagnostic markers of Alzheimer's disease. Additionally, the effects of sarcopenia are not evenly distributed throughout the body. Further research may be needed to determine specific affected muscles for early diagnosis. However,

understanding the relationship between sarcopenia and dysphagia in Alzheimer's disease models allows for more accurate treatment plans regarding nutrient intake, exercise, and early diagnostic criteria.

Sources Cited:


1. Sidney, S.; Go, A.S.; Jaffe, M.G.; Solomon, M.D.; Ambrosy, A.P.; Rana, J.S. Association Between Aging of the US Population and Heart Disease Mortality From 2011 to 2017. *JAMA Cardiol.* **2019**, *4*, 1280–1286, doi:10.1001/jamacardio.2019.4187.
2. Todd, S.; Barr, S.; Roberts, M.; Passmore, A.P. Survival in Dementia and Predictors of Mortality: A Review. *Int. J. Geriatr. Psychiatry* **2013**, *28*, 1109–1124, doi:https://doi.org/10.1002/gps.3946.
3. Waldo, D.R.; Sonnefeld, S.T.; McKusick, D.R.; Arnett, R.H. Health Expenditures by Age Group, 1977 and 1987. *Health Care Financ. Rev.* **1989**, *10*, 111–120.
4. Wodchis, W.P.; Austin, P.; Henry, David; Corallo, Ashley; Newman, Alice Little Ado (yet) About Much (Money). **2012**, 27.
5. Bloom, D.E.; Chatterji, S.; Kowal, P.; Lloyd-Sherlock, P.; McKee, M.; Rechel, B.; Rosenberg, L.; Smith, J.P. Macroeconomic Implications of Population Ageing and Selected Policy Responses. *The Lancet* **2015**, *385*, 649–657, doi:10.1016/S0140-6736(14)61464-1.
6. Lee, J.Y.; Muratov, S.; Tarride, J.-E.; Holbrook, A.M. Managing High-Cost Healthcare Users: The International Search for Effective Evidence-Supported Strategies. *J. Am. Geriatr. Soc.* **2018**, *66*, 1002–1008, doi:https://doi.org/10.1111/jgs.15257.
7. Wimo, A.; Winblad, B.; Aguero-Torres, H.; von Strauss, E. The Magnitude of Dementia Occurrence in the World. *Alzheimer Dis. Assoc. Disord.* **2003**, *17*, 63–67.
8. Tom, S.E.; Hubbard, R.A.; Crane, P.K.; Haneuse, S.J.; Bowen, J.; McCormick, W.C.; McCurry, S.; Larson, E.B. Characterization of Dementia and Alzheimer's Disease in an Older Population: Updated Incidence and Life Expectancy With and Without Dementia. *Am. J. Public Health* **2014**, *105*, 408–413, doi:10.2105/AJPH.2014.301935.

9. Jönsson, L.; Wimo, A. The Cost of Dementia in Europe: A Review of the Evidence, and Methodological Considerations. *PharmacoEconomics* **2009**, *27*, 391–403, doi:10.2165/00019053-200927050-00004.
10. Thies, W.; Bleiler, L. 2013 Alzheimer's Disease Facts and Figures. *Alzheimers Dement.* **2013**, *9*, 208–245, doi:https://doi.org/10.1016/j.jalz.2013.02.003.
11. Association, A.P. *Diagnostic and Statistical Manual of Mental Disorders (DSM-5®)*; American Psychiatric Pub, 2013; ISBN 978-0-89042-557-2.
12. Seçil, Y.; Arıcı, Ş.; İncesu, T.K.; Gürgör, N.; Beckmann, Y.; Ertekin, C. Dysphagia in Alzheimer's Disease. *Neurophysiol. Clin. Neurophysiol.* **2016**, *46*, 171–178, doi:10.1016/j.neucli.2015.12.007.
13. Kukull, W.A.; Brenner, D.E.; Speck, C.E.; Nochlin, D.; Bowen, J.; McCormick, W.; Teri, L.; Pfanschmidt, M.L.; Larson, E.B. Causes of Death Associated with Alzheimer Disease: Variation by Level of Cognitive Impairment Before Death. *J. Am. Geriatr. Soc.* **1994**, *42*, 723–726, doi:https://doi.org/10.1111/j.1532-5415.1994.tb06531.x.
14. Burns, J.M.; Johnson, D.K.; Watts, A.; Swerdlow, R.H.; Brooks, W.M. Reduced Lean Mass in Early Alzheimer Disease and Its Association With Brain Atrophy. *Arch. Neurol.* **2010**, *67*, 428–433, doi:10.1001/archneurol.2010.38.
15. White, H.; Pieper, C.; Schmader, K. The Association of Weight Change in Alzheimer's Disease with Severity of Disease and Mortality: A Longitudinal Analysis. *J. Am. Geriatr. Soc.* **1998**, *46*, 1223–1227, doi:https://doi.org/10.1111/j.1532-5415.1998.tb04537.x.
16. McKhann, G.; Drachman, D.; Folstein, M.; Katzman, R.; Price, D.; Stadlan, E.M. Clinical Diagnosis of Alzheimer's Disease: Report of the NINCDS-ADRDA Work Group* under

the Auspices of Department of Health and Human Services Task Force on Alzheimer's Disease. *Neurology* **1984**, *34*, 939–939, doi:10.1212/WNL.34.7.939.

17. Stewart, R.; Masaki, K.; Xue, Q.-L.; Peila, R.; Petrovitch, H.; White, L.R.; Launer, L.J. A 32-Year Prospective Study of Change in Body Weight and Incident Dementia: The Honolulu-Asia Aging Study. *Arch. Neurol.* **2005**, *62*, 55–60, doi:10.1001/archneur.62.1.55.
18. Ogawa, Y.; Kaneko, Y.; Sato, T.; Shimizu, S.; Kanetaka, H.; Hanyu, H. Sarcopenia and Muscle Functions at Various Stages of Alzheimer Disease. *Front. Neurol.* **2018**, *9*, doi:10.3389/fneur.2018.00710.
19. Scisciola, L.; Fontanella, R.A.; Surina; Cataldo, V.; Paolisso, G.; Barbieri, M. Sarcopenia and Cognitive Function: Role of Myokines in Muscle Brain Cross-Talk. *Life* **2021**, *11*, 173, doi:10.3390/life11020173.
20. Cabett Cipolli, G.; Sanches Yassuda, M.; Aprahamian, I. Sarcopenia Is Associated with Cognitive Impairment in Older Adults: A Systematic Review and Meta-Analysis. *J. Nutr. Health Aging* **2019**, *23*, 525–531, doi:10.1007/s12603-019-1188-8.
21. Johnson, D.K.; Wilkins, C.H.; Morris, J.C. Accelerated Weight Loss May Precede Diagnosis in Alzheimer Disease. *Arch. Neurol.* **2006**, *63*, 1312–1317, doi:10.1001/archneur.63.9.1312.
22. Short, K.; Nair, K. Mechanisms of Sarcopenia of Aging. *J. Endocrinol. Invest.* **1999**, *22*, 95–105.
23. Fisher, A.L. Of Worms and Women: Sarcopenia and Its Role in Disability and Mortality. *J. Am. Geriatr. Soc.* **2004**, *52*, 1185–1190, doi:https://doi.org/10.1111/j.1532-5415.2004.52320.x.

24. Cruz-Jentoft, A.J.; Kiesswetter, E.; Drey, M.; Sieber, C.C. Nutrition, Frailty, and Sarcopenia. *Aging Clin. Exp. Res.* **2017**, *29*, 43–48, doi:10.1007/s40520-016-0709-0.

25. Bauer, J.; Biolo, G.; Cederholm, T.; Cesari, M.; Cruz-Jentoft, A.J.; Morley, J.E.; Phillips, S.; Sieber, C.; Stehle, P.; Teta, D.; et al. Evidence-Based Recommendations for Optimal Dietary Protein Intake in Older People: A Position Paper From the PROT-AGE Study Group. *J. Am. Med. Dir. Assoc.* **2013**, *14*, 542–559, doi:10.1016/j.jamda.2013.05.021.

26. Yoo, J.-I.; Lee, K.-H.; Choi, Y.; Lee, J.; Park, Y.-G. Poor Dietary Protein Intake in Elderly Population with Sarcopenia and Osteosarcopenia: A Nationwide Population-Based Study. *J. Bone Metab.* **2020**, *27*, 301–310, doi:10.11005/jbm.2020.27.4.301.

27. Morley, J.E.; Argiles, J.M.; Evans, W.J.; Bhasin, S.; Cella, D.; Deutz, N.E.P.; Doehner, W.; Fearon, K.C.H.; Ferrucci, L.; Hellerstein, M.K.; et al. Nutritional Recommendations for the Management of Sarcopenia. *J. Am. Med. Dir. Assoc.* **2010**, *11*, 391–396, doi:10.1016/j.jamda.2010.04.014.

28. Rondanelli, M.; Faliva, M.; Monteferrario, F.; Peroni, G.; Repaci, E.; Allieri, F.; Perna, S. Novel Insights on Nutrient Management of Sarcopenia in Elderly. *BioMed Res. Int.* **2015**, *2015*, e524948, doi:10.1155/2015/524948.

29. Deschenes, M.R. Motor Unit and Neuromuscular Junction Remodeling with Aging. *Curr. Aging Sci.* **2011**, *4*, 209–220, doi:10.2174/1874609811104030209.

30. Zhang, Y.; Thompson, R.; Zhang, H.; Xu, H. APP Processing in Alzheimer's Disease. *Mol. Brain* **2011**, *4*, 3, doi:10.1186/1756-6606-4-3.

31. Rosenberg, R.N.; Lambracht-Washington, D.; Yu, G.; Xia, W. Genomics of Alzheimer Disease: A Review. *JAMA Neurol.* **2016**, *73*, 867–874, doi:10.1001/jamaneurol.2016.0301.

32. Younkin, S.G. The Role of Aβ42 in Alzheimer's Disease. *J. Physiol.-Paris* **1998**, *92*, 289–292, doi:10.1016/S0928-4257(98)80035-1.

33. Rissman, R.A.; Trojanowski, J.Q.; Shaw, L.M.; Aisen, P.S. Longitudinal Plasma Amyloid Beta as a Biomarker of Alzheimer's Disease. *J. Neural Transm.* **2012**, *119*, 843–850, doi:10.1007/s00702-012-0772-4.

34. Jacobsen, J.S.; Wu, C.-C.; Redwine, J.M.; Comery, T.A.; Arias, R.; Bowlby, M.; Martone, R.; Morrison, J.H.; Pangalos, M.N.; Reinhart, P.H.; et al. Early-Onset Behavioral and Synaptic Deficits in a Mouse Model of Alzheimer's Disease. *Proc. Natl. Acad. Sci.* **2006**, *103*, 5161–5166, doi:10.1073/pnas.0600948103.

35. McGowan, E.; Eriksen, J.; Hutton, M. A Decade of Modeling Alzheimer's Disease in Transgenic Mice. *Trends Genet.* **2006**, *22*, 281–289, doi:10.1016/j.tig.2006.03.007.

36. Zheng, H.; Jiang, M.; Trumbauer, M.E.; Sirinathsinghji, D.J.S.; Hopkins, R.; Smith, D.W.; Heavens, R.P.; Dawson, G.R.; Boyce, S.; Conner, M.W.; et al. β-Amyloid Precursor Protein-Deficient Mice Show Reactive Gliosis and Decreased Locomotor Activity. *Cell* **1995**, *81*, 525–531, doi:10.1016/0092-8674(95)90073-X.

37. Phinney, A.L.; Calhoun, M.E.; Wolfer, D.P.; Lipp, H.-P.; Zheng, H.; Jucker, M. No Hippocampal Neuron or Synaptic Bouton Loss in Learning-Impaired Aged β-Amyloid Precursor Protein-Null Mice. *Neuroscience* **1999**, *90*, 1207–1216, doi:10.1016/S0306-4522(98)00645-9.

38. Kelleher, R.J.; Shen, J. Presenilin-1 Mutations and Alzheimer's Disease. *Proc. Natl. Acad. Sci. U. S. A.* **2017**, *114*, 629–631, doi:10.1073/pnas.1619574114.

39. Goldman, J.S.; Hahn, S.E.; Catania, J.W.; Larusse-Eckert, S.; Butson, M.B.; Rumbaugh, M.; Strecker, M.N.; Roberts, J.S.; Burke, W.; Mayeux, R.; et al. Genetic Counseling and

Testing for Alzheimer Disease: Joint Practice Guidelines of the American College of Medical Genetics and the National Society of Genetic Counselors. *Genet. Med.* **2011**, *13*, 597–605, doi:10.1097/GIM.0b013e31821d69b8.

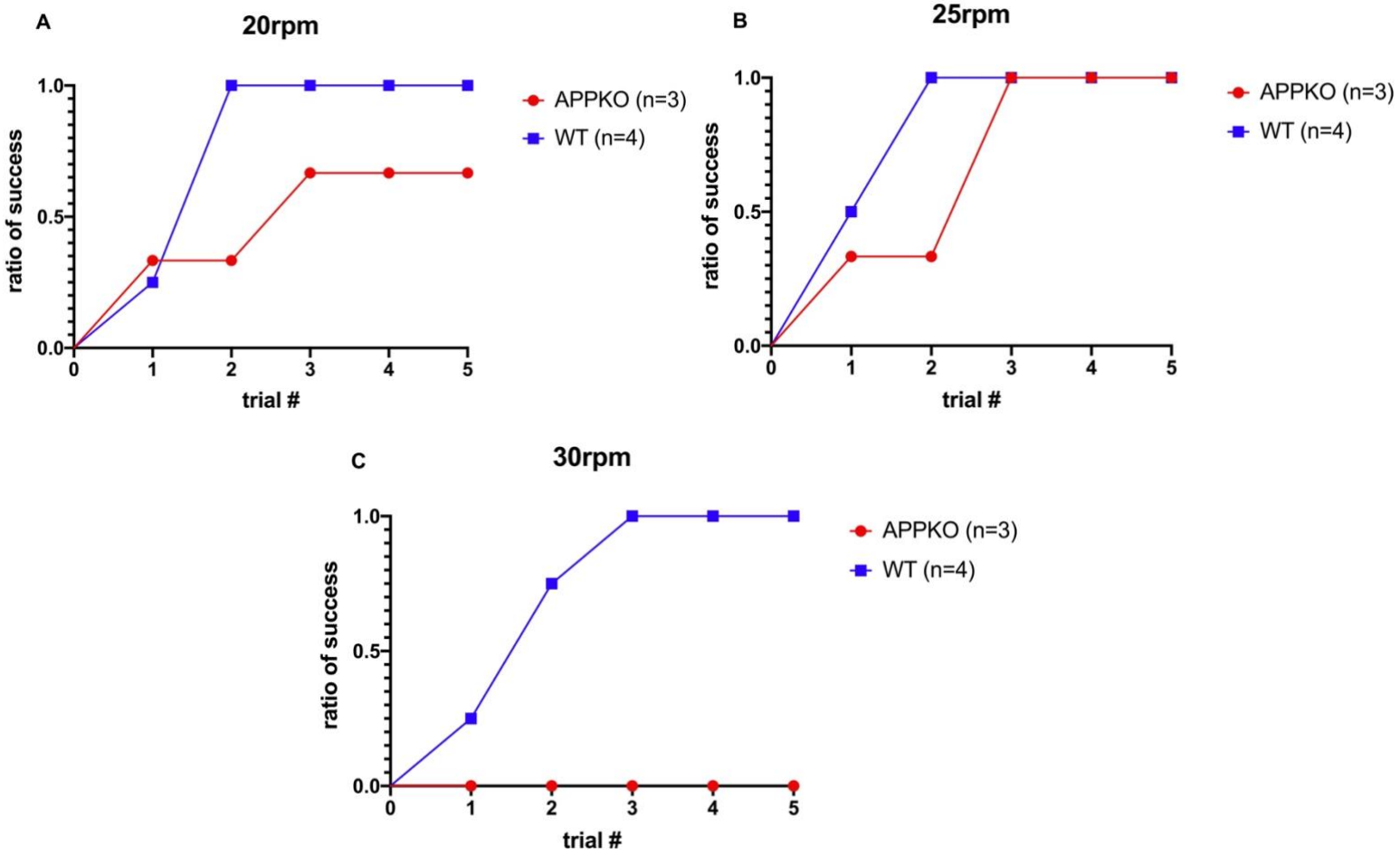
40. Radde, R.; Bolmont, T.; Kaeser, S.A.; Coomaraswamy, J.; Lindau, D.; Stoltze, L.; Calhoun, M.E.; Jäggi, F.; Wolburg, H.; Gengler, S.; et al. Abeta42-Driven Cerebral Amyloidosis in Transgenic Mice Reveals Early and Robust Pathology. *EMBO Rep.* **2006**, *7*, 940–946, doi:10.1038/sj.embor.7400784.
41. Rupp, N.J.; Wegenast-Braun, B.M.; Radde, R.; Calhoun, M.E.; Jucker, M. Early Onset Amyloid Lesions Lead to Severe Neuritic Abnormalities and Local, but Not Global Neuron Loss in APPPS1 Transgenic Mice. *Neurobiol. Aging* **2011**, *32*, 2324.e1–6, doi:10.1016/j.neurobiolaging.2010.08.014.
42. Encarnacion-Rivera, L.; Foltz, S.; Hartzell, H.C.; Choo, H. Myosoft: An Automated Muscle Histology Analysis Tool Using Machine Learning Algorithm Utilizing FIJI/ImageJ Software. *PLOS ONE* **2020**, *15*, e0229041, doi:10.1371/journal.pone.0229041.
43. Bachmanov, A.A.; Reed, D.R.; Beauchamp, G.K.; Tordoff, M.G. Food Intake, Water Intake, and Drinking Spout Side Preference of 28 Mouse Strains. 9.
44. Possidente, B.; Birnbaum, S. Circadian Rhythms for Food and Water Consumption in the Mouse, Mus Musculus. *Physiol. Behav.* **1979**, *22*, 657–660, doi:10.1016/0031-9384(79)90226-9.
45. Possidente, B.; Hegmann, J.P.; Elder, B.; Carlson, L. Dissociation of Circadian Rhythms for Food and Water Consumption in Mice. *Physiol. Behav.* **1980**, *25*, 279–281, doi:10.1016/0031-9384(80)90217-6.

46. Southam, K.A.; Stennard, F.; Pavez, C.; Small, D.H. Knockout of Amyloid β Protein Precursor (APP) Expression Alters Synaptogenesis, Neurite Branching and Axonal Morphology of Hippocampal Neurons. *Neurochem. Res.* **2019**, *44*, 1346–1355, doi:10.1007/s11064-018-2512-0.

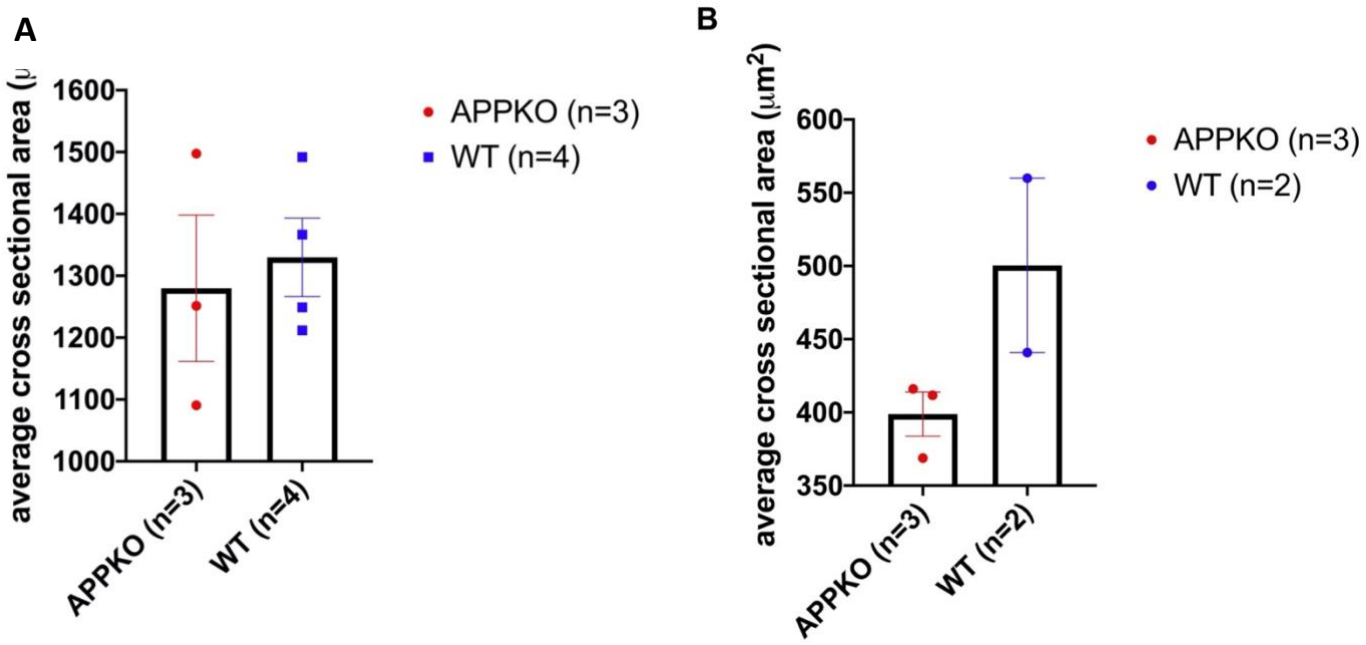
47. Aydin, D.; Weyer, S.W.; Müller, U.C. Functions of the APP Gene Family in the Nervous System: Insights from Mouse Models. *Exp. Brain Res.* **2012**, *217*, 423–434, doi:10.1007/s00221-011-2861-2.

48. Yang, L.; Wang, Z.; Wang, B.; Justice, N.J.; Zheng, H. Amyloid Precursor Protein Regulates Cav1.2 L-Type Calcium Channel Levels and Function to Influence GABAergic Short-Term Plasticity. *J. Neurosci.* **2009**, *29*, 15660–15668, doi:10.1523/JNEUROSCI.4104-09.2009.

## Supplemental Figures

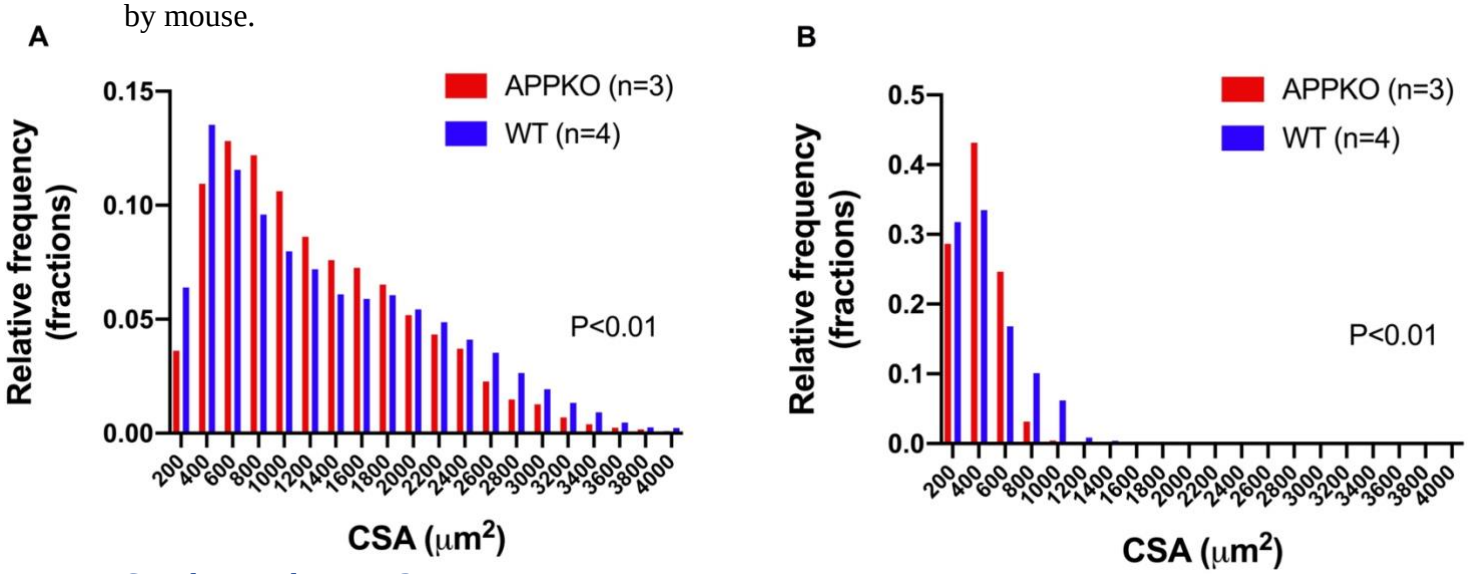



Supplemental Figure 1: **Comparison of rotarod success rates at 20, 25 and 30 rpm.** a) results for 20rpm. b) results for 25 rpm. c) results for 30 rpm. Success rate was determined as the percentage of mice who had reached the maximum trial time of 300 seconds per trial. 17-week-old male mice were tested for five trials at each speed over three separate days.



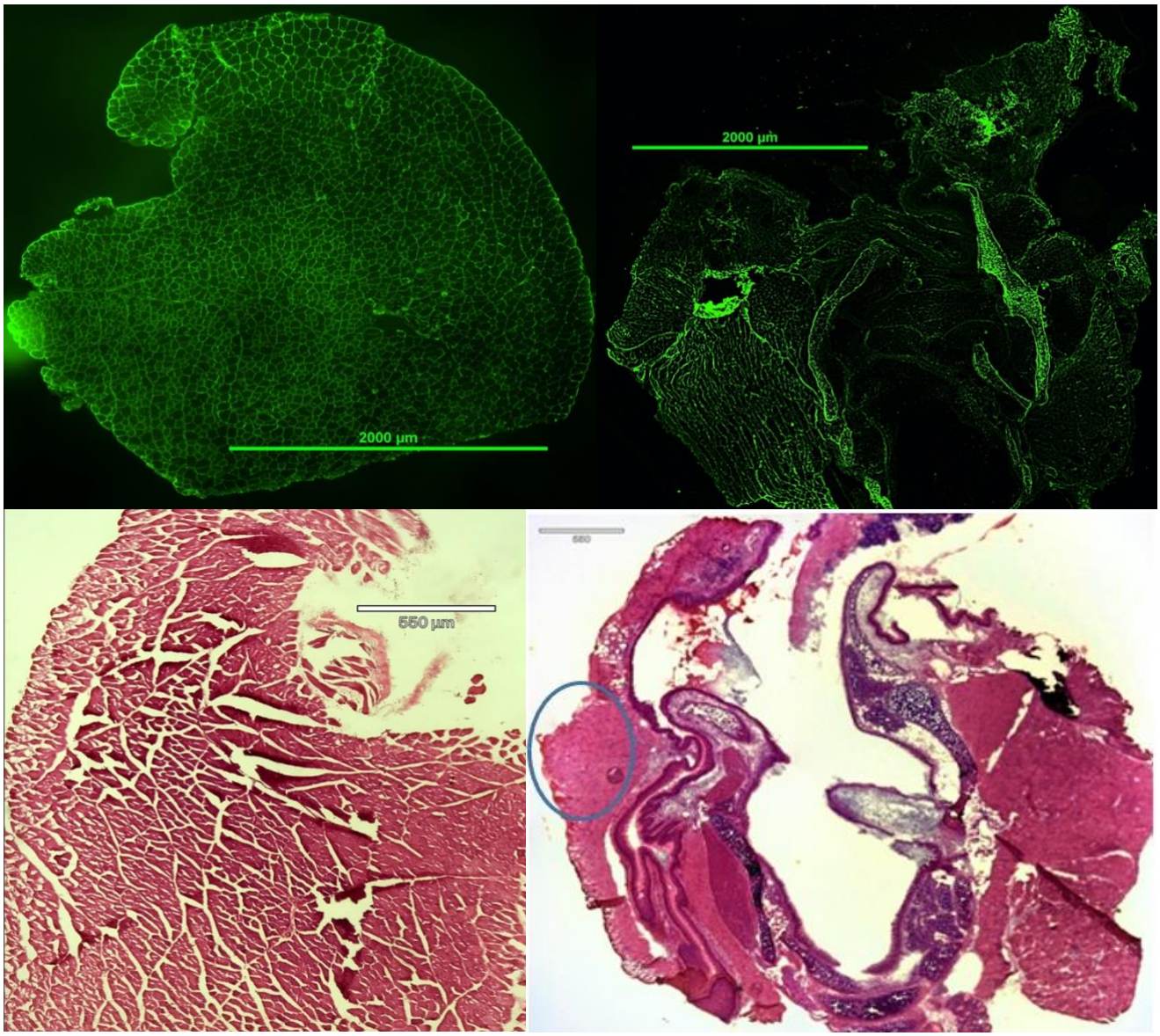

Supplemental Figure 2: **TA and pharynx area are equivalent in WT vs APPKO mice**

APPKO mice exhibit no difference in average muscle fiber size compared to WT when grouped by mouse.




Supplemental Figure 3:

**TA and pharynx area are equivalent in WT vs APPKO mice.** a) APPKO mice exhibit significantly smaller TA area compared to WT. b) APPKO mice exhibit significantly smaller pharynx area compared to WT. Two tailed t tests were conducted between groups. Bars represent SEM.




Supplemental Figure 4. **Representative images of TA and pharynx sections.** a) Whole section laminin-stained image of TA (tibialis anterior) muscle section b) Whole section laminin stained image of pharynx image. c) Whole section H&E-stained image of TA muscle section. d) Whole section H&E-stained image of pharynx, target area, CP (cricopharyngeal bar) area used for analysis circled. Muscles were cryosectioned at 18 weeks and stained with anti-laminin antibody (green fluorescence).