Impact of age and exercise on intron retention

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## Abstract

Several studies have shown that aging affects the pre-mRNA splicing machinery. Similarly, studies suggest physical exercise can slow-down or reverse aging-induced effects on the human body. This study investigates intron retention in the vastus lateralis muscle of young and old adults; and how it is affected by ~12 week of resistance exercise training . RNA-Seq data from five different resistance exercise studies comprising of old and young adult humans are included in this study.

## Introduction

Alternative splicing is a mechanism in mRNA processing in which protein-coding and non-protein coding parts of the RNA are alternatively skipped. This results in transcriptomic and proteomic diversity that can determine cellular plasticity as it influences an organism’s ability to adapt to changes (Holly et al. 2013). Alternative splicing is responsible for the proteomic diversity observed in living beings (Angarola and Anczuków 2021) ; a diversity that could lead to dysfunction if the biological system breaks down (Bhadra et al. 2020) . Basically, alternative splicing regulates life and death decisions (Schwerk and Schulze-Osthoff 2005). This mechanism is tissue\_specific and increases with age (Latorre and Harries 2017 ; Holly et al. 2013 ) due to an alteration in the expression of splicing factors Stegeman and Weake (2017) as a resilience strategy to counteract aging-induced damages and loss of function (Ferrucci et al. 2022 ; Ubaida-Mohien et al. 2019b). There are three broad types of alternative splicing; exon skipping, alternative use of splice sites, and intron retention (Nishida et al. 2015 ; Zheng et al. 2020 ). Of specific interest to this study is intron retention.

Intron retention (IR) occurs when a full intron, or parts of an intron in a mature messenger RNA (mRNA) is retained during splicing, resulting in the presence of an unprocessed sequence in the mRNA. Eukaryotes are said to have two distinct pre-mRNA splicing machinery; the major spliceosome and the minor spliceosome . The major spliceosome removes over 99% of introns while the minor spliceosome removes evolutionarily conserved introns (Inoue et al. 2021). mRNAs with retained introns play roles in normal physiology and in disease conditions (Wong et al. 2016; Ge and Porse 2014). This leads either to the degradation of the IR-containing transcripts through nonsense-mediated decay or the action of nucleases; or to frameshifts that cause the generation of alternative protein isoforms (Baralle and Romano 2023 ; Zheng et al. 2020 ; Mauger et al. 2016; Wong and Schmitz 2022 ; Monteuuis et al. 2019 ). IR affects about 80% of human protein-coding genes (Middleton et al. 2017 ; Braunschweig et al. 2014 ) and is the main form of alternative splicing that is affected by age (Kodama et al. 2024 ; Mariotti et al. 2022; Bhadra et al. 2020) . IR occurs mostly at the 3` end of transcripts(Wong et al. 2013 ; Braunschweig et al. 2014 ), and mostly in transcripts that lack physiological relevance in cells and tissues they are detected in (Braunschweig et al. 2014) . Wong et al. (2013) reports that high IR levels correlated with the down-regulation of correctly spliced mRNA transcripts in myelocytes and granulocytes . Short genes with high mRNA levels tend to be inefficiently spliced compared to long genes with high mRNA levels . That is, there is a positive correlation between gene length, expression level and SE Saudemont et al. (2017) .

IR used to be considered a result of mis-splicing which render transcripts non-functional (Wong et al. 2016) but is increasingly being viewed as a mechanism of gene regulation. This is because it tends to increasingly occur in response to specific developmental cues and serve as a strategy for rapid mobilization of some mRNAs for protein translation (Ong and Adusumalli 2020). Intron retention is often measured as splicing efficiency which describes the amount of introns retained in a mature mRNA. Splicing efficiency is cell and tissue specific (Nishida et al. 2015; Baralle and Romano 2023) but tends to be similar between the introns of a gene [Sánchez-Escabias et al. (2022)] . This suggests that a balanced study of intron retention should be comparing same introns in same genes and same tissues across the subjects of interest.

Besides aging, poor splicing efficiency has been linked to to human diseases like alpha thalassemia [Nelson et al. (2005)] , inflammatory bowel disease [Häsler et al. (2011)] , neurodegenerative diseases [Sznajder et al. (2018); Ong and Adusumalli (2020) ] and the risk of certain cancers (Dvinge and Bradley 2015 ; Inoue et al. 2021 ; Shah et al. 2022). IR is a potential biomarker or driver of the aging process (Baralle and Romano 2023 ; Adusumalli et al. 2019 ). There is a gap in knowledge about how the decline in splicing efficiency can be minimised or reversed.

### Aging and progressive resistance exercise training

Aging is the progressive transformation of young organisms into aged ones. It involves changes across several physiological processes and is characterized by a progressive decline in most biological functions, and results to reduced vitality, increased risk of diseases and eventually death (López-Otín et al. 2013; Keshavarz et al. 2023 ; Gyenis et al. 2023) . It is the greatest cause of disease and death worldwide (Schaum et al. 2020). Physiologically, aging is associated with the induction of different stress response pathways in different cell, tissue and specie types (Stegeman and Weake 2017; Welle et al. 2003). Aging and aging-like phenotypes are associated with increased splicing alterations (Angarola and Anczuków 2021 ; García-Ruiz et al. 2023 ; Nishida et al. 2015 ; Mariotti et al. 2022; Stegeman and Weake 2017; Pabis et al. 2024) . Aging is often assessed using the concept of a “biological clock” that is organ, system and sex specific (Nie et al. 2022). A significant part of aging-focused research is focused on interventions that could slow-down aging or its impacts.

One of the known effects of aging is the decline in skeletal muscle mass and strength. The muscle accounts for about half the mass of the body. Physical exercise is an anti-aging intervention targeted at maintaining not just muscle health, but general well-being. It counteracts the effects of aging on mitochondrial health and can offset aging-related changes to the splicing machinery (Ubaida-Mohien et al. 2019a, 2022). Progressive resistance exercise training (RT) describes the type of physical exercise training where the skeletal muscle is exercised against progressively increased types of resistance (Liu and Latham 2009). The benefits of RT include improvements in muscle strength, muscle mass and bone density. RT could thus be a potent preventive or treatment strategy for improving aging-related functional disabilities associated with the skeletal muscle (Peterson et al. 2011 ; Kryger and Andersen 2007 ; Lu et al. 2021 ).

This study is aimed at investigating differentially spliced introns between the young and the old, and how they are affected by about 12 weeks of RT. It also hopes to determine the age range with the most abherrent splicing efficiency.

We hypothesize that since intron retention is a mechanism by which the cells modify gene expression in response to muscular stress, there would be age-related decline in splicing efficiency. We also hypothesize that 12 weeks of RT would improve upon age-related splicing efficincy while eliciting a unique set of retained introns in response to muscular stress.

## Materials and methods

Five datasets are included in the study. Three datasets were from the TrainOme group and had either young, or old individuals. Publicly available datasets from (**robinson2017?**) Kulkarni et al. (2020) were obtained which fitted the criteria of paired-end Illumina RNA-Seq data from vastus lateralis muscle of human subjects . It contained both young and old participants.

| Dataset name | Number of participants | Number of samples | Age range (in years) | Linked Publication |
| --- | --- | --- | --- | --- |
| COPD | 53 | 65 | 56-79 | Mølmen et al. (2021) |
| Volume | 25 | 50 | 20-37 | Khan et al. (2020) |
| Contratrain | 26 |  | 19-34 | Hamarsland et al (in preparation) |
| SRP102542 | 52 | 52 | 19-30 (Young cohort)  65-78 (Old cohort) | Robinson et al. (2017) |
| SRP280348 | 86 | 86 | 18-27 (Young cohort)  64-80(Old cohort) | Kulkarni et al. (2020) |

The benjamin and hochberg method Benjamini and Hochberg (1995) was used to control for false discovery rates

### Methodology

RNA-SEQ data processing

The quality of the reads were checked using FASTQC(v0.11.9). STAR (2.7.9a) was used to map reads to the human reference genome (GRCh38 version 40) using the 2-pass alignment method described by Veeneman et al. (2016) which advances quantification and discovery of splicing events. RSEM (1.3.3) (Li and Dewey 2011) was used to generate transcript and gene level counts while SpliceQ Melo Costa et al. (2021) was used to quantify splicing efficiency.

Using preexercise data alone, we sought to identify differentially spliced introns by age, sex and age group. For this, we built two generalized linear mixed beta regression models. One captured age as a continuous variable and modeled the interaction between age and sex as fixed effects, while study and participant were used as random effect having individual intercepts

The second model, used age as a categorical variable where the participants were grouped into young, and old. Similar to the first model, the interaction of age\_group and sex was used as fixed effects,

The differentially spliced introns based on age of preexercise data was filtered for adjusted pvalues at or below 0.05. Those that had an absolute log2fc of 1 were also filtered to determine those with significant differential expression

# Results

To investigate the impact of age and RT on intron retention, we investigated intron retention in RNA sequence data from vastus lateralis of 141 individuals from four different RT studies . The participants were divided into two age groups with young comprising 65 individuals aged between 19 to 37 , the old age group consisted of 76 participants aged between 57 and 79 .

We measured intron retention as splicing efficiency using SpliceQ (**demelocosta2021?**). mis-splicing occurs more in non-protein coding genes than in protein-coding genes García-Ruiz et al. (2023), this disagrees with the results we obtained . We however take cognisance of the limitations in RNA-Seq’s reported ability to correctly quantify non-coding RNAs Stokes et al. (2023)

Saudemont et al. (2017) stipulated a mutation-selection-drift theory that suggests shorter, intron-poor and lowly expressed genes show a poorer splicing efficiency

There appears to be a progressive decrease in Splicing efficiency from young to middle aged, and then old

detect differentially spliced introns between young and old participants, At baseline, using the filter characteristics of adjusted p.values =< 0.05, only age had differentially spliced introns, sex and interaction between sex and age showed no ds introns. age alone revealed 1786 out of 18541 introns were differentially spliced between old and young participants. When this data was further queried to detected those with absolute log2fc of 1, it showed that 511 were significantly spliced differently between old and young participants. 3 introns were differentially sploiced by sex

Seven introns were shown to be differentially expressed by age when used as a continous variable

Of the 1693 ds introns captured in the full dataset, 135 were changed postexercise. Based on the Estimate values, they were all improvements

## Discussion

IR is a physiological mechanism that controls gene expression (Wong et al. 2016 ; Schmitz et al. 2017 ) increase in IR is a signature of the aging process Bhadra et al. (2020)

Tissues and organs are altered differently by aging and aging-related processes Baralle and Romano (2023) .

### Shortcoming

This study did not group RT into conditions of volume of exercise done. The benefits of RT are known to be volume-dependent and the 4 studies involved varying RT conditions

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