EVIDENCE FOR SEX-SPECIFIC INTRAMUSCULAR CHANGES ASSOCIATED TO PHYSICAL WEAKNESS IN OLDER ADULTS

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INTRODUCTION AND AIM

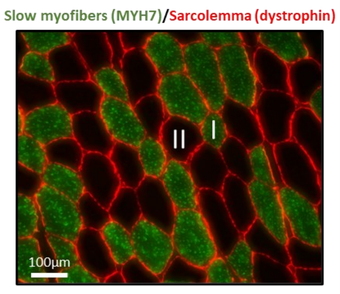
- Physical weakness is a key component of frailty, and frailty is more prevalent in females compared to males.
- Studies suggest the existence of sex-specific mechanisms underlying frailty, but human studies on intramuscular molecular changes associated to physical weakness are scarce.
- Therefore, our aim was to investigate the intramuscular parameters that differentiate fit from weak older adults, for each sex separately.

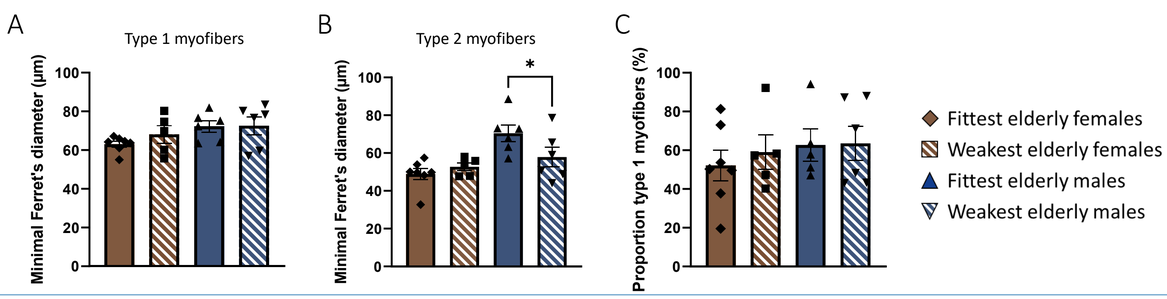
METHODS

Older male (n=28, 79.7 ± 3.5 yrs) and female (n=26, 80.2 ± 3.1 yrs) adults with varied fitness levels (Fried frailty index ranging from 0 to 4) were recruited. The male and female participants were subsequently ranked according to three frailty-related physical performance criteria to identify the weakest (n=8) and fittest (n=8) participants. *Vastus lateralis* muscle biopsies were used for RNA-sequencing and immunohistochemistry, and fittest vs. weakest groups were compared for each sex separately.

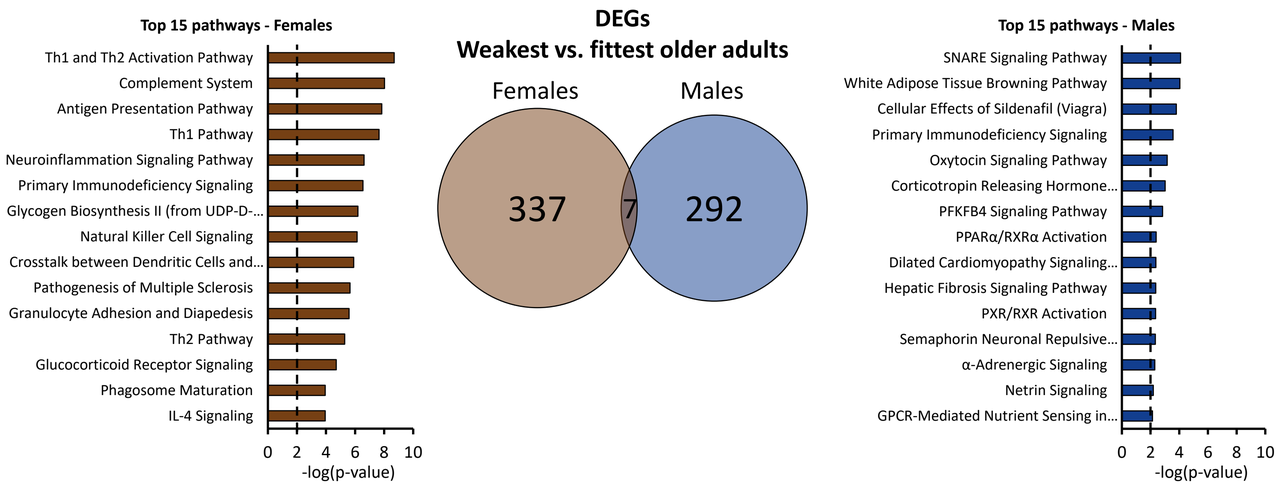
DIAMETER OF TYPE 2 MYOFIBERS IS DECREASED IN WEAK OLDER MALES ONLY

- The diameter of the type 1 myofibers was not different in the fittest vs. weakest groups of both sexes (A).
- The diameter of the type 2 myofibers was bigger in the fittest (70.4 ± 4.4 µm) compared to the weakest (57.9 ± 5.2) older males ($p < 0.05$), but no difference was found between the fittest (48.9 ± 2.9 µm) and weakest (52.7 ± 2.1 µm) older females (B).
- The proportion of the type 1 myofibers was not different in fittest vs. weakest groups of both sexes (C).



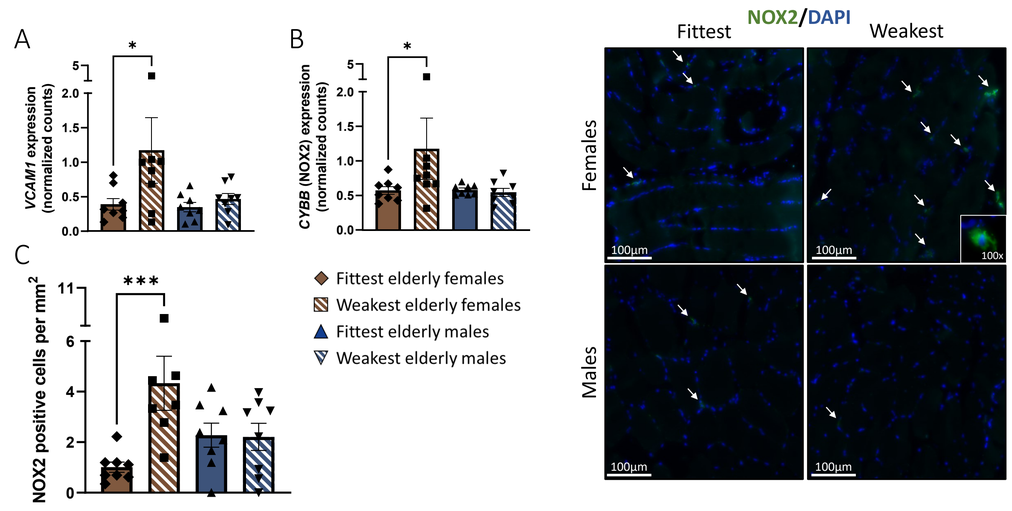


PHYSICAL WEAKNESS ASSOCIATED CHANGES IN SKELETAL MUSCLE TRANSCRIPTOME ARE SEX-SPECIFIC



- In females 344 differentially expressed genes (DEGs) and in males 299 DEGs were found. Strikingly, only 7 of these DEGs were shared between the two sexes, indicating highly sex-specific physical weakness associated changes in the muscle transcriptome (middle panel).
- In females, 45 pathways were significantly differentially expressed, and the top ranked pathways were involved in immune cell infiltration and inflammation (e.g., th1 and th2 activation pathway, complement system, natural killer cell signaling; left panel).
- In males, pathway analysis revealed a smaller number of pathways, namely 20, which, in contrast to females, were involved in a scattered set of different biological processes, e.g., membrane trafficking (SNARE signaling pathway), energy regulation (white adipose tissue browning pathway) and vasodilation (cellular effects of sildenafil; right panel).

FEMALE PHYSICAL WEAKNESS IS CHARACTERIZED BY INFILTRATION OF NOX2 EXPRESSING IMMUNE CELLS

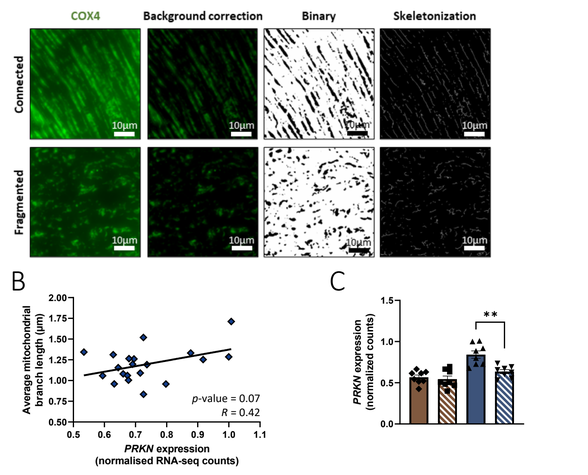


- Among the top ranked female pathways, well-known genes were found, such as *VCAM-1*, which mediates adhesion of immune cells and immune cells infiltration (A).
- *CYBB* was also one of the top DEGs, and encodes for NOX2, a subunit of the NADPH oxidase complex that is expressed in inflammatory cells such as macrophages and neutrophils, and mediates production of reactive oxygen species (B).
- Immunohistochemical staining of *CYBB* (NOX2) confirmed an increased infiltration of NOX2 expressing immune cells in the weakest (4.3 ± 1.0 NOX2 positive cells per mm2) vs. fittest (1.0 ± 0.2 NOX2 positive cells per mm2) females, whilst this difference was not observed in the weakest (2.2 ± 0.5 NOX2 positive cells per mm2) vs. fittest (2.3 ± 0.5 NOX2 positive cells per mm2) males (C).

MALE PHYSICAL WEAKNESS IS CHARACTERIZED BY A DECREASED PARKIN EXPRESSION

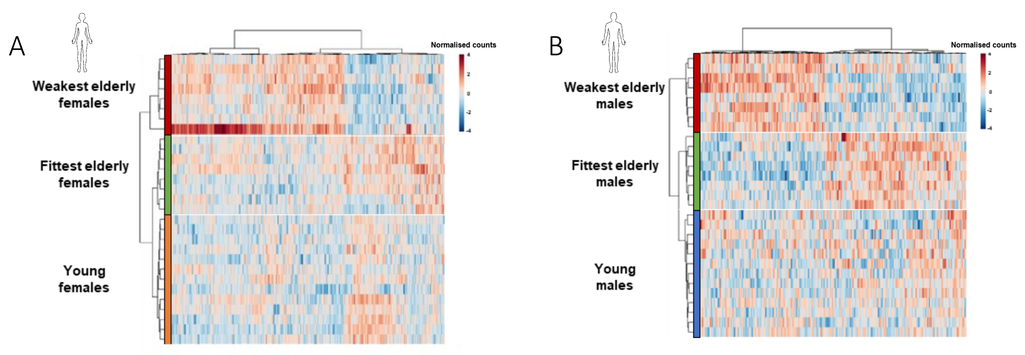
A

Top 15 DEGs males	Males -log(p-value)	Males Log2(FC)	Females -log(p-value)	Females Log2(FC)
RP11-144C15.2	5.6	-0.9	0.3	-0.1
TTC7B	5.2	-0.5	1.3	-0.2
PRKN	4.4	-0.4	0.2	-0.1
C16orf89	4.4	0.7	0.5	0.2
AC136616.1	4.4	0.6	0.1	0.0
IKBKB	4.1	-0.5	1.0	-0.2
SNURF	4.1	0.8	0.0	0.0
WASF3	3.9	-0.5	0.3	0.1
TESC	3.9	0.7	0.5	0.2
LIN52	3.9	0.6	0.5	-0.2
SVIL	3.8	-0.4	0.1	0.0
MESP1	3.8	0.7	0.1	0.0
CHI3L2	3.7	0.5	1.4	0.3
USP31	3.6	-0.6	1.1	-0.3
MLXIP	3.6	-0.4	0.1	0.0



- Since male pathways did not represent a uniform set of biological processes we investigated the top DEGs instead, among which *PRKN* was observed (A).
- Average mitochondrial branch length tended to positively correlate with *PRKN* expression (B).
- *PRKN* expression was decreased in the weakest vs. fittest males, but not in females (C).

CHANGES IN MUSCLE TRANSCRIPTOME ASSOCIATED TO PHYSICAL WEAKNESS ARE NOT NECESSARILY A CONTINUUM OF AGING



- Young male (n=13) and female (n=13) subjects were recruited as well.
- Strikingly, the fittest older adults and young adults were clustered together in both sexes. Suggesting that in regard to the DEGs associated to physical weakness, the fittest older adults maintained an expression pattern similar to young adults (A-B).
- This suggests that changes in the transcriptome that are associated to physical weakness, are not necessarily associated to aging as well.

CONCLUSIONS

We conclude that intramuscular features associated to physical weakness are highly sex-specific in older adults. Weak older females were characterized by an increased expression of inflammatory pathways and infiltration of NOX2 expressing immune cells, concomitant with an increased *VCAM1* expression. Weak older males were characterized by a shrinkage of type 2 (fast) myofibers and decreased expression of *PRKN*. The novel findings of our study underline that weakness associated changes in muscle are sex-specific in older adults and we therefore recommend that sex differences are taken into account in research on frailty, as they could have a large impact on the development of (pharmaceutical) interventions against frailty.

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