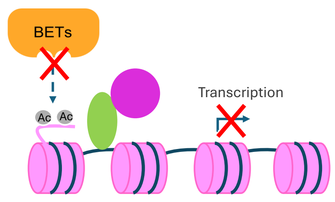
BET (Bromodomain and Extra-terminal domain) inhibitors rewire metabolism in the aged skeletal muscle

Lorenzo Nevi[1], Silvia, Gaino[1], Alice Passerini[1], Cinzia Bottino[1], Ummu Guven[1], Giulio Pavesi[1], Alessia S. Cento[2] , Claudia Fornelli[2], Vittorio Sartorelli[3], Marco Segatto[4], Fabio Penna[2], Giuseppina Caretti[1]

(1)Department of Biosciences, University of Milan, Milan, Italy
(2)Department of Clinical and Biological Sciences, University of Torino, Torino, Italy
(3) NIAMS, NIH, Bethesda, MD, USA
(4) Department of Biosciences and Territory, University of Molise, Italy

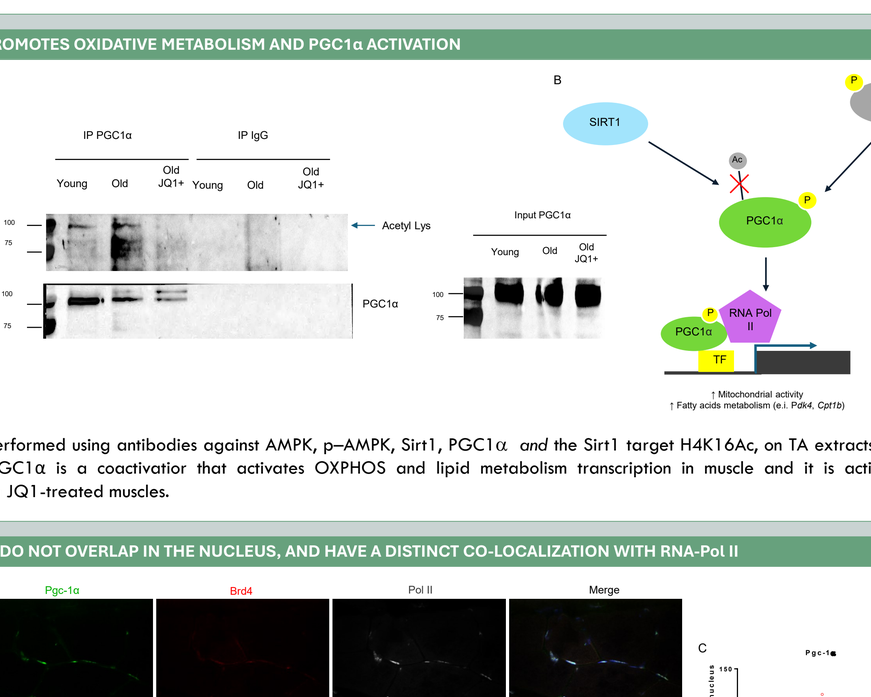
BACKGROUND

Aging is associated with a progressive decline in muscle mass and strength, observed among healthy adults, with an acceleration in the rate of decline past middle age. The pathological loss of muscle mass associated with aging, known as sarcopenia, negatively affects quality of life and leads to an increased occurrence of falls, hospitalization, and decreased independence. Previous reports from our group have shown that the BET protein BRD4 plays a role in promoting muscle wasting in cancer cachexia and muscular dystrophy. Because of BRD4 role in regulating muscle wasting, we evaluated the impact of pharmacological blockade of BET proteins in the skeletal muscle of 24-month-old mice.



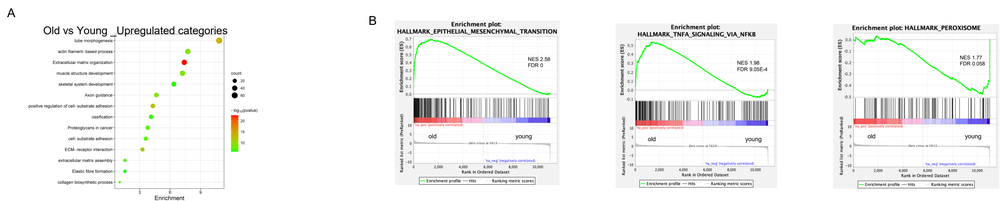
AIM AND METHODS

We evaluated the impact of BET pharmacological blockade in the muscle of old mice, by treating the mice with the BET inhibitor JQ1.



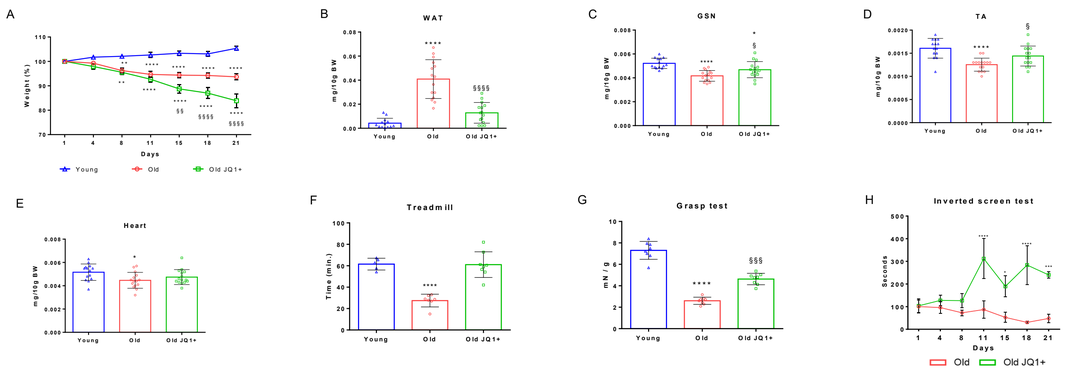
RESULTS

Figure 1 – AGING ALTERS THE EXPRESSION OF GENE CATEGORIES, IN 24 MONTHS OLD MICE



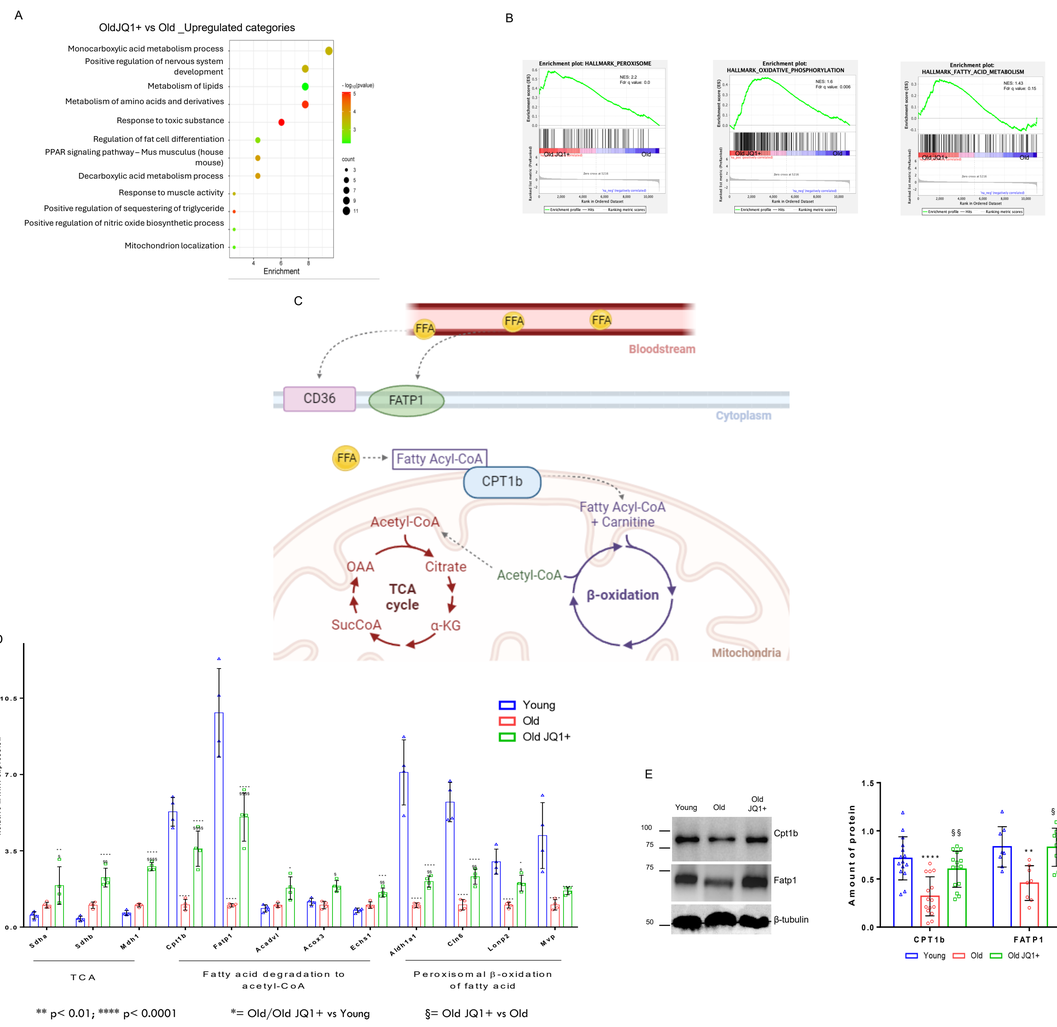
RNA-seq was performed on TA muscles of young (3 months) and old (24 months) mice.
A) Gene categories of genes upregulated in the old muscles. B) GSEA analysis of upregulated and downregulated transcripts.

FIGURE 2 - JQ1+ TREATMENT INDUCES WAT LOSS WITHOUT AFFECTING MUSCLE MASS AND IMPROVES SKELETAL MUSCLE FUNCTION



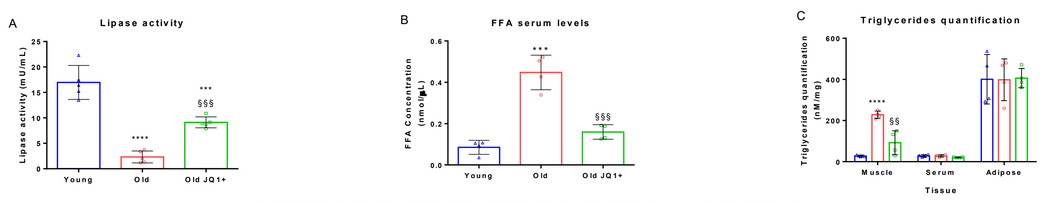
A) Body weight was measured every 3 days in the 3 experimental groups and was expressed as a percentage of the initial weight. B-E) White adipose tissue, gastrocnemius, tibialis anterior and heart were weighed after sacrifice. F-H)Treadmill, grip an inverted screen test show an amelioration in muscle function, following JQ1 treatment.

FIGURE 3 - JQ1+ TREATMENT UPREGULATES BETA OXIDATION, IN THE OLD MUSCLE



A) and B) Gene Ontology and GSEA analysis of RNA-seq data from Old/JQ1 versus control Old TAs revealed categories of genes that were up-regulated following JQ1 treatment, including oxidative phosphorylation and beta oxidation related genes. C) Scheme of fatty acid metabolism in skeletal muscle. D) qRT-PCR validation of targets identified in RNA-seq assays, assayed in young, old and Old/JQ1 treated mice. E) Immunoblot analysis showing increased levels of PDK4, a marker for the switch from glycolysis to beta oxdiation, and the FFA transporter Fatp1 and Cpt1b (Carnitine Palmitoyltransferase 1B).

FIGURE 5 - JQ1+ TREATMENT PROMOTES FFA UPTAKE AND THE MODULATION OF SEVERAL METABOLITES, IN THE OLD MUSCLE



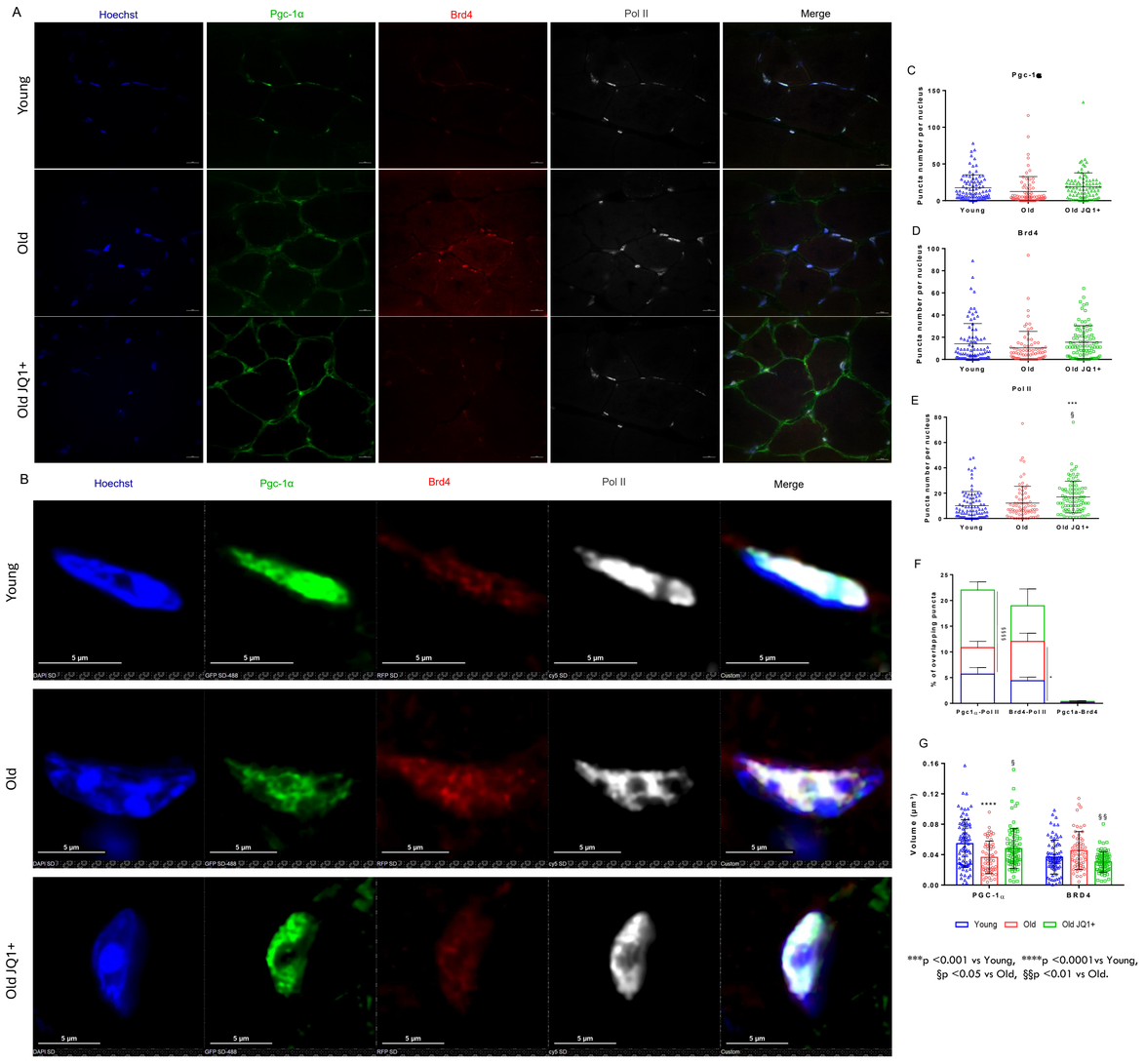
* p< 0.05; ** p< 0.01; *** p< 0.001; **** p< 0.000 *= Old/Old JQ1+ vs Young §= Old JQ1+ vs Old

A-C) FFA, triglycerides and lipase activity were measured using commercial kits.

FIGURE 6 - JQ1+ TREATMENT PROMOTES OXIDATIVE METABOLISM AND PGC1α ACTIVATION

A) Immunoblot analysis was performed using antibodies against AMPK, p–AMPK, Sirt1, PGC1α *and* the Sirt1 target H4K16Ac, on TA extracts from the 3 experimental groups. B) PGC1α is a coactivatior that activates OXPHOS and lipid metabolism transcription in muscle and it is activated by deacetylation and by AMPK in JQ1-treated muscles.

FIGURE 7 - BRD4 AND PGC-1 α DO NOT OVERLAP IN THE NUCLEUS, AND HAVE A DISTINCT CO-LOCALIZATION WITH RNA-Pol II



A) TA muscles from the 3 experimental groups were sectioned and used for immunofluorescence analysis by confocal microscopy spinning disk. Photos were taken with magnification100X (Young N=4; Old N=4; Old JQ1+ N=4); B) one representative nucleus is shown. Pgc-1α in shown in green, Brd4 in red, RNA PolII in white and nuclei in blue. C-G Using NSI NIkon software, dots were analyzed for total or partial overlapping, number of dots in the nucleus, and nuclear dot size for Pgc-1α, BRD4 and RNA Pol II.

***p <0.001 vs Young, ****p <0.0001vs Young, §p <0.05 vs Old, §§p <0.01 vs Old.

CONCLUSIONS

1) Mice treated with JQ1$^+$ showed enhanced muscle strength compared to mice treated with the inactive enantiomer JQ1$^-$.
2) JQ1$^+$ treatment induces a loss of body mass without affecting muscle mass. In fact, the loss of body weight is due to a decrease in white adipose tissue.
3) Old mice treated with JQ1$^+$ showed a higher number of oxidative fibers compared to the mice treated with JQ1$^-$
4) JQ1-mediated increase in Sirt1 levels and AMPK activation leads to an increased activation of PGC-1a, which co-activates the transcription of mitochondrial activity and lipid metabolism related genes.
5) We observed a reduced fibrotic level and inflammatory infiltrate in mice treated with JQ1$^+$ compared to mice treated with JQ1$^-$, that may also be beneficial.
6) BRD4 and PGC-1α are present in nuclear puncta, but do not colocalize in the nucleus.