Deciphering urolithin A and B activities in Muscle:
Transcriptomic modulation in human primary myotubes

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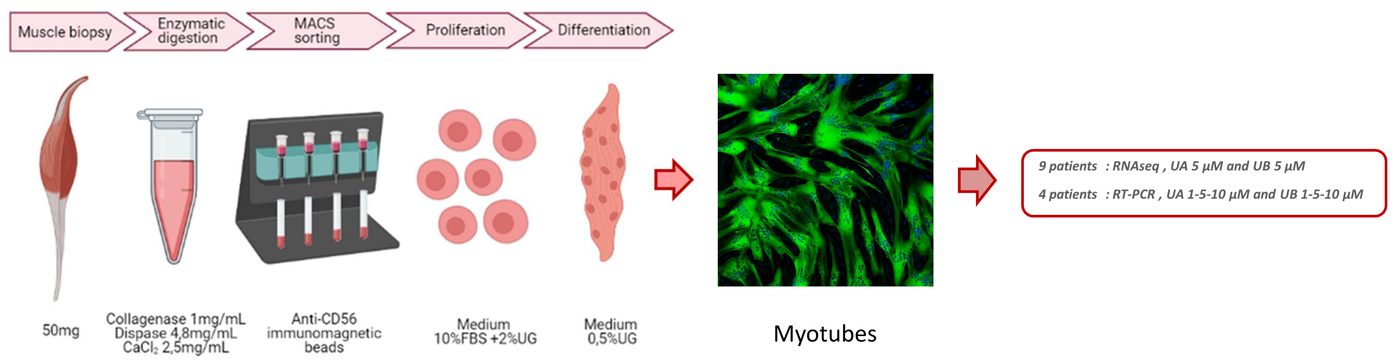
PURPOSE. **Urolithins** are intestinal bacterial metabolites of ellagic acid, from pomegranate and nuts. They modulate **oxidative-regulated pathways** and display **anti-inflammatory**, **antioxidative** properties. Several studies indicate that they could be inducers of **muscle strengthening**. The aim of this *in vitro* study was *to investigate their mechanisms of action at plasma concentrations on primary human myotubes.*

METHODS. Urolithin A and B (UA and UB, 1-10 µg/mL) were evaluated separately on **primary human muscle CD56+ cells**, isolated from the *vastus lateralis* of 6 men and 3 women (aged range 55 to 96-y) and differentiated into myotubes. mRNA-sequencing results after 24h treatment of these 9 patients was studied by **DESeq2 analysis** (R software). Modulation of several target genes was then validated by **RT-qPCR** on 4 patients.



RESULTS

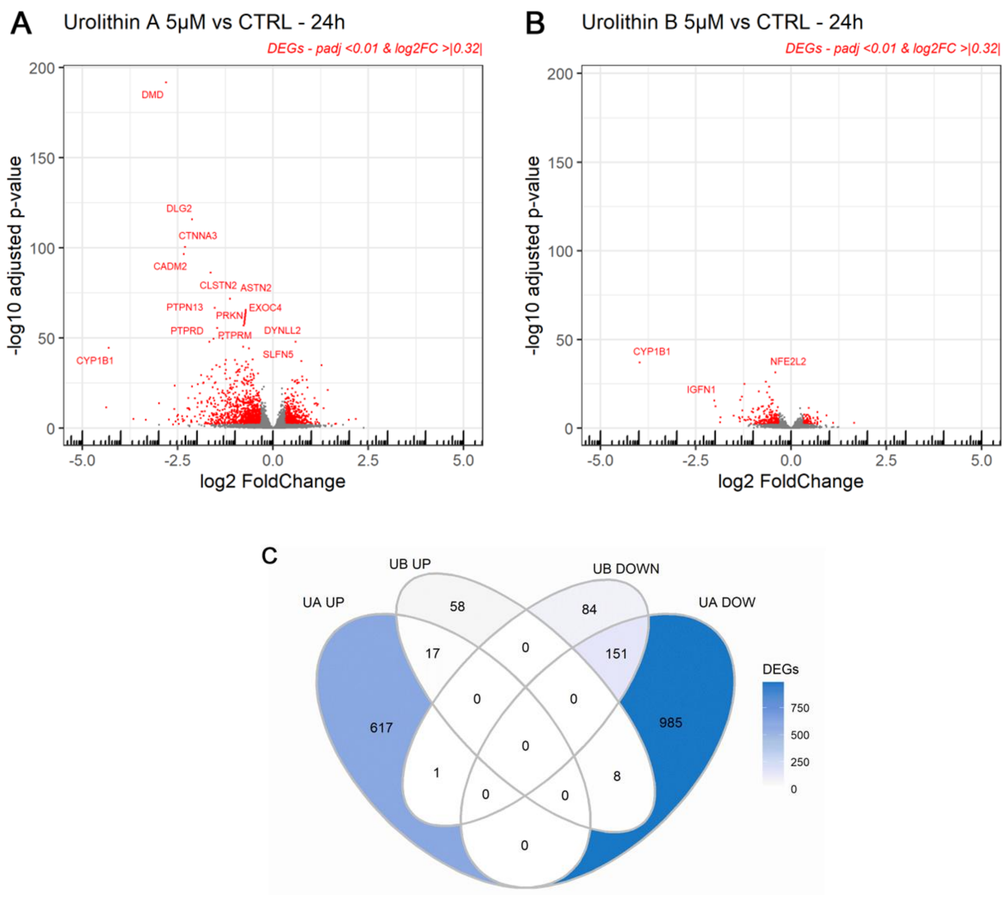


Figure 1
Volcano plots (A-B) and Venn diagram (C) of UA 5µM and UB 5µM after 24 h treatment on primary human muscle CD56+ cells : After 24h of treatment at 5 µM, UA and UB significantly modified the expression of **1779** and **319** genes, respectively (adjusted p-value of 0.01 and Log2FoldChange |>0.32|).

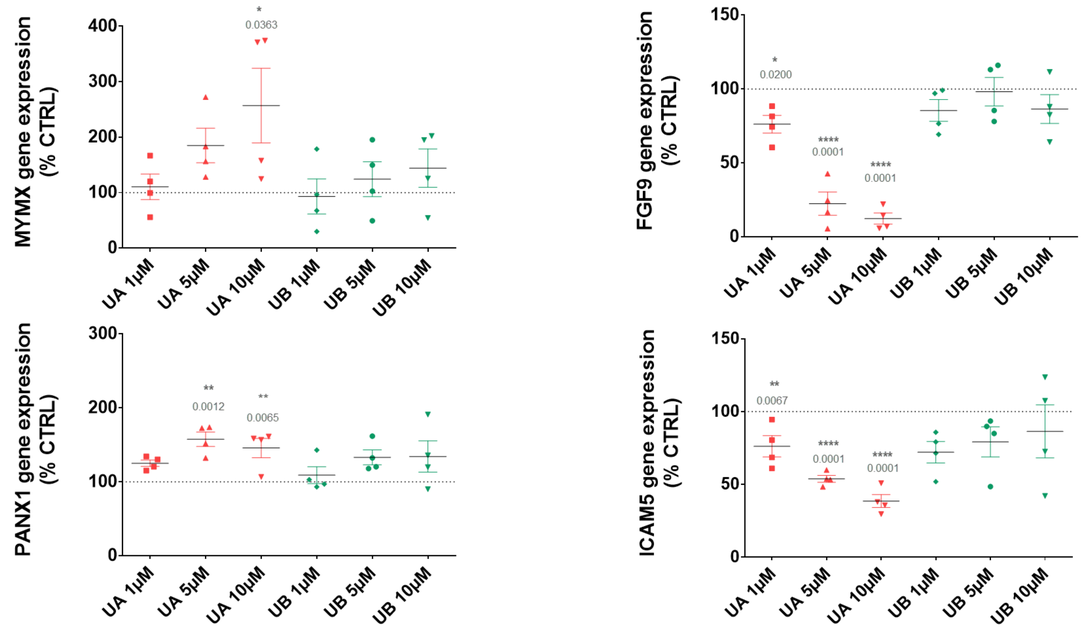


Figure 2
Modulation of genes involved in myoblast to myotube differentiation. Among the most regulated genes in the RNA-Seq analysis, **UA** (5 µM) ***increased*** the expression of **MYMX** (+70%), **PANX1** (+50%) and conversely ***decreased*** **FGF9** (-75%) and **ICAM5** (-52%). We confirmed the modulation of these by RT-qPCR, as illustrated in the graphs above.

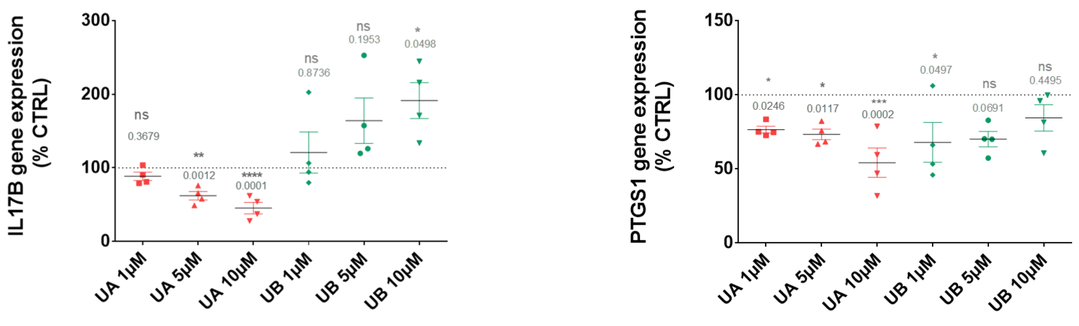


Figure 3
Modulation of genes involved in the inflammatory process : In the RNA-Seq analysis, **PTGS1** was ***decreased*** by 41% by **UA** (5 µM) and by 43% by **UB** (5 µM). UA and UB had the **opposite effect** on **IL17B**, a cytokine involved in tissue repair but its role in muscle is still to be defined. IL17B was ***decreased*** by 49% by **UA** and conversely ***upregulated*** by 45% by **UB**. We confirmed the modulation of these by RT-qPCR, as illustrated in the graphs above.

CONCLUSIONS. **At plasma concentrations, UA seems more active than UB. It promotes the differentiation process of myoblasts to myotubes. In parallel, urolithins present anti-inflammatory properties, mainly by reducing the PGE_2 synthesis via PTGS1. Our data provide a better understanding of urolithin activities and highlight the importance of the gut microbiome in muscle health.**