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A Multi-Omics Approach to Investigating the Role of Fecal miRNAs in the Gut Microbiota Response to Physical Exercise: A Pilot Study

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INTRODUCTION & AIM

Regular physical activity improves overall health and reduces the risk of non-communicable diseases.
Emerging evidence suggests that exercise induces beneficial shifts in gut microbiota composition.
However, the **molecular mediators** driving this interaction remain unclear. Fecal **microRNAs (miRNAs)** may act as messengers linking **exercise**, **inflammation**, and **microbial regulation**.

To evaluate the **effects of physical exercise** on fecal miRNA expression and explore their relationship with **gut microbiota composition** and **inflammatory profiles**.

METHOD

Participants

Subjects: 27 healthy adults (20–45 years)
Recruitment: Faculty of Sport Sciences & Physiotherapy
Activity levels:
Trained: ≥3 sessions/week, 60–90 min, moderate–high intensity
Sedentary: <1 session/week
Assessments: IPAQ, anthropometry, plasma IL-6 & LPS
***CEIm-Comunidad de Madrid:* **47/734814.9/18**

Gut Microbiota Analysis

Sequencing: 16S rRNA (V3–V4) via Illumina MiSeq
Data analysis: Microbial associations with IL-6 & LPS assessed using MaAsLin2

Clustering

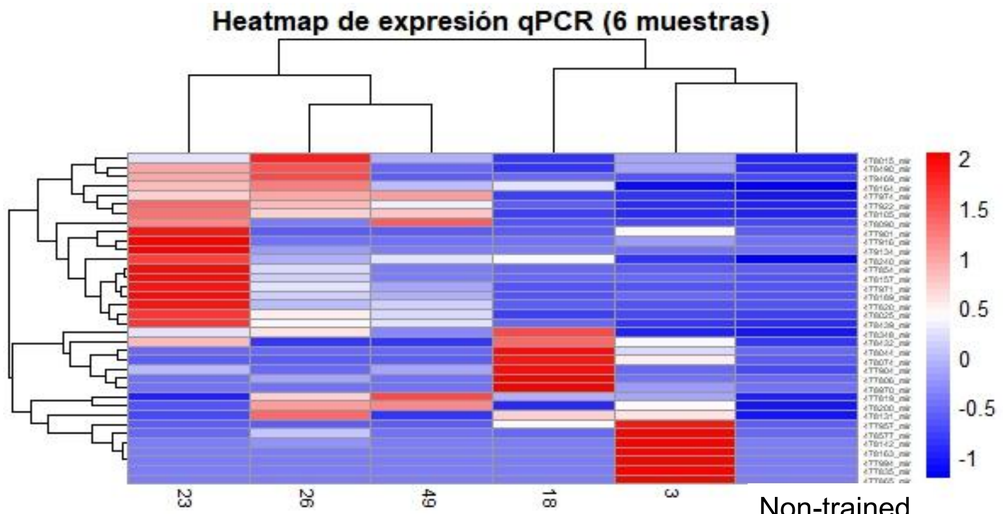
Method: Unsupervised hierarchical clustering (Ward's algorithm) to identify inflamatory profiles

Fecal miRNA Profiling

Platform: TaqMan™ Array Cards (386 targets, ThermoFisher)
Sample selection: 6 trained (2 per cluster) + 1 sedentary
Bioinformatics:
Differential expression: DESeq2; 36 significantly regulated miRNAs ($p < 0.05$)
Visualization: Heatmap of expression patterns
Target prediction: Validated target genes via multiMiR (R package)
Functional enrichment: GO & KEGG pathway analysis using clusterProfiler

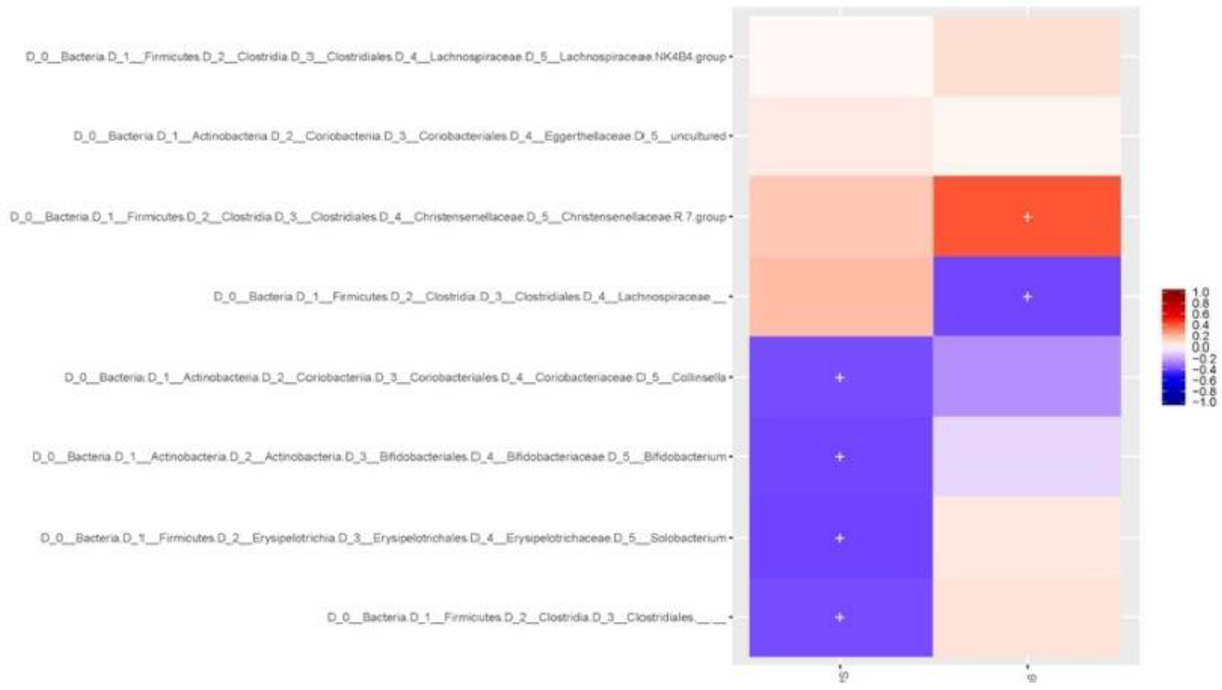
***The integration of miRNA and microbiota datasets is currently ongoing to identify shared mechanistic pathways.*

Figure 3. *36 Fecal miRNAs were quantified using TaqMan Array Cards (ThermoFisher) assessing 386 miRNA when comparing 5 trained (from different inflammatory profiles) vs 1 non-trained men.*



RESULTS & DISCUSSION

MaAsLin2 analysis showed that *Christensenellaceae R-7* group and *Lachnospiraceae NK4A136* group were positively associated with lower IL-6 and LPS levels, suggesting an **anti-inflammatory microbial signature** in trained individuals. *Bifidobacterium, Collinsella,* and *Solobacterium* were negatively correlated with these markers.



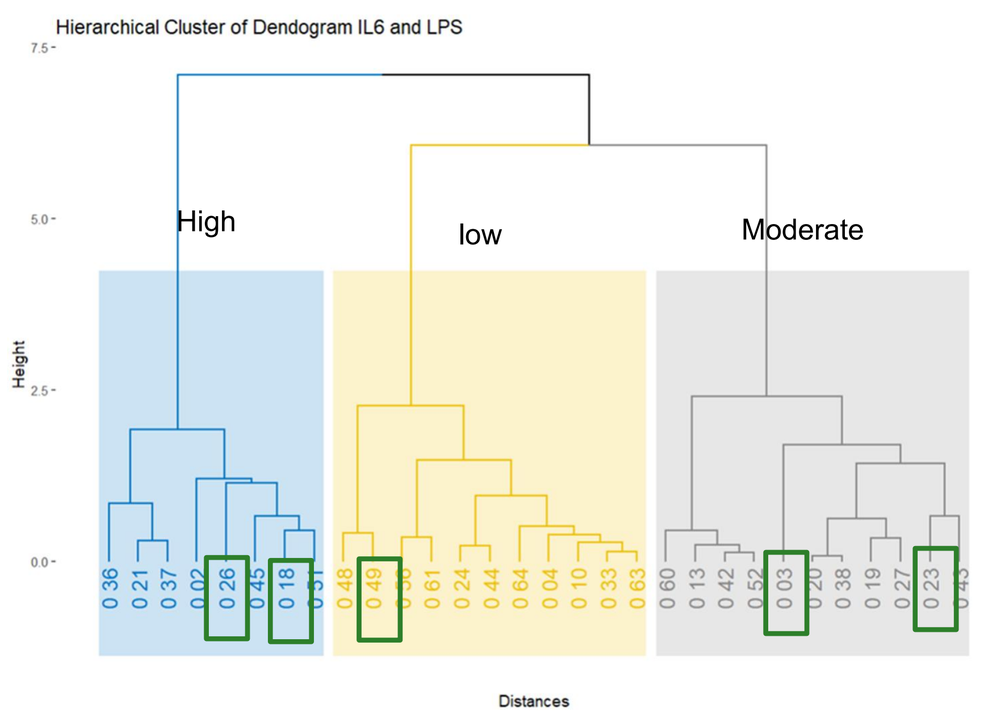


Figure 2. *Participants were clustered by IL-6 and LPS plasma levels into three inflammatory profiles (low, moderate, high).*

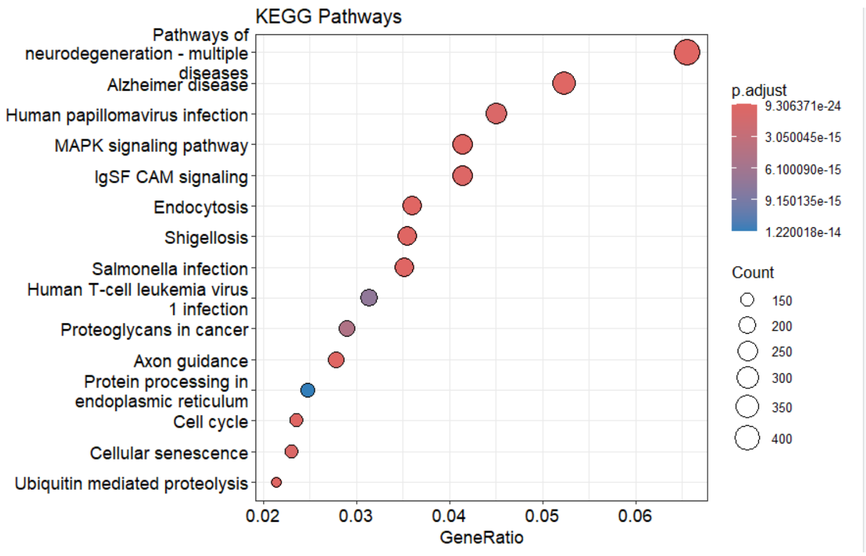


Figure4. *Main KEGG pathways were identified based on the 36 differentially expressed miRNAs between trained and untrained individuals.*

CONCLUSION

These preliminary findings suggest that regular exercise modulates the expression of specific fecal miRNAs, potentially influencing host–microbiota interactions. Integration of demographic, inflammatory, microbiota, and transcriptomic data in larger cohorts is required to further elucidate the mechanisms underlying the interplay between physical activity, fecal miRNAs, and gut microbial dynamics.