

Exercise and Probiotic-Induced Epigenetic and Exosomal Modulations: A 12-Week Intervention Study in Healthy Individual

Proia, P.¹, Naselli, F.², Paladino, L.³, Volpes, S.², Cardinale, P.S.², Caruso Bavisotto⁴, C., Pagliaro, A.¹, Alioto, A.¹, Boatta, A.^{1,5,6}, Messina, G.⁵, Cappello, F.⁴, Baldassano, S.², Barone, R.⁴.

¹Sport and Exercise Sciences Research Unit, Department of Psychology, Educational Sciences and Human Movement, University of Palermo, Italy

²Department of Biological, Chemical and Pharmaceutical Sciences and Technologies, University of Palermo, Palermo, Italy

³Department of Theoretical and Applied Sciences (DiSTA), eCampus University, 22060 Novedrate, Italy.

⁴Section of Human Anatomy, Department of Biomedicine, Neuroscience and Advanced Diagnostics (BIND), University of Palermo, 90127 Palermo, Italy

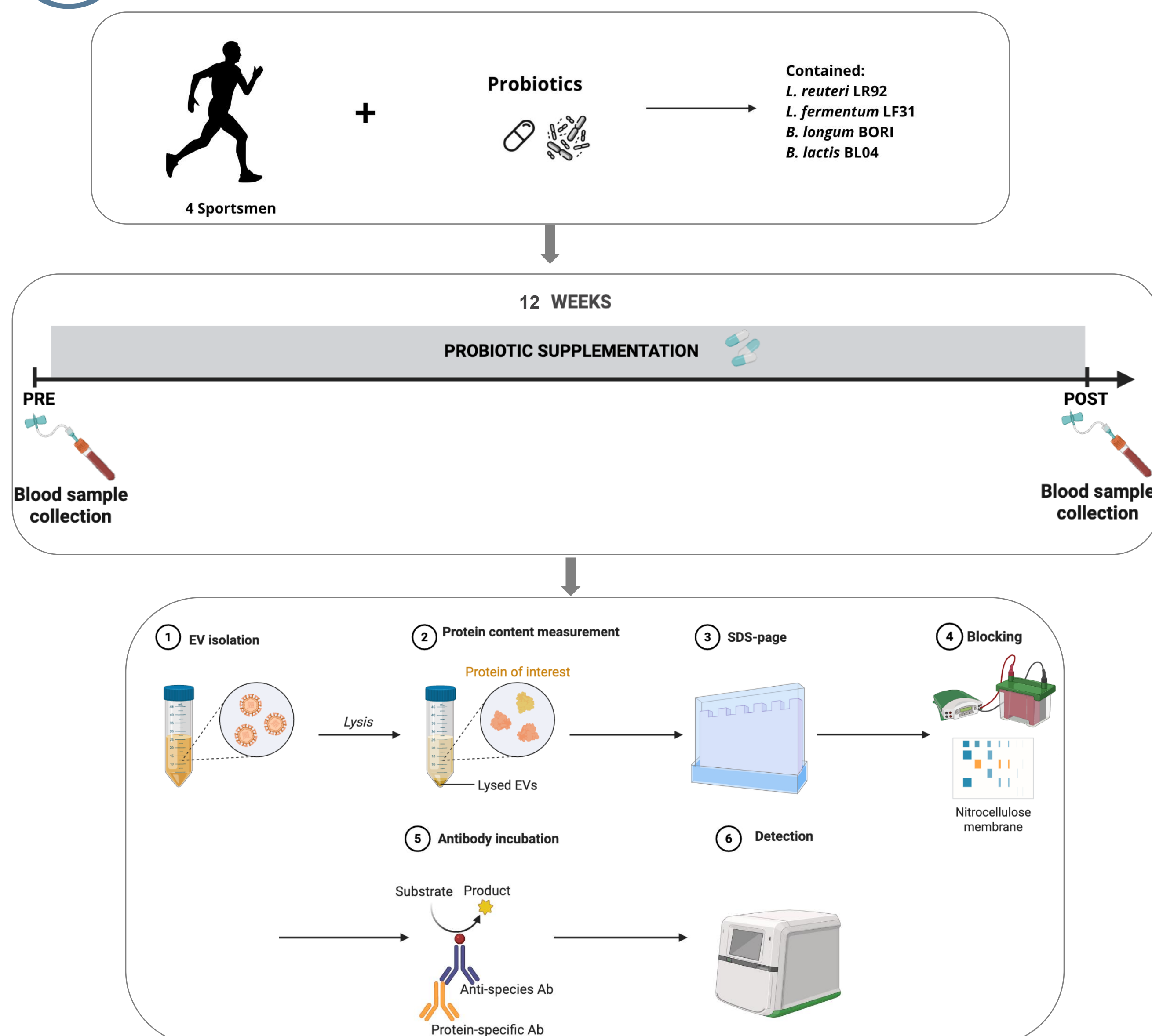
⁵Department of Human Sciences and Promotion of Quality of Life, San Raffaele Roma Open University

⁶Institute for Biomedical Research and Innovation, CNR via U. La Malfa 153, 90146 Palermo.

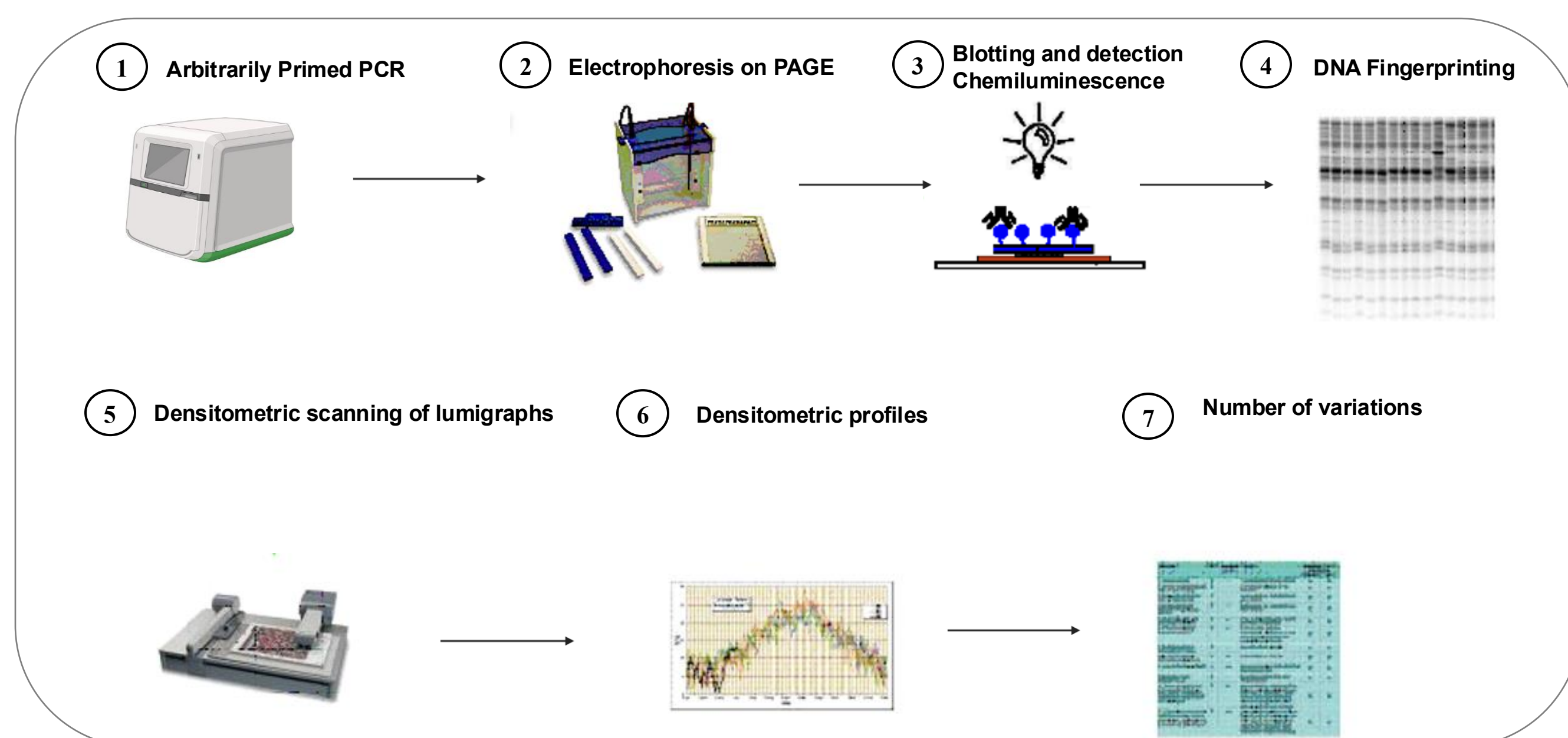
1 Introduction

Physical activity (PA) is known to influence gene regulation through mechanisms such as DNA methylation and non-coding RNA modulation, as well as intercellular communication via exosomes. Nutritional interventions, particularly probiotics like *Lactobacillus fermentum*, may also modulate the pathways that are involved in these mechanisms. The aims study was to investigated the combined effects of a 12-weeks training program and probiotic supplementation on epigenetic modifications and molecular changes, starting from exosomal Hsp60, Hsp70 and Hsp90 expressions.

2 Methods

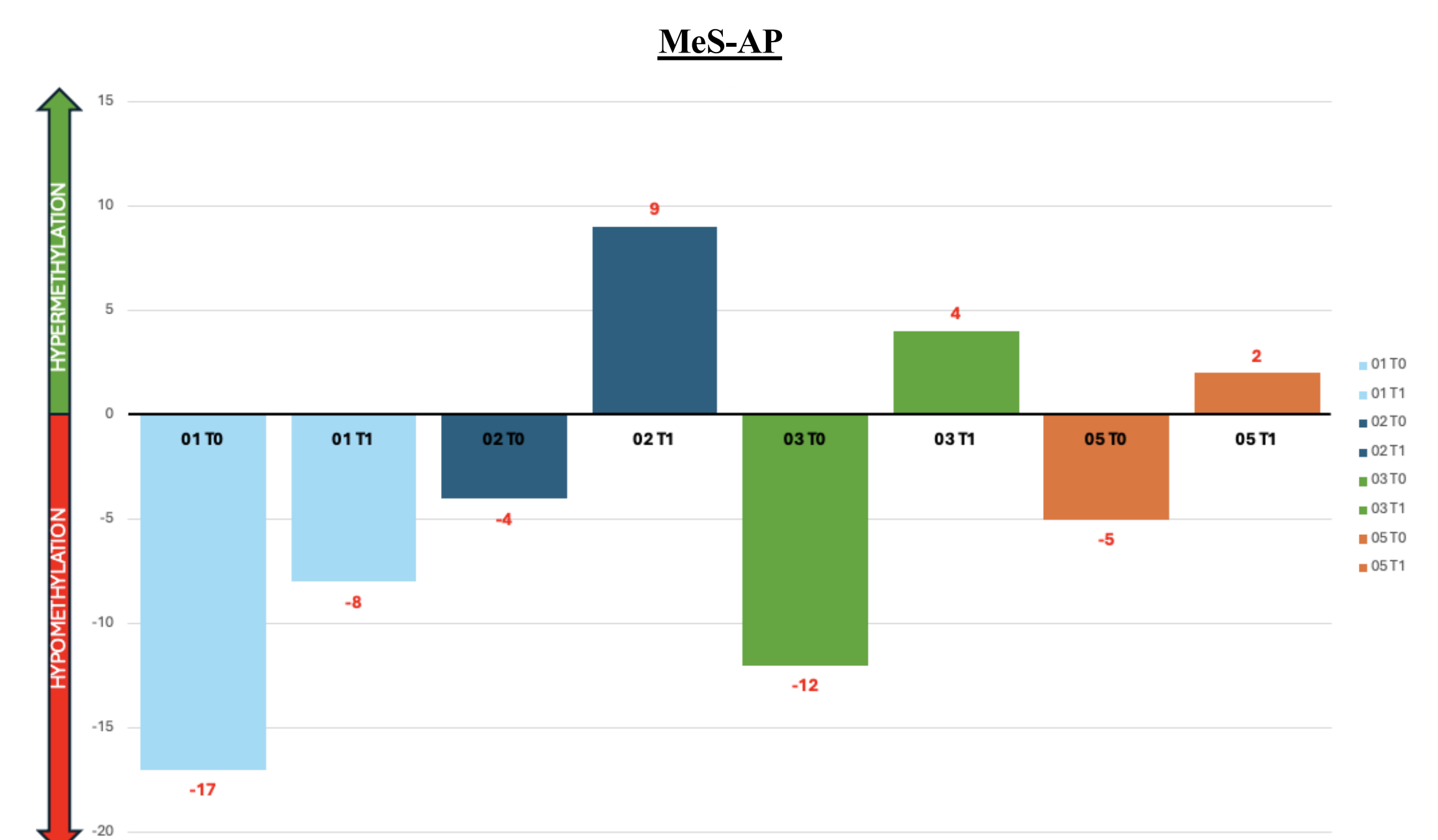
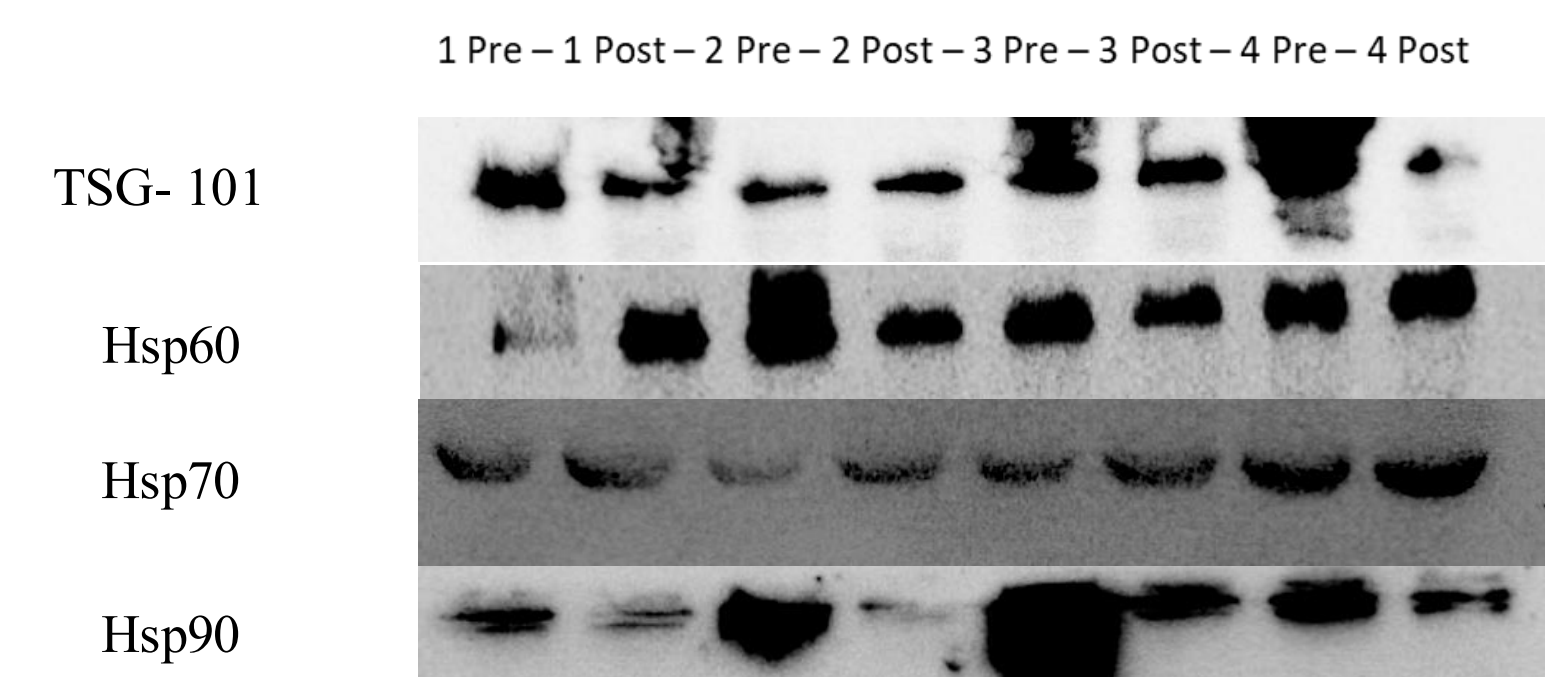


The expression of Hsp60, Hsp70 and Hsp90 within the exosomes was analyzed through western blot analysis on the exosome isolated from serum following the procedure above described.



Simultaneously, epigenetic analysis was performed via MeSAP-PCR to assess the possible genomewide changes in DNA methylation.

3 Results



4 Discussions

Significant differences in epigenetic profiles were observed. Athletes showed baseline hypomethylation, with subject 01 showing -17 demethylation points; after 12-weeks of probiotic supplementation this was reduced to -8, indicating a shift toward hypermethylation. Similar trends were seen in other athletes: subject 02 (from -4 to +9), subject 03 (from -12 to +4), and subject 05 (from -5 to +2). In parallel, TSG-101 was used as marker of exosomes and also highlighted the effects of probiotics in the modulation of heat shock proteins expression. Particularly, there were an increse of Hsp70 and Hsp90 levels, while Hsp60 showed variable changes.

5 Conclusions

Physical activity and probiotics act synergistically as non-pharmacological modulators of epigenetic and exosomal pathways. Physical activity induces hypomethylation, while probiotics shift the balance toward hypermethylation, increasing stability and reducing inflammation. Probiotic intake also enhances Hsp70 and Hsp90 expressions in plasma exosomes, supporting intercellular communication and stress-response signaling. These findings suggest that the combination of 2-3 well-selected probiotic strains may enhances adaptive response to physiological stress. Further studies are needed to assess the impact on performance and recovery.

References

1. Raleigh, S. M. (Ed.). (2021). Epigenetics of Exercise and Sports: Concepts, Methods, and Current Research.
2. Barone, R. et al. (2016) Skeletal muscle Heat shock protein 60 increases after endurance training and induces peroxisome proliferator-activated receptor gamma coactivator 1 α 1 expression. Sci Rep 6, 19781.
3. Sausa M, Paladino L, Scalia F, Zummo FP, Vergilio G, Rappa F, Cappello F, Gratie MI, Proia P, Di Felice V, et al. Lactobacillus fermentum LF31 Supplementation Reversed Atrophy Fibers in a Model of Myopathy Through the Modulation of IL-6, TNF- α , and Hsp60 Levels Enhancing Muscle Regeneration. Nutrients. 2025; 17(9):1550. <https://doi.org/10.3390/nu17091550>