

Investigating circulating Extracellular Vesicles in response to physical exercise

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Physical exercise represents a highly complex perturbation of homeostasis in a large number of tissues, largely consequential of the increasing metabolic demands of contracting skeletal muscle. When regularly performed, it produces physiological adaptations associated with improved health and longevity (Whitham M et al. (2018) *Cell Metab* 27, 237–251). Although exercise's benefits are well-established, the molecular mechanisms underlying exercise adaptations remain ill-defined and are actively being investigated. Extracellular vesicles are a major source of regulatory signals that play a key role in cell-to-cell communication and have recently emerged as a potential tool through which the muscle communicates with other tissues and organs (Maggio S et al. (2023) *Int J Mol Sci* 24, 3039). For this reason, the present project aims to investigate and characterize circulating EVs released in response to high intensity and duration exercise. To isolate EVs, serum and plasma samples were collected from ultramarathoners pre- and post-race and then processed with Size Exclusion Chromatography (SEC). The obtained SEC fractions were characterized by Nanoparticle Tracking Analysis to check particle counting and size distribution, which resulted comparable with those reported in literature as previously published in Maggio S et al. (2023) *Int J Mol Sci* 24, 3039. Dot blot assay, employed for EV marker detection, showed that EV surface protein CD9, muscle-related marker CAV3 and stress-related marker HSP60 expression increased in post-race in most marathoners. These findings suggest that EVs could be involved in the systemic responses to high intensity and duration exercise. Circulating EVs could therefore represent an innovative source of exercise biomarkers which could give a helpful insight on the physiological activation state in response to physical exercise.