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LONGITUDINAL STUDY IN LOW AND HIGH FUNCTIONING OLDER HEALTHY ADULTS: PRELIMINARY DATA ON PLASMA METABOLOMICS AND MUSCLE DNA METHYLATION

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Ambulatory disability and loss of independence are major concerns in older adults, with 21% of Americans ≥65 reporting serious mobility difficulty. (1) Habitual walking speed and standard physical function measures reflect healthy aging and predict mobility disability and mortality, and low-functioning (LF) older adults decline faster than high-functioning (HF) peers, though the biological drivers remain unclear. (2) Aging disrupts systemic and cellular iron homeostasis, leading to iron accumulation (i.e., ferritin a major iron storage protein) that contributes to age-related conditions like sarcopenia. (3) Cells possess quality control mechanisms to preserve function in the presence of iron overload and oxidative stress, which involve the export of damaged organelles and unwanted material, and extracellular vesicles (EVs) may play a role in these mechanisms. (4,5) Because unloading and microgravity rapidly induce muscle deconditioning, (6) and mitochondrial stress, we leverage microgravity exposure (spaceflight and simulated microgravity) as a proof-of-concept accelerator of ambulatory disability-relevant biology, while establishing clinically feasible methods to isolate EVs directly from small skeletal muscle biopsies for downstream cargo profiling.Clinical muscle EV isolation. EVs were isolated from approximately 20 mg of frozen human skeletal muscle using nanographene immunomagnetic particles (NanoPoms) (7) conjugated to antibodies to EV-specific surface proteins (CD9, CD81, and CD63). Particle size and morphology were verified by Nanoparticle tracking analysis (NTA) and TEM. Immunoblotting confirmed the presence of intravesicular (Alix, TSG101) and membrane (CD81, CD63) EV markers. (Microgravity EV transcriptomics). Muscle tissue chips derived from young, active and older, sedentary individuals were cultured during an International Space Station exposure, and EVs were isolated from conditioned media collected during the final 24 hours of spaceflight and compared to matched ground controls. EVs from conditioned media were enriched using the same NanoPoms magnetic capture-release (7,8) workflow, followed by RNA extraction and transcriptomic profiling using the FREYA pipeline aligned to the telomere-to-telomere human genome. ExoView profiling assessed tetraspanin markers (CD9, CD63, CD81). NanoPom beads yielded 2 × 10^9 to 1 × 10^10 particles from 15–30 mg of undigested tissue, with particles in the 100–150 nm size range typical of small EVs. These EVs contained characteristic markers, CD81, CD63, Alix, and TSG101, validated by Western blot, with EV-like morphology by TEM and size/-concentration by NTA. In microgravity experiments, TEM and NTA revealed spaceflight-associated alterations in EV morphology, particle concentration, zeta potential, and RNA yield, and ExoView profiling showed age- and activity-specific modulation of CD9, CD63, and CD81. Transcriptomic analysis identified 99 significantly altered transcripts in spaceflight EVs, with age-dependent shifts in stress response, gene regulation, and mitochondrial pathways. Spaceflight EVs exhibited reduced expression of key mitochondrial genes (PHB2, RHOT1, COX5B, ACO2), alongside downregulation of lipid metabolism, mitochondrial organization, and oxygen transport pathways, whereas ground-only EVs were enriched for mitochondrial communication markers. In conclusion, EVs can be reproducibly isolated from small amounts of whole skeletal muscle tissue in older adults, enabling clinically grounded studies of local EV cargo linked to physical function (LF vs HF) and pathways relevant to ambulatory disability. In parallel, microgravity exposure acts as an accelerator platform that surfaces EV transcriptomic signatures consistent with mitochondrial stress and functional decline biology. Ongoing work will optimize EV purity, quantify mitochondrial complex proteins and ferritin in control and participant muscle EVs, extend analyses across LF and HF biopsies, integrate parallel plasma EV profiling, and correlate EV cargo with functional measures and metabolic adaptation under microgravity.

Keywords: *Extracellular Vesicles, nanographene immunomagnetic particles, aging, space flight, EV transcriptomics*