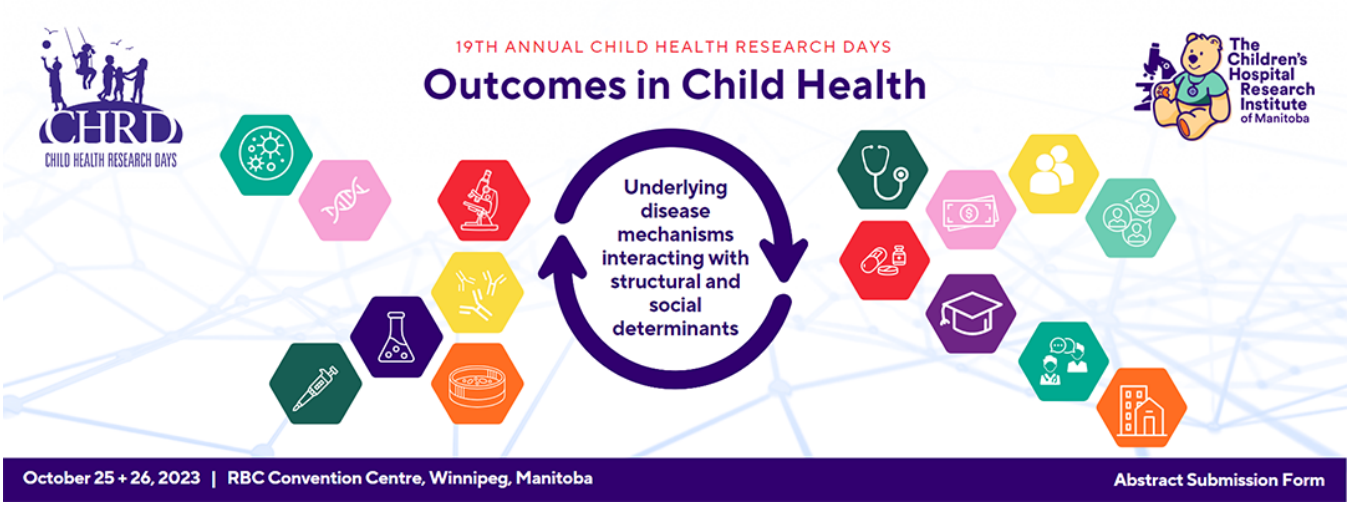

CHRD 2023: Abstract Submission Form

Submitter Name
Patience Obi

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Presenter Status
PhD Student

Research Category
Basic Science

Role in the project
Design
Perform Experiments
Analyze Data
Write Abstract

Title
Identification of putative protein targets that mediate enhanced mitochondrial biogenesis in skeletal muscle-derived extracellular vesicles after chronic contractile activity

Background
Extracellular vesicles (EVs) are membrane-bound nanoparticles encapsulating biological cargo, and mediate cellular communication. We have previously shown that skeletal muscle-derived EVs released post-chronic contractile activity (CCA) increased mitochondrial biogenesis (MitoB) in murine myoblasts, but the underlying mechanisms are unknown.

Objective
Here, we investigated the localization of EV protein cargo post-CCA (luminal vs. membrane-bound) and identified putative EV protein targets (using proteomics) that mediate this effect.

Methods
C2C12 murine myotubes were electrically paced (3 hrs/day x 4 days @ 14V, IonOptix). EVs were isolated from conditioned media using differential ultracentrifugation. CCA-EVs were treated with 100 µg/mL Proteinase K (ProK) + 0.1% Triton X-100 (Tr), and then co-cultured with myoblasts for 4 days. Mitochondrial respiration was measured (Seahorse XFe24, Agilent) and data analyzed using Student's t-tests. Liquid chromatography-mass spectrometry (LC-MS/MS) proteomic analysis was performed on control-EVs and CCA-EVs and data analyzed using bioinformatic tools (FunRich, Ingenuity Pathway Analysis, STRING).

Results
CCA-EVs increased basal oxygen consumption rate (OCR) by 52% (p=0.0493, N=6), and maximal OCR by 28% vs. PBS (p=0.0320, N=6). Treatment with ProK + Tr ameliorated this effect (p=0.0292; p=0.0007, N=6). Using LC-MS/MS, a total of 2363 proteins were identified, of which 62 proteins were significantly altered, with 46 increased and 16 decreased in CCA-EVs vs. control-EVs. Pathway analysis identified Cap1-Pfn1-Actn1-Itga6 as significantly altered. These four proteins were also in Top-100 proteins identified in ExoCarta and Vesiclepedia databases. Interestingly, Cap1 and Pfn1 have been reported to mediate mitochondrial function and are both membrane-bound.

Conclusion
Our data show that the CCA-EVs increased basal and maximal OCR, and the effect was mediated by membrane-bound and/or EV corona protein cargo. Additionally, the Cap1-Pfn1-Actn1-Itga6 pathway was significantly increased in CCA-EVs, with all four proteins enriched in EV databases. Our next step will be to validate putative targets to identify proteins regulating the pro-metabolic effects of CCA-EVs.

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