Investigating exosomal microRNA and lipidomic profile in response to acute endurance exercise in males and females

S. Alexander,[1] S. Lamon,[1] J. McMullen,[2] Y.K. Tham[2] and G.D. Wadley,[1] [1]Institute for Physical Activity and Nutrition, Deakin University, 221 Burwood Highway, Burwood, VIC 3125, Australia and [2]Cardiac Hypertrophy, Baker Heart and Diabetes Institute, 75 Commercial Road, Melbourne, VIC 3004, Australia.

Introduction: Exercise has many well-established health benefits, including reduction of disease and all-cause mortality. Despite not being directly involved in muscular contraction, tissues such as the liver and the brain display adaptations to exercise, including increased mitochondrial function and number. One postulated mechanism by which this occurs is *via* tissue cross-talk, a process mediated by exosomes. Exosomes are small, membranous vesicles that are ubiquitously expressed by nearly all cells in the human body. Exosomes transport nucleic acids, proteins and lipids to distal tissues and influence the recipient cell structure and function. Exosome number increase after an acute bout of endurance exercise (Fruhbeis *et al.*, 2015; Whitham *et al.*, 2018). The current study aims to examine how cycling at 70% VO2peak for 60 mins influences exosomal miRNA and lipid abundance in men and women.

Methodology: Sixteen males (age 23.6 ± 3.7, mean ± SD) and eight females (age 23.0 ± 3.4), cycled for 60-min at 70% predetermined VO_2peak. Blood samples were taken before, immediately after, and three hours after exercise from the ante-cubital vein. Exosomes were isolated from separated plasma and treated with RNase to prevent potential contamination by free nucleic acids. Immunoblotting was performed to validate the presence and purity of exosomes. Exosomal lipid composition was analysed with mass spectrometry. Expression levels of exosomal miR-1, -16, -23a/b and -133a/b were established by qPCR, using cel-miR-39 as an exogenous control. The data were analysed using one-way ANOVA.

Results/Conclusions: Immunoblotting of exosomal marker proteins confirmed the presence of exosomes within the extracted fraction. Lipidomic analysis revealed co-isolation of cholesterol esters and diacylglycerol within the exosome-enriched fraction. Because of this, it was deemed inappropriate for further down-stream lipidomic analysis using the current exosome isolation procedure. miR-1, -16, -23a/b and -133a/b were present in exosomes from males and females. No significant changes were observed between rest, exercise and recovery samples in any species. These muscle-enriched miRNA species are regulators of mitochondrial biogenesis, vascularisation and myoblast differentiation and proliferation in skeletal and cardiac muscle tissue. More studies are needed to confirm the effect of exercise on exosomal miRNAs and how they contribute to tissue adaptation.

Fruhbeis C, Helmig S, Tug S, Simon P, Kraemer-Albers E-M. (2015) *J Extracell Vesicles*, **4**, 28239.

Whitham M, Parker BL, Friedrichsen M, Hingst JR, Hjorth M, Hughes WE, Egan CL, Cron L, Watt KI, Kuchel RP. (2018) *Cell Metab*, **27**, 237-51.