Abstract: 47O

The abundance of cell-free mitochondria are increased in human blood following an acute bout of endurance exercise

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Until recently, mitochondria had been considered as organelles localised exclusively inside cells, where they play important roles in multiple biological processes in most tissues. However, recent studies have demonstrated the extracellular presence of intact mitochondria in human plasma (Al Amir Dache *et al.* 2020, Stephens *et al.* 2020, Stier 2021). The mechanisms by which these cell-free mitochondria can appear in blood include transfer via extracellular vesicles (EVs) (Stephens, Grant *et al.* 2020) and secretion by activated platelets (Boudreau *et al.* 2014), although their physiological roles and function are largely unknown. Endurance exercise is known to result in the secretion of various molecules such as EVs, growth factors and cytokines into the circulation (Whitham *et al.* 2018). Therefore, the aim of the study was to determine if an acute bout of endurance exercise in humans leads to an increase in cell-free mitochondria in the circulation. The study was approved by the Deakin University Human Research Ethics Committee (DUHREC 2021-223 and 2022-219) and conforms to the Declaration of Helsinki. Written, informed consent was obtained from all participants before commencing sampling procedures and exercise trials. A pilot study was conducted, where we isolated cell-free mitochondria from 1 ml of human plasma via immunoprecipitation of an outer mitochondrial membrane protein and we subsequently detected the presence of the mitochondrial protein ATP5A by western blot. A second pilot study found that 93% of the mitochondria isolated from human plasma were intact with the maximal citrate synthase enzyme activity being 489 ± 223 $pmol.min^{-1}.ml^{-1}$ plasma (mean ± SD; n= 2 participants). Twelve healthy male participants (age 25.6 ± 3.6 years, BMI 25.5 ± 3.7 $kg.m^{-2}$, VO_{2peak} 42.2 ± 6.1 $ml.kg^{-1}.min^{-1}$, Mean ± SD, n=12) then cycled on a bicycle ergometer at 70% VO_2peak for 60 minutes. Mitochondria were isolated from 1 ml of plasma collected pre-exercise and immediately post-exercise. The abundance of mitochondrial protein ATP5A increased approximately 2.1-fold post-exercise (p=0.007, Wilcoxon matched-pairs non-parametric test). In conclusion, we detected intact mitochondria in human plasma and their abundance appears to increase following moderate intensity endurance exercise. Further examination of other mitochondrial proteins and measures of mitochondrial content including the use of electron microscopy and/or mitochondria-specific probes are required to confirm these findings.

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