Bioenergetics of peripheral blood mononuclear cells from endurance-trained and untrained healthy young adults analysed using extracellular flux assays

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Background and objectives. Skeletal muscle mitochondrial amount and function is positively affected by endurance exercise and training status[1]. However, to routinely assess mitochondrial function in response to exercise in humans, practical and non-invasive measurements of mitochondrial function are needed. We used near-infrared spectroscopy as a non-invasive method to assess the effect of training status on *in vivo* mitochondrial function[2]. In addition, we study peripheral blood mononuclear cells (PBMCs), since PBMCs are relatively easily obtained from individuals and the immune system is involved in regulating the response of the body to exercise. Therefore we optimized and validated brightfield imaging coupled to extracellular flux measurements in PBMCs to study the effect of training status on bioenergetic PBMC profiles. **Methods.** Extracellular flux measurements were performed using the Seahorse XFe Analyzer. For validation of our novel imaging approach in analyzing PBMC bioenergetics, PBMCs were isolated from human buffy coats obtained from healthy blood donors and plated on coated Seahorse XFe96 cell culture microplates (0.5 – 3.0 x 10^5 cells/well). Brightfield images were taken using Cytation 1 and analyzed using ImagePro and ImageJ software. To study the effect of training status on bioenergetic PBMC profiles, eight endurance-trained (VO_2max >57 ml/kg/min) and eight untrained (VO_2max <47 ml/kg/min) young male adults were included. **Results and conclusions** Linear relationships were identified between plated cell number and pixel number (R^2 = 0.9834, $P < 0.001$) and between calculated cell number (based on pixel number) and basal oxygen consumption rate (OCR) (R^2 = 0.9491, $P < 0.001$) in multiple buffy coats, demonstrating reproducibility and suitability of our method for analyzing mitochondrial function in PBMCs over different days over a wide cell number range. Findings from our human trial on endurance-trained individuals indicated that PBMCs from endurance-trained individuals showed significantly lower coupling efficiency values ($P = 0.0289$) as well as higher proton leak compared to untrained individuals. No differences in basal respiratory OCR, maximal respiratory OCR or spare respiratory capacity in PBMCs from endurance-trained and untrained individuals were observed ($P > 0.05$). Our findings reveal a possible lower ATP production capacity of PBMCs from endurance-trained individuals and could contribute to further understanding how PBMC bioenergetics contribute to immune function and whole-body performance.