

## Increase in Mitochondrial content after Electrical Pulse Stimulation is dependent on duration of stimulation

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Category: Masters

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### ABSTRACT

We have previously shown that human skeletal muscle myotubes cultured *in vitro*, retain *in vivo* characteristics of the donors. Recent studies indicate that electrical pulse stimulation (EPS) can be used as an exercise mimetic in a cell culture model, and could be beneficial to understand molecular mechanisms underlying exercise training. **Purpose:** The purpose of this study was to compare acute, moderate and long duration EPS treatments on mitochondrial and lipid content in cultured myotubes. **Methods:** EPS stimulation was applied to human myotubes cultured from sedentary donors under three conditions: Acute (bipolar pulses of 100 Hz for 200 ms every 5<sup>th</sup> second; 30V for 60 min) and chronic stimulation (single bipolar pulses of 2 ms; 30V, 1Hz continuously for 24 h or 48 h). Mitochondrial and lipid contents were measured by primary antibody for complex IV and bodipy green dye, respectively, using immunohistochemistry techniques. Fluoroskan ascent microplate reader was used to quantify fluorescence signals. OXPHOS proteins were measured using western immunoblotting. **Results:** There was no change in lipid or mitochondrial content as assessed by immunohistochemistry after acute EPS stimulation. Chronic stimulation resulted in a significant increase in the mitochondrial content after 24 h (from  $0.183 \pm 0.02$  AU to  $0.350 \pm 0.03$  AU;  $p=0.008$ ) and 48 h (from  $0.290 \pm 0.01$  AU to  $0.337 \pm 0.01$  AU;  $p=0.02$ ) of continuous EPS stimulation. OXPHOS proteins increased after 48 h of EPS. There was also a significant increase in lipid content after 48 h of EPS stimulation (from  $0.210 \pm 0.01$  AU to  $0.256 \pm 0.01$  AU;  $p=0.02$ ). **Conclusion:** These findings suggest that 48 h of chronic EPS results in an increase in both mitochondrial and lipid contents in human myotubes. The concomitant increase in lipid and mitochondrial content after exercise mimetic EPS stimulation supports the elevated level of intramyocellular lipid and mitochondrial content evident in endurance trained athletes.