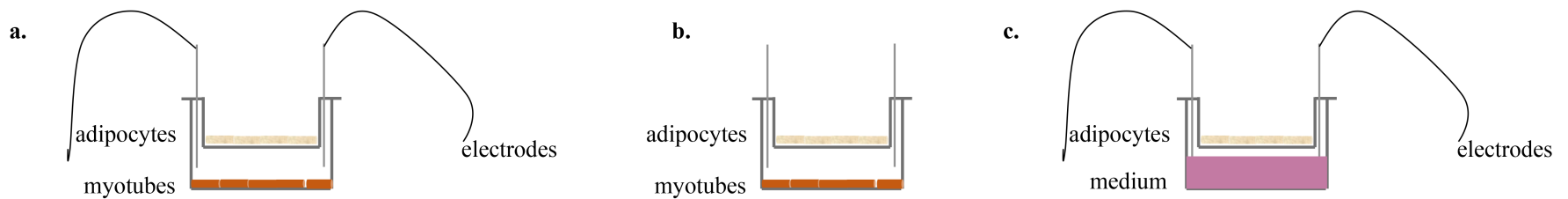


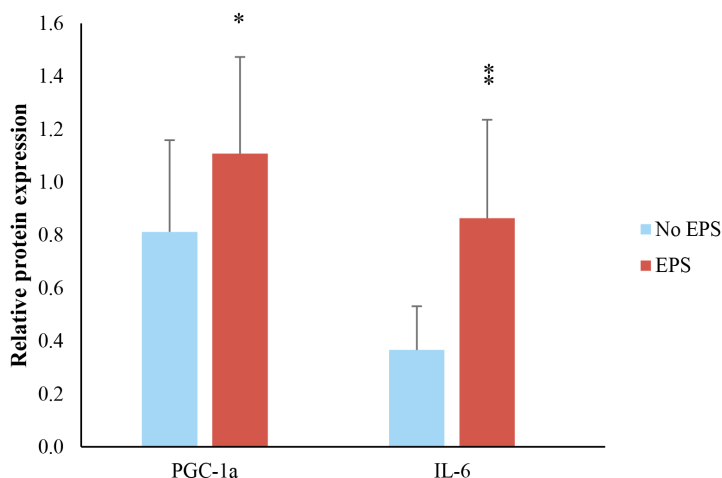
**INTRODUCTION:** The crosstalk between the exercising muscle and the adipose tissue, mediated by myokines and metabolites derived from both tissues during exercise, has created a controversy between animal and human studies with respect to the impact of exercise on the browning process. **AIM:** Our aim was to elucidate the interaction of myotubes with white adipocytes, with respect to browning, during muscle contraction.

**METHODS:** We allowed C2C12 myotubes and 3T3-L1 adipocytes to interact *in vitro* under electrical pulse stimulation (EPS) mimicking muscle contraction (Figure 1), while monitoring the consequent effect in expression of thermogenic genes. Immunoblotting was used to measure protein expression levels.

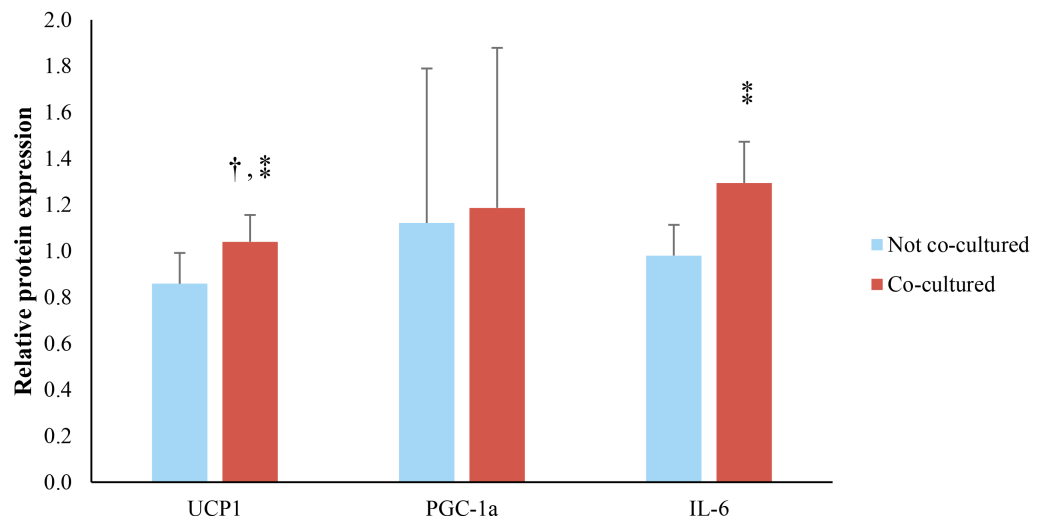


**Fig.1:** Experimental set up. Schematic representation of the three experimental conditions used: **a)** co-culture of both cell types and EPS applied to the myotubes; **b)** 3T3-L1 adipocytes were co-cultured with the C2C12 myotubes in absence of EPS; **c)** EPS was applied to the well filled with medium, with only the presence of 3T3-L1 cells inserts

**RESULTS:** In the co-cultured C2C12 cells, EPS increased the expression of PGC-1 $\alpha$  ( $p=0.129$ ;  $d=0.73$ ) and IL-6 ( $p=0.09$ ;  $d=1.13$ ) protein levels (Figure 2). When EPS was applied, we found that co-culturing led to significant increase in UCP1 ( $p=0.044$ ;  $d=1.29$ ) and elevation of IL-6 ( $p=0.097$ ;  $d=1.13$ ) protein expression in the 3T3-L1 adipocytes. The expression of PGC-1 $\alpha$  increased by EPS but was not significantly elevated after co-culturing ( $p=0.448$ ;  $d=0.08$ )(Figure 3).



**Fig.2:** PGC-1 $\alpha$  and IL-6 relative protein expression in C2C12 myotubes co-cultured with 3T3-L1 with and without EPS in relation to C2C12 differentiated and untreated cells. Graphs represent mean $\pm$ SD; asterisks indicate (\*) and large (\*) effect sizes.



**Fig.3:** UCP1, PGC-1 $\alpha$  and IL-6 relative protein expression in 3T3-L1 adipocytes with EPS co-cultured with and without C2C12 myotubes in relation to 3T3-L1 adipocytes co-cultured with C2C12 without EPS. Graphs represent mean $\pm$ SD; † indicates statistically significant difference ( $p<0.05$ ); \* indicates large effect sizes.

**CONCLUSIONS:** *In vitro* co-culturing of C2C12 myotubes and 3T3-L1 adipocytes under the stimuli of EPS leads to increased expression of thermogenic proteins. These findings indicate a crosstalk between adipocytes and contracting muscle cells, resulting in changes in the expression pattern of proteins related to browning of adipose tissue.