Contraction induces a cyclic promoter hypomethylation in mouse skeletal muscle

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Background: Muscle contraction drives adaptive responses to improve metabolic efficiency, oxidative capacity and, contractile activity by altering gene expression. In most cases, DNA methylation levels inversely correlate with gene expression. After an acute bout of exercise, transient DNA hypomethylation occurs and this may be an early event in contraction-induced activation of gene expression (Barrès *et al.*, 2012). The enzymatic machinery involved has not been identified.

Objective: To establish an *ex vivo* contraction model to study DNA methylation dynamics and the enzymes involved.

Methods: Mice were anaesthetized by intraperitoneal injection with Ketamine/Xylazine (100 and 10 mg/kg/body weight respectively) and soleus muscles removed. Soleus muscles were incubated in oxygenated (95% O2/5%CO2) Krebs-Henseleit bicarbonate buffer at 30°C (containing 8 mM mannitol, 11 mM glucose, and 0.1% BSA) and stimulated to contract with trains of 25 Hz stimuli (5 min of contraction repeated every 10 min) for 60min. Thereafter, muscles were rested in the same oxygenated buffer at 30°C for 0, 15, 30, 45, 90, 180 and 270 min after the contraction bout. Contralateral uncontracted muscles incubated for the same length of time as the contracted muscles were used as non-contracted controls. Muscles were frozen in liquid nitrogen and analysed for DNA methylation and gene expression by MethylDNA capture followed by quantitative PCR (qPCR) and qPCR, respectively. Statistical differences ($P<0.05$) were determined using a two-tailed paired Student's t-test (stimulated *vs* non-contracted).

Results: DNA methylation analysis showed a cyclic demethylation of promoters of genes involved in oxidative metabolism and mitochondrial biogenesis after contraction: peroxisome proliferator-activated receptor δ (*Pparδ*), pyruvate dehydrogenase kinase isoenzyme 4 (*Pdk4*), citrate synthase (*CS*) and peroxisome proliferator activated receptor γ coactivator 1-α (*Pgc-1α*). Transient demethylations were observed on the promoters of these genes at 30 min and 270 min ($P<0.05$ for *Pparδ, Pgc-1α,* and $P=0.06$ for *Pdk4*) after contraction. Analysis of mRNA expression levels showed an increase in *Pparδ, Pgc-1α, Pdk4* and *CS* at 180 mins after contraction ($P<0.05$ for *Pparδ, Pgc-1α,* and $P=0.05$ for *Pdk4*).

Conclusions: We have established an isolated muscle contraction protocol and confirmed that contraction induces rapid promoter hypomethylation. Our results further suggest a cyclic methylation process. We propose that DNA hypomethylation is an early event in contraction-induced gene activation. We will test this hypothesis by selectively blocking the enzymatic machinery involved in DNA methylation.

Barrès R, Yan J, Egan B, Treebak JT, Rasmussen M, Fritz T, Caidahl K, Krook A, O'Gorman DJ, Zierath JR. (2012) Acute exercise remodels promoter methylation in human skeletal muscle. *Cell Metabolism* **15:** 405-11.