## Abstract:

Macrophages play a central role in skeletal muscle repair. Macrophages secrete pro- and anti-inflammatory cytokines important for successful regeneration of muscle fibers. Age related muscle loss has been associated with increased systemic levels of pro-inflammatory cytokines similar to those expressed by macrophages. It is speculated that an increased inflammatory environment in local tissue impairs muscle regeneration in elderly, contributing to reductions in muscle mass. Resistance training is believed to enhance the anti-inflammatory properties of the muscle, protecting against or counteracting an inflammatory environment.

**Purpose**: The primary aim of this study was to investigate local macrophage content following an acute bout of unaccustomed exercise and after a 12 week training intervention in young and elderly subjects. Secondarily, we wanted to develop a method for detection of macrophage subtypes in human muscle cross sections.

**Methods:** Data from two separate studies were analyzed. Study 1: *Acute study*. 27 elderly men (mean ± SD: age = 70.3 ± 6.6) performed one bout of unilateral leg extension consisting of 5 x 12 concentric repetitions (70% 1RM) followed by 4 x 6 eccentric repetitions (110% 1RM). 4 biopsies from the vastus lateralis were analyzed: one sample prior (PRE) to the training bout and 3 samples in the days following the training bout (1day, 4days and 7days). *Study 2*: *training study*. 10 young (mean ± SD: age = 22.4 ± 1.8) and 10 elderly men (mean ± SD: age = 66.6 ± 4.2) were subjected to 36 training sessions (12 weeks with 3 sessions/week). Biopsies were taken before the training intervention (PRE) and following the training intervention (POST). For both studies, immunohistochemistry was performed for total macrophages (CD68+), anti-inflammatory macrophages (CD68+CD163+) and pro-inflammatory macrophages (CD68+CD163-). Additionally, iNOS and TNF-α was tested as possible markers for pro-inflammatory macrophages.

**Results:** *Acute study:* Infiltration of CD68+ cells increased significantly within 24 hours (0.037 vs 0.055 cells/fiber, P = 0.046). Gradual increases were observed during the following days, with highest detected counts 7 days following the training bout (0.104 cells/fiber, P < 0.001). CD68+CD163+ cells were significantly elevated on day 4 (0.047 cells/fiber) and day 7 (0.069 cells/fiber) when compared to PRE (0.020 cells/fiber) and day 1 (0.029 cells/fiber) . CD68+CD163- baseline results (0.014 cells/fiber) cells tended to increase on day 4 (0.022, P = 0.051) and significantly increased on day 7 (0.026 cells/fiber, P = 0.014). *Training study:* Local changes in CD68+ cells were observed for the OLD group (0.047 cells/fiber vs 0.068 cells/fiber, P = 0.042) but not for the young group post exercise. No changes were observed over time for CD68+CD163+ cells in any of the groups, but in general OLD muscle contained more CD68+CD163+ cells compared to young muscle (P = 0.020). At baseline, young muscle contained more CD68+CD163- cells than muscle in elderly (0.019 cells/fiber vs 0.009 cells/fiber, P = 0.002).

**Conclusion**: These findings illustrate an increased local macrophage content following one physiological bout of resistance training. In contrast to our expectations, there was generally no sign of local accumulated macrophage content following a 12 week training intervention. Differences in pro- and anti-inflammatory macrophage content when comparing young and elderly may play a role for changes in muscle structure/quality as seen with progressing age. Finally, no successful staining protocol was evident using iNOS or TNF-α for pro-inflammatory macrophages.