Exercise-Induced STAT3 Signaling in the Heart

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ABSTRACT: Exercise elicits a transient increase in the production of interleukin-6 (IL-6) in contracting skeletal muscle, and subsequent release into circulation. IL-6 binding to its receptor (IL-6R or sIL-6R, see Figure 1) induces activation of the JAK2/STAT3 pathway in target tissues. Previous studies suggest IL-6 signaling confers protection against myocardial ischemia reperfusion injury, but rely on ischemic preconditioning or pharmacologic administration of IL-6. The purpose of this study was to characterize the IL-6 signaling response in the heart following steady state exercise. Male wild type (C57; n=32) and IL-6 knockout (IL-6$^{-/-}$, n=32) aged 8 weeks were divided into sedentary and exercised groups. Exercised mice performed 3 consecutive days treadmill running at 18 m*min-1 for 1 hour/day. Mice were sacrificed pre-, post-, 30 or 60 min post-exercise. Serum, gastroc and heart were collected and snap frozen. Serum IL-6 was measured via ELISA. Western Blotting for (s)IL-6R, (P)-STAT3, and (P)-Akt was conducted. Exercise induced an increase in serum IL-6, myocardial IL-6R and sIL-6R (all $p<0.05$) expression in C57 animals. No increase was present in IL-6$^{-/-}$. The nuclear fraction P-STAT3/STAT3 ratio increased similarly in both C57 and IL-6$^{-/-}$ gastroc (time main effect) and heart (not significant). Cytosolic P-Akt/Akt decreased post-exercise in both groups ($p<0.05$). It is possible that the time frame of sample collection was insufficient to capture downstream signaling (nuclear translocation of P-STAT3). Interestingly, elimination of a single IL-6 Family cytokine was insufficient to blunt the exercise response, which warrants further investigation, especially as it pertains to cardioprotection. Funded by Auburn University IGP – JQ.

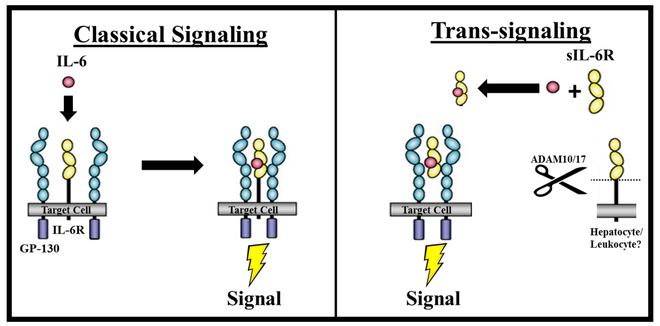
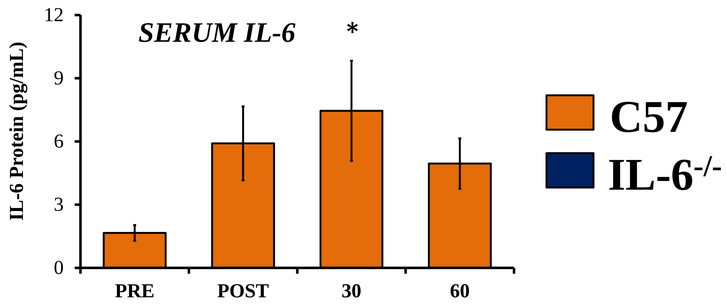
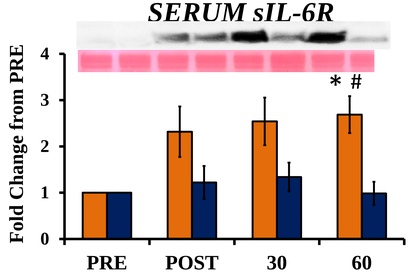
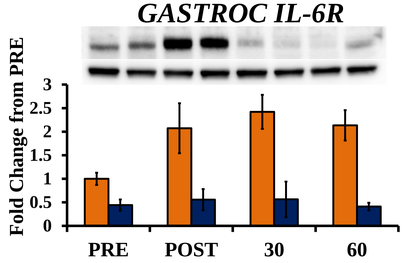
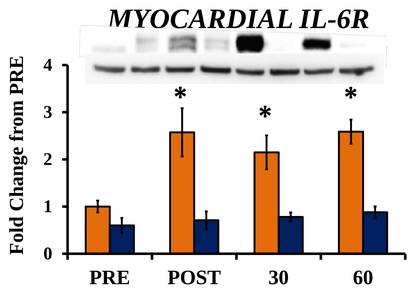


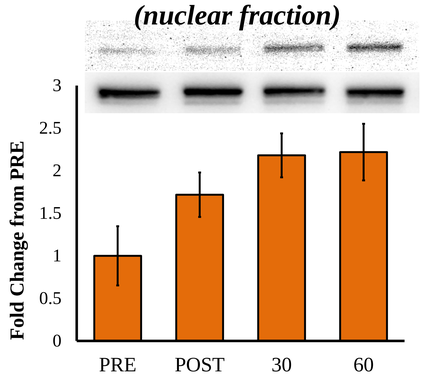
Figure 1. Diagram of Classical vs. Trans-Signaling. Illustrates importance of IL-6R & sIL-6R, respectively. Modified from Rose-John (2006). Cytokine Growth Factor. IL-6 Trans-signaling in IBD.

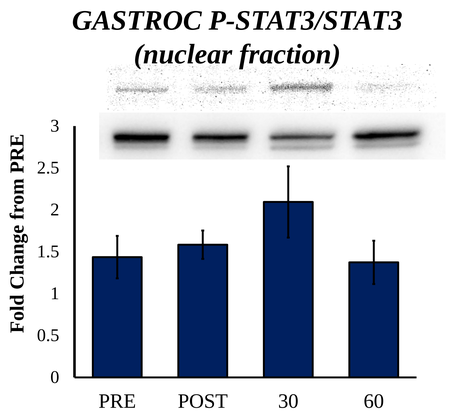


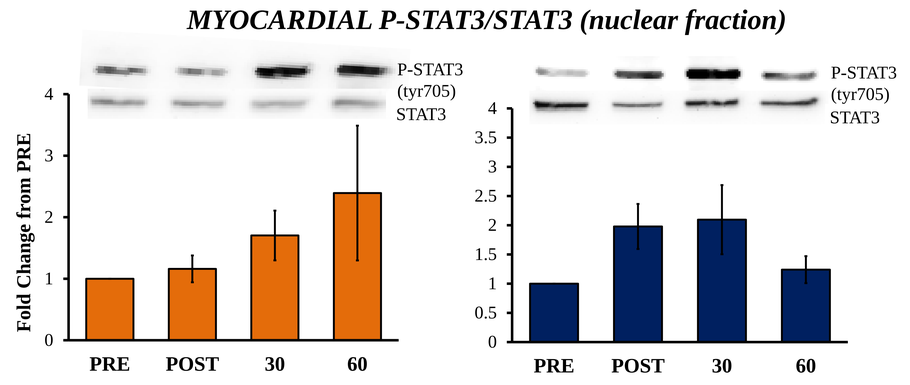


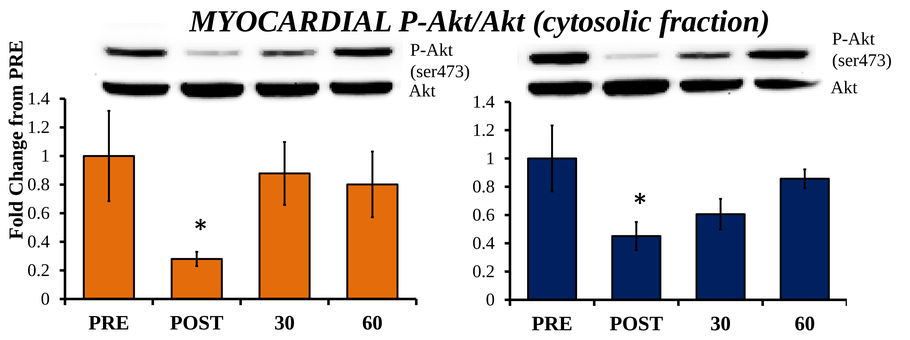












METHODS: Wild Type and Interleukin-6 knockout mice (n=64; C57 = 32 and IL-6$^{-/-}$ = 32, respectively) were habituated to treadmill exercise over 4 days, rested for 1 day, and completed 3 steady state exercise sessions for 60 min*day^{-1} at 18 m*min^{-1} and 0% grade. Mice were sacrificed pre-, post-, 0, and 30 minutes post exercise. Serum, gastrocnemius and myocardial samples were harvested analysis. A commercially available ELISA was used to measure the serum concentration of IL-6 (Invitrogen). Serum and whole muscle homogenates of gastroc and heart were used for western blotting of IL-6R and sIL-6R, respectively. Cytosolic and Nuclear fractions were isolated from gastroc and heart samples to measure STAT3 signaling. Standard Western Blotting techniques were used probe for tyr705-(P)-STAT3, total STAT3, ser473-(P)-Akt and total Akt. Blots were imaged with ECL substrate and quantified using ImageJ.

RESULTS: ELISA and Western Blotting results for serum, gastrocnemius, and myocardial samples. A significant increase in serum IL-6 protein and sIL-6R was evident at 60 minutes post-exercise. Similarly, and increase in gastrocnemius and myocardial IL-6R was seen with exercise. Increases in receptor expression was only seen in C57 mice. No changes were seen in IL-6$^{-/-}$ mice. Nuclear expression of P-STAT3, normalized to total STAT3, was measured as an indicator of IL-6 signaling. A non significant increase was seen in both C57 and IL-6$^{-/-}$ mice, in the heart (not significant) and gastroc (time main effect). Myocardial phospho-Akt, a downstream protein in the PI3K pathway, was measured. A significant post-exercise reduction in P-Akt/Akt ratio was seen in both groups immediately post-exercise, which rebounded to basal levels within 1 hour.

CONCLUSIONS: Exercise induced an upregulation of IL-6R in serum, gastroc, and heart of the C57 animals only, while no differences were seen in the downstream pathways, as evidenced by similar responses in P-STAT3 and P-Akt in heart and skeletal muscle. It is likely that an alternative IL-6 Family cytokine (LIF, CT-1, OSM, CNTF) could compensate for the genetic loss of IL-6 in the IL-6$^{-/-}$ mouse, but this theory needs to be tested. Subsequent gene expression and protein analysis for products of IL-6 signaling, iNOS and COX-2, and alternative IL-6 Family cytokines are necessary to clarify the acute response to exercise stimulus. Ongoing experiments will investigate the effects of exercise-induced IL-6 on ischemia reperfusion injury.