

# The Effect of Physical Activity on Stress-induced Cardiac Fibrosis

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## Abstract

**INTRODUCTION.** This study examined whether routine physical activity limits stress-induced tissue remodeling processes that lead to cardiac fibrosis. The study also explored whether the cardiac urocortin 2/corticotropin releasing factor receptor 2 $\beta$  pathway was activated during physical activity and involved in reducing fibrotic processes. **METHODS.** C57BL/6J male mice were divided into four groups (n=8/group); sedentary/control, voluntary running/control, sedentary/stress and voluntary running/stress. Voluntary running groups were given 24 hr unlimited access to a running wheel in the home cage for 9 weeks. During the 9<sup>th</sup> week, stress groups were exposed to a 5 day resident-intruder social stress paradigm that models human post traumatic stress outcomes. Ventricular cardiac tissue was collected for analysis. **RESULTS.** Mice ran an average of 4.75  $\pm$  1 km each night. Interestingly, running behavior essentially ceased following stress. Running distance dropped to 0.31 km following the 1<sup>st</sup> stress day. Some habituation to stress occurred, as running distance increased to 1.12 km by the 5<sup>th</sup> day of stress but remained significantly lower than pre-stress running distances and distances recorded in non-stressed mice. Quantitative RT-PCR showed small changes in ventricular urocortin 2 and CRF-R2 $\beta$  expression in the running groups. TGF- $\beta$ , a signaling molecule known to induce fibrosis, had comparable expression levels across groups over controls. **CONCLUSION.** Further work is planned to fully characterize urocortin 2/ CRF-R2 $\beta$  and fibrotic processes. Our running data lead us in a new direction, as we have stumbled upon a paradigm that will be useful to study underlying mechanisms by which stress exposure impairs physical activity behavior.

## Introduction

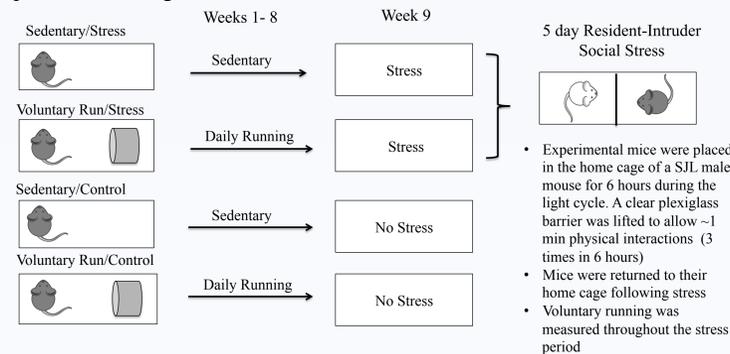
Frequent or prolonged activation of the physiological stress response has been linked to a number of cardiovascular pathologies including hypertension, cardiac arrhythmia, myocardial infarction and hypertrophy. Disruption of normal myocardial and vascular tissue structure is one precipitant of these disease states and has been found to occur following exposure to stress. For example, chronic stress has been shown to lead to the development of fibrosis, a condition of excessive deposition of extracellular matrix of proteins such as collagen in coronary vessel walls and myocardium (5) (12). Recent gene expression studies have suggested that these architectural changes are reflective of tissue repair mechanisms initiated in response to stress-induced cardiac injury (3). This remodeling leads to stiffness and reduced compliance of blood vessels and myocardium, thus hampering normal cardiovascular function.

The primary aim of the current project was to examine whether routine physical activity is a viable means of mitigating the fibrotic effects of stress. Exercise training has been shown to protect against fibrosis associated with the natural aging process, as well as in surgical or genetic models of cardiovascular disease (7). While physical activity is known to reduce various physiological and behavioral responses to stress (11), to date, there is a lack of research examining whether physical activity could limit stress-induced tissue remodeling processes that lead to fibrosis. A secondary aim was to take the first steps to explore a potential and novel underlying mechanism, wherein a cardiac urocortin 2/ corticotropin releasing factor receptor 2 $\beta$  pathway may be activated during physical activity and involved in reducing fibrotic processes.

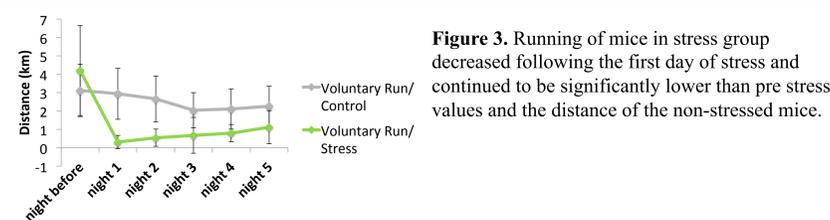
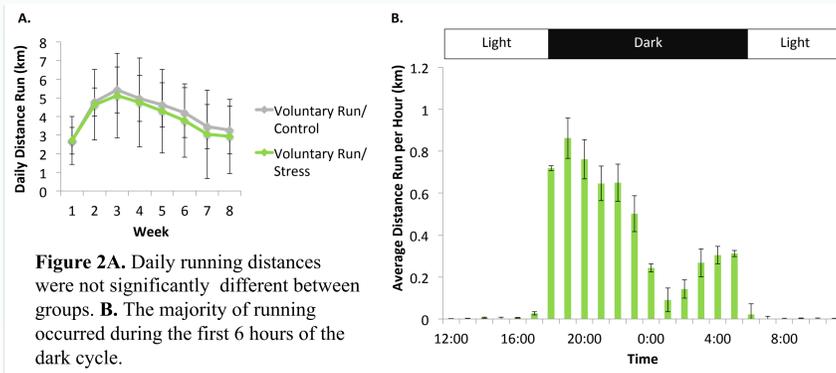
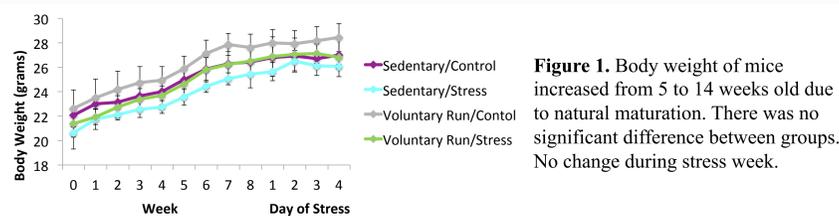
## Methods

C57BL/6J male mice (5 weeks old) were divided into four groups (n=8/group); sedentary control, voluntary running control, sedentary/stress and voluntary running/stress. Mice in the voluntary running groups were given 24 hr unlimited access to a running wheel in the home cage for 9 weeks with wheel running activity recorded continuously. During the 9<sup>th</sup> week of running, mice in the stress groups were exposed to a 5 day resident-intruder social stress paradigm that is considered to model human post traumatic stress outcomes and has been shown to induce cardiovascular fibrosis. Ventricular cardiac tissue was collected for protein and RNA expression analysis at the end of the experiment.

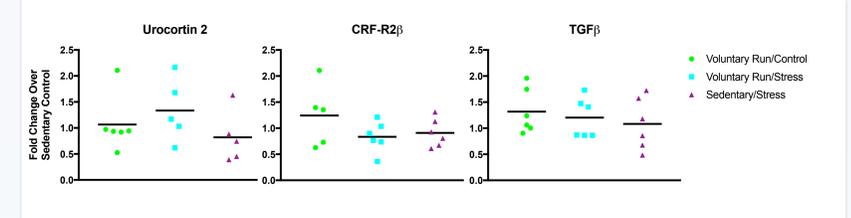
### Experimental Design



## Results



**Figure 4.** Quantitative RT-PCR showed small changes in ventricular urocortin 2 and CRF-R2 $\beta$  expression in the running groups. TGF- $\beta$  had comparable expression levels across groups.



## Summary and Conclusion

- We found that mice in the voluntary running groups ran an average of 4.75  $\pm$  1 km each day. The majority of running bouts occurred within the first 6 hr of the dark cycle. These data are consistent with previous reports of running behavior in C57BL/6J male mice.
- Interestingly, running behavior essentially ceased in mice exposed stress. Running distance dropped to 0.31 km following the first day of stress, compared to 2.93 km in controls. Some habituation to the stress occurred, as running distance increased to 1.12 km by the 5<sup>th</sup> day of stress but was still significantly lower than pre-stress running distances and those recorded in non-stressed mice.
- Quantitative RT-PCR showed only small changes in ventricular urocortin 2 and CRF-R2 $\beta$  expression in the running groups and little change in stress groups. These data suggest that this cardiac pathway may not be activated in response to this stress paradigm. Though, it is possible that changes may have occurred at early time points such as following the first or second day of stress.
- TGF- $\beta$ , a signaling molecule known to induce fibrosis, had comparable expression levels across groups over controls, which indicates that it may not be released in response to our stressor. Though, earlier time points should be examined.

### Future Work

- Examine ventricular urocortin 2 and CRF-R2 $\beta$  expression at earlier time points in the stress paradigm
- Perform Western blots to assess levels of specific proteins (collagen type 1 and fibroblast specific protein) that are indicative of fibrosis.
- Examine the impact of stress on voluntary running

## Selected References

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