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Effect of Shear Stress on ecNOS Expression and Dilation in Soleus Feed Arteries

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Abstract

Shear stress causes artery dilation and increased expression of endothelial cell nitric oxide synthase (ecNOS) in coronary and placental arteries. We sought to determine the importance of shear stress in maintaining normal dilation and normal levels of ecNOS in rat soleus feed arteries (SFA). SFA were isolated from male Sprague-Dawley rats and cannulated for in vitro microscopy (Fig. 6). SFA were exposed to no shear stress, low shear stress, or high shear stress conditions for 4 hours. After 4 hours, endothelium-dependent dilation (acetylcholine: ACh) and endothelium-independent dilation (sodium nitroprusside: SNP) were tested. Arteries were then uncannulated, mRNA was isolated, and RT-PCR for ecNOS mRNA was performed to determine whether shear stress altered ecNOS gene expression. Shear stress did not alter dilation to ACh, but dilation to SNP was greater in the high shear stress arteries. ecNOS mRNA content was greater in high shear stress arteries than low shear stress arteries. These data indicate that altered wall shear stress conditions alter ecNOS gene expression and vascular smooth muscle cell function.

Hypotheses

We hypothesized the following:
- Endothelium dependent dilation will be reduced by a period of reduced shear stress
- Endothelium independent dilation will not be altered by a period of reduced shear stress
- ecNOS gene expression will be reduced by a period of reduced shear stress

Introduction

Control of blood flow is accomplished by regulating the diameter of the arteries and arterioles feeding different organs. Various neural, hormonal, chemical and mechanical mechanisms contribute to the constriction and dilation of arteries. Shear stress, the frictional force between the blood and the endothelial cells, is one of these mechanical mechanisms. Shear stress has been found to have immediate effects, such as endothelial cell secretion of nitric oxide (NO), and long term effects, such as increased expression of endothelial cell nitric oxide synthase (ecNOS) in coronary arterioles and placental arteries (2,3). However, the role of shear stress in ecNOS expression in skeletal muscle arteries is unknown.

Acknowledgements

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Results

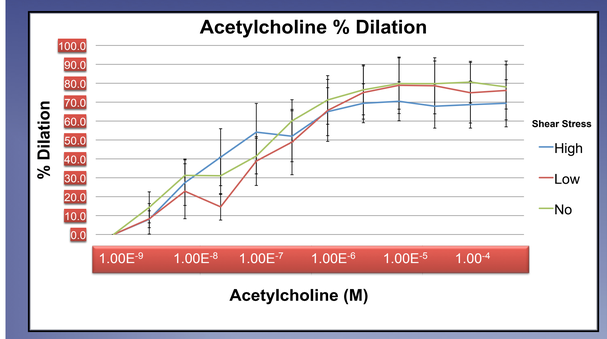

Figure 1: Soleus feed arteries under different shear stress conditions all responded similarly to acetylcholine (endothelium dependent dilator).

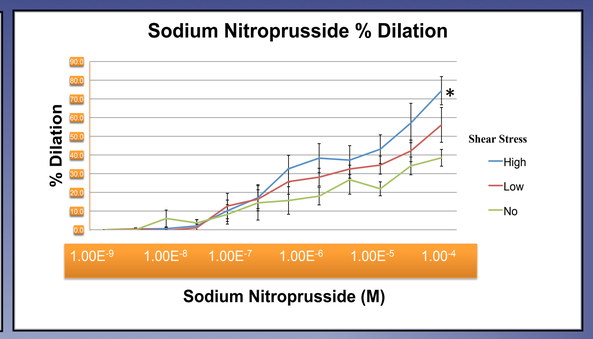

Figure 2: Endothelium independent dilation is reduced by decreased shear stress. (*) High shear stress different than low shear stress (P<0.05)

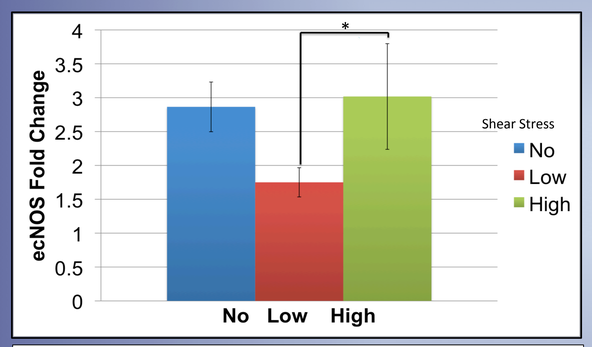

Figure 3: Soleus feed arteries under high shear stress demonstrate an increase in ecNOS mRNA expression

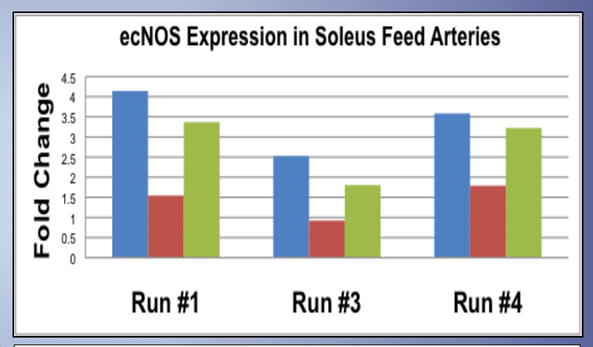

Figure 4: Soleus feed arteries under high shear stress demonstrate increased ecNOS mRNA expression (RT-PCR runs 1,3,4)

Methods

- Soleus feed arteries were isolated from male Sprague-Dawley rats.
- Artery average maximal diameter was 200.6 ± 7.9 um.
- Feed arteries developed average spontaneous tone of 42.2 ± 3.4%.
- Artery diameter was measured using video microscopy (Fig.6)
- High, low, and no flow were induced in individual arteries for 4 hrs to create shear stress.
  - High flow = 200 uL/min
  - High shear stress = 135 dynes/cm^2
  - Low flow = 5 uL/min
  - Low shear stress = 25 dynes/cm^2
- Dose response curves in half long increments (10^{-9} M to 10^{-4} M) of the following drugs were performed:
  - Acetylcholine (ACh): endothelium dependent dilator
  - Sodium nitroprusside (SNP): endothelium independent dilator
- mRNA was isolated from individual soleus feed arteries
- RT-PCR of mRNA from individual soleus feed arteries was used to measure ecNOS expression (Fig. 5).

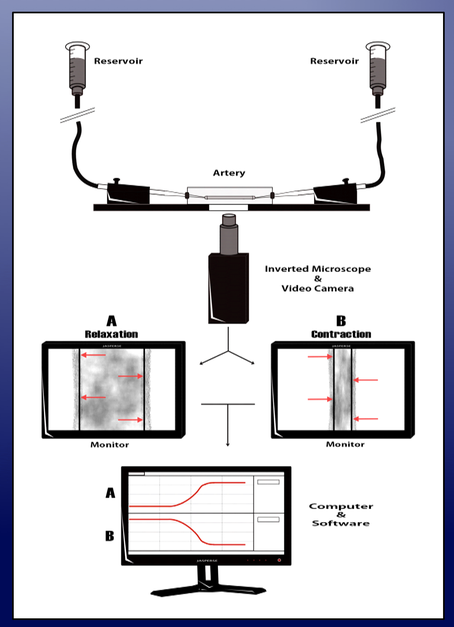

Figure 6: Video microscopy of cannulated arteries

Conclusions

In Soleus Feed Arteries:
1. Low shear stress (reduced flow) did not alter endothelium dependent dilation (Fig.1).
2. Low shear stress (reduced flow) reduced endothelium independent dilation (Fig. 2).
3. High shear stress (high flow) increased the expression of ecNOS mRNA relative to no flow (Fig. 3).

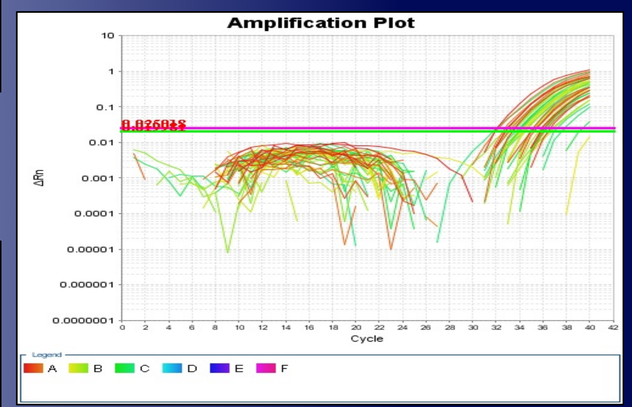

Figure 5: RT-PCR ecNOS and GAPDH (control) amplification plot

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