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Effect of Shear Stress Direction on Endothelial Function and eNOS Phosphorylation in Soleus Feed Arteries

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Introduction

Blood flow feeding tissues and organs is closely regulated in order to meet metabolic and functional needs. Control of blood flow is accomplished by regulating the diameter of the arteries and arterioles feeding different organs. Several neural, hormonal, chemical and mechanical mechanisms contribute to the constriction and dilation of arteries. Shear stress, the frictional force created by streaming blood on the endothelial layer of arteries, is one of these mechanical mechanisms (1). Shear stress causes both acute and long term effects on endothelial cells (1,2,5).

Blood in arteries typically flows away from the heart towards organs (causing antegrade shear stress) during cardiac contraction and briefly flows back toward the heart (causing retrograde shear stress) during cardiac filling. Retrograde flow occurs more often in some disease situations, and studies have shown that retrograde shear stress decreases endothelial cell function (3,4). The specific mechanisms for endothelial dysfunction are unknown, but altered mechanisms could include impaired cell signaling pathways. The most important endothelial cell dilatory signaling pathway is the production of nitric oxide (NO). Retrograde shear stress causes endothelial cells to secrete NO, and increased rates of shear stress cause increased expression and phosphorylation of nitric oxide synthase (eNOS). Regulatory phosphorylation of eNOS can potentially occur on at least four sites: Ser 1177, Ser 116, Ser 635 and Thr 497 (3). The most well characterized of these is Ser 1177, which is phosphorylated by a by PI3K/AKT shear dependent pathway. Regulating phosphorylation of eNOS is critical to endothelial health and maintaining cardiovascular equilibrium. Using rat soleus muscle feed arteries, we seek to determine the effects of changes in shear stress direction on both endothelial cell function and phosphorylation of eNOS at the Ser 1177 site.

Hypotheses

We hypothesized the following:

- Soleus feed artery endothelium function will be impaired by constant retrograde shear stress or by alternating periods of retrograde shear stress.
- Ser 1177 phosphorylation of eNOS will be reduced by constant retrograde shear stress or by alternating periods of retrograde shear stress.

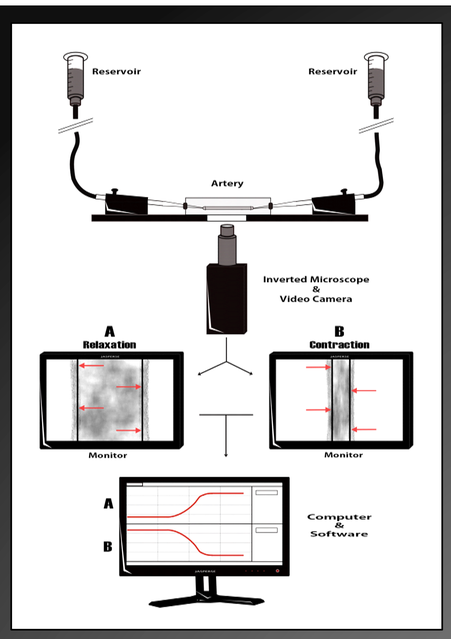

Figure 5: Video microscopy methods for observing artery diameter

Results

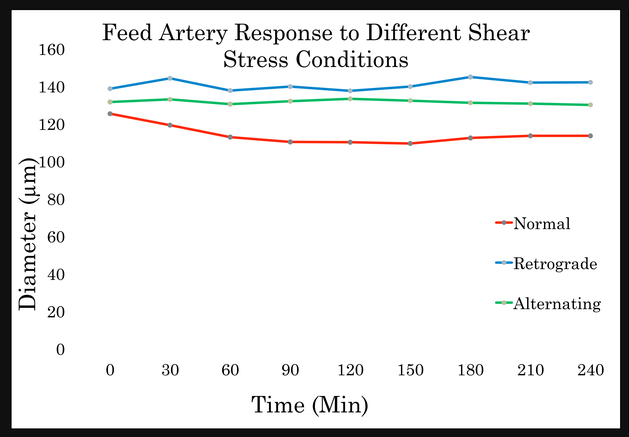

Figure 1: Soleus feed artery diameter was not altered by changes in shear stress direction over a flow period of 4 hrs.

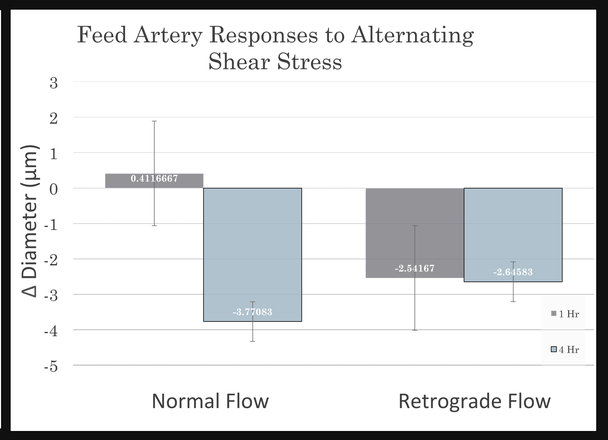

Figure 2: Directional changes in shear stress impaired normal endothelial cell function (flow-induced dilation) of soleus feed arteries over time. (n=12)

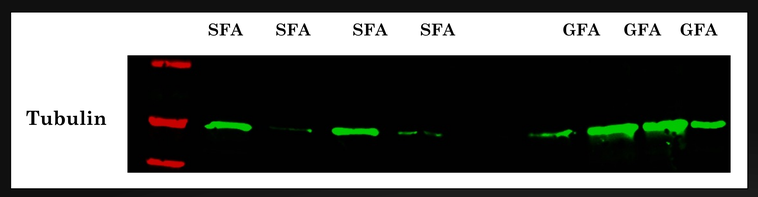

Figure 3: Western Blot Analysis of Tubulin (50 KDa) protein expression in soleus (SFA) and gastrocnemius (GFA) feed arteries

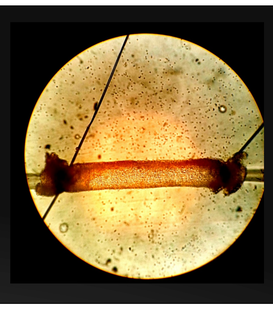

Figure 4: Microphotograph of feed artery cannulated between micropipettes for in vitro flow experiment

Methods

- Soleus feed arteries from male Sprague-Dawley rats were isolated and cannulated for in vitro videomicroscopy (~36 arteries) (Figure 6).
- Artery average maximal diameter was 200.6 $\pm$ 7.9 um.
- Feed arteries developed a minimum average spontaneous tone of 20 $\pm$ 5.0%
- Artery diameter was measured using video microscopy (Figure 5)
- Normal, Retrograde, and Alternating flow was induced in individual arteries for 4 hrs to create shear stress.
  - Normal Flow:
    - Flow = 5 uL/min
    - Shear stress = 25 dynes/cm^2
  - Retrograde flow = 5 uL/min
    - Flow = 5 uL/min
    - Shear stress = 25 dynes/cm^2
  - Alternating Flow (10 min normal direction, 5 min retrograde direction alternating for 4 hrs
    - Flow = 5 uL/min
    - Shear stress = 25 dynes/cm^2
- Shear stress (τ)values were calculated using: $\tau = 4\eta Q/\pi r^3$ (1)
  η = fluid viscosity, Q = blood flow, r = artery radius
- Microvessel Western Immunoblotting Analysis technique is being developed in order to determine and quantify total eNOS and p-eNOS^{ser1177} protein in soleus feed arteries

Conclusions

1. Retrograde shear stress did not alter average diameter of feed arteries over a four hour period.
2. Alternating normal/retrograde shear for four hours converted normal flow-induced dilation to flow-induced constriction.
3. Analysis of the effect of retrograde shear stress on Ser 1177 phosphorylation of eNOS is still in process.

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