Title: The Role of Protein Kinase C in Free Fatty Acid-Induced Endothelial Dysfunction

Specific Aims:
1. To determine the effects of free fatty acids (FFA) on Vascular Endothelial Growth Factor (VEGF) signaling via VEGF-receptor-2 (VEGFR2).
2. To ascertain whether FFA induce protein kinase C (PKC) activation in human umbilical vein endothelial cells (HUVEC), bovine aortic endothelial cells (BAEC), and murine aortic tissue.

Hypothesis: Elevated levels of FFA and the cellular responses in liver and skeletal muscle are central to the signaling defects of insulin resistance (IR) syndromes. Given the increased risk of cardiovascular disease in individuals with IR and the deficiency in skeletal and cardiac muscle collateral formation in this group, it is likely that FFA impair endothelial responses to angiogenic growth factors. Our hypothesis is that, through activation of PKC, FFA induce an endothelial “VEGF-resistance” state, resulting in defective VEGFR2-mediated signaling.

Methods Used: To investigate VEGFR2 signaling, BAEC were incubated with 300µM linoleic acid (LA), palmitic acid (PA), or vehicle for 6 hours and stimulated with 50ng/mL VEGF. Lysates were immunoprecipitated for total VEGFR2, and the product immunoblotted for phosphotyrosine as a marker of activation. BAEC exposed to PA were also stimulated with VEGF, lysed, and immunoblotted for eNOS, Akt, and ERK 1/2 phosphorylation, downstream mediators of the VEGFR2 signal. To determine the role of PKC, HUVEC and BAEC were exposed to LA or vehicle, lysed, and immunoblotted for phosphorylated (active) PKC, using treatment with the phorbol ester PMA as positive control. To investigate membrane translocation as an additional marker of PKC activation, BAEC treated with LA were lysed in a non-detergent buffer, homogenized, and ultracentrifuged at 100,000 x g. The supernatant was collected as the cytosolic fraction, and the pellet resuspended in detergent buffer and homogenized to provide the membrane fraction. Fractions were analyzed by Western methods to determine the membrane:cytosol ratio of PKC. In vivo experiments were performed using wild-type B6 mice fed a high-fat (60% fat) diet for 4-6 weeks, with a normal-diet (10% fat) group as controls. Aortae were harvested and total lysates immunoblotted for phospho-PKC.

Results: In BAEC, FFA exposure led to a 50-70% reduction in VEGFR2 phosphorylation, as well as reduced phosphorylation of eNOS, Akt, and ERK 1/2. HUVEC and BAEC treated with LA demonstrated increased levels of phospho-PKC. Activation of the PKC-βI isoform with LA treatment was evidenced by enhancement of the membrane:cytosol ratio by 42%. Aortic tissue of mice fed a high-fat diet revealed phospho-PKC levels 207% of that of the normal-diet group.

Conclusions: Treatment with FFA led to significantly decreased VEGFR2 activation and defects in its downstream signaling via eNOS, Akt, and ERK 1/2. In vitro FFA exposure was associated with increased PKC activation in human and bovine endothelium, as was elevated dietary intake in murine aorta. The connection of elevated FFA to increased endothelial PKC activation may, in part, elucidate a mechanism by which FFA lead to poor collateralization in IR states via impairment of VEGF signaling.