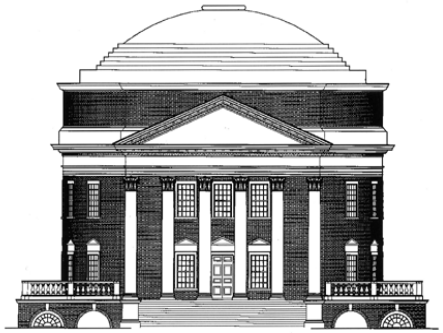
Brian H. Annex, M.D.
Professor of Medicine
University of Virginia



Rotunda at the
University of Virginia
Charlottesville, 1826

THE ROBERT M. BERNE CARDIOVASCULAR RESEARCH CENTER

"Angiogenic Signals in Diabetic Complications"

Thomas M. Coffman, M.D. P.I., Duke University complication = nephropathy
Co-PI's
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Atherothrombotic Diseases in US

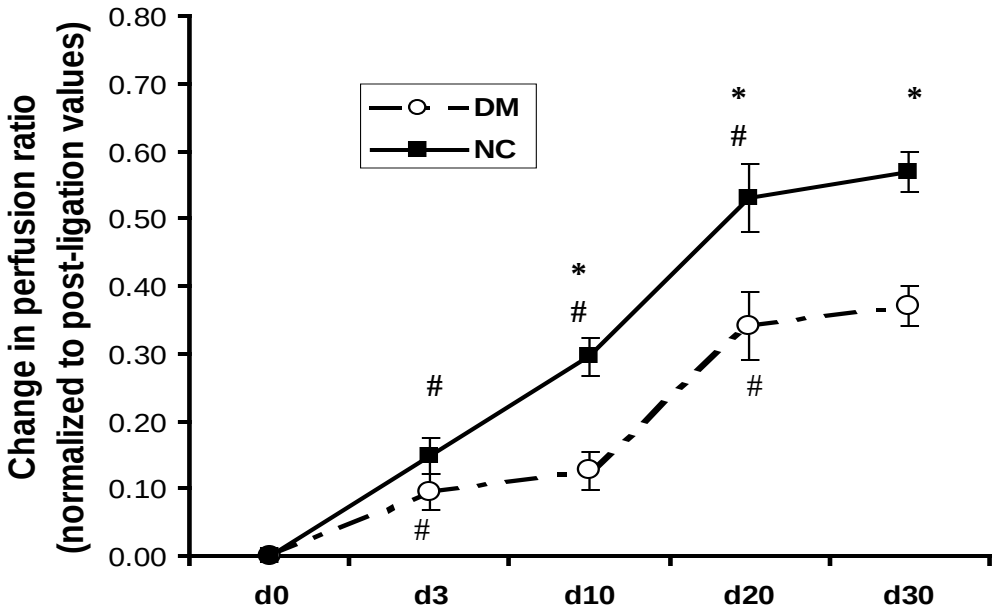
	Prevalence (millions)	Incidence (millions)
Coronary heart disease	**13.2**	**1.2**
Cerebrovascular disease	**4.8**	**0.7**
Peripheral arterial disease	**8.0–12.0**	**—**

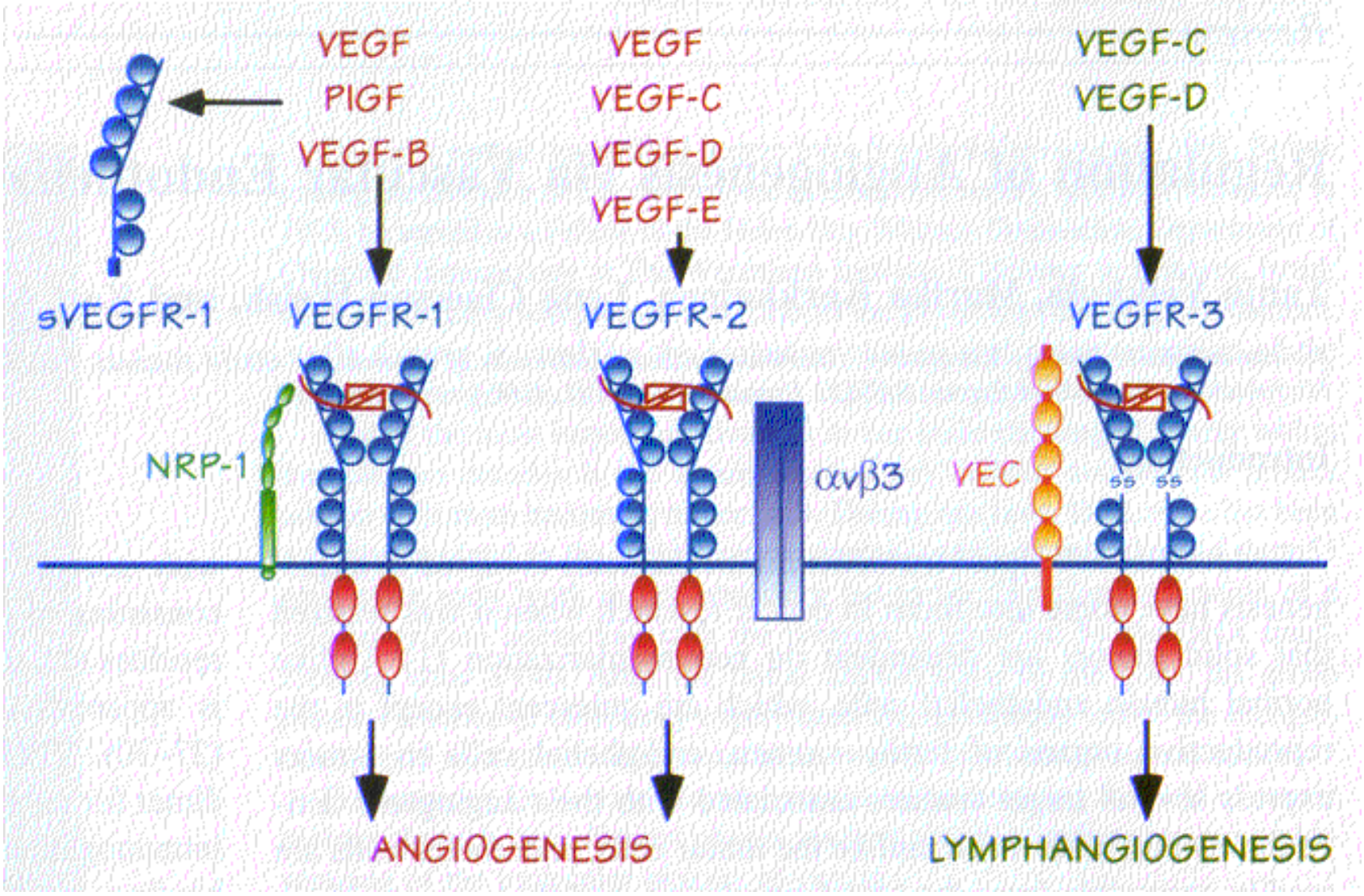
AHA. *Heart Disease and Stroke Statistics—2004 Update.* Dallas, Tex.; 2003

Peripheral Arterial Obstructive Disease (PAD) and Diabetes Mellitus (DM)

- **After adjusting for age, together with smoking, DM accounts for >80% of the excess risk for developing PAD.**
- **In patients with PAD, DM is not merely a risk factor but DM profoundly and negatively impacts the natural history of PAD.**

Mice Fed a HF Diet Have Impaired Perfusion Recovery Compared to Age Matched C57Black6 Mice

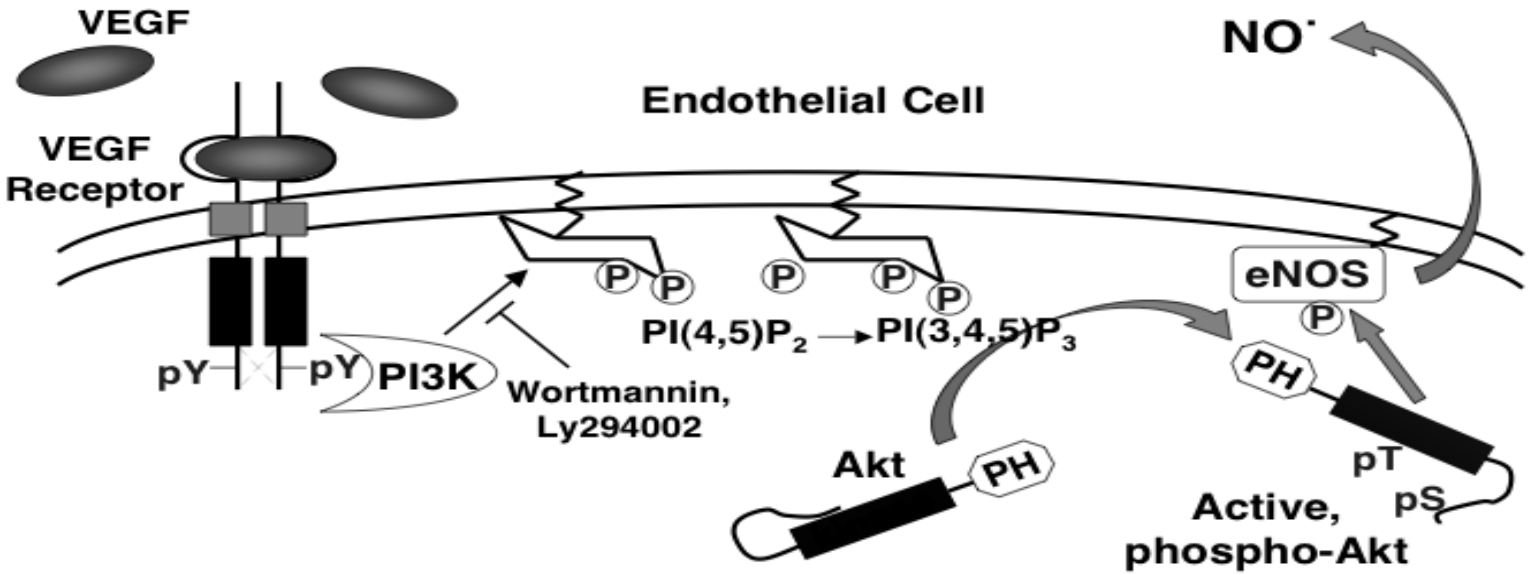




Veikkola et al., *Cancer Res* 2000; 60:203-212

Data now suggest that R1 and R2 are not biologically or functionally equal.

A VEGF Signaling Pathway



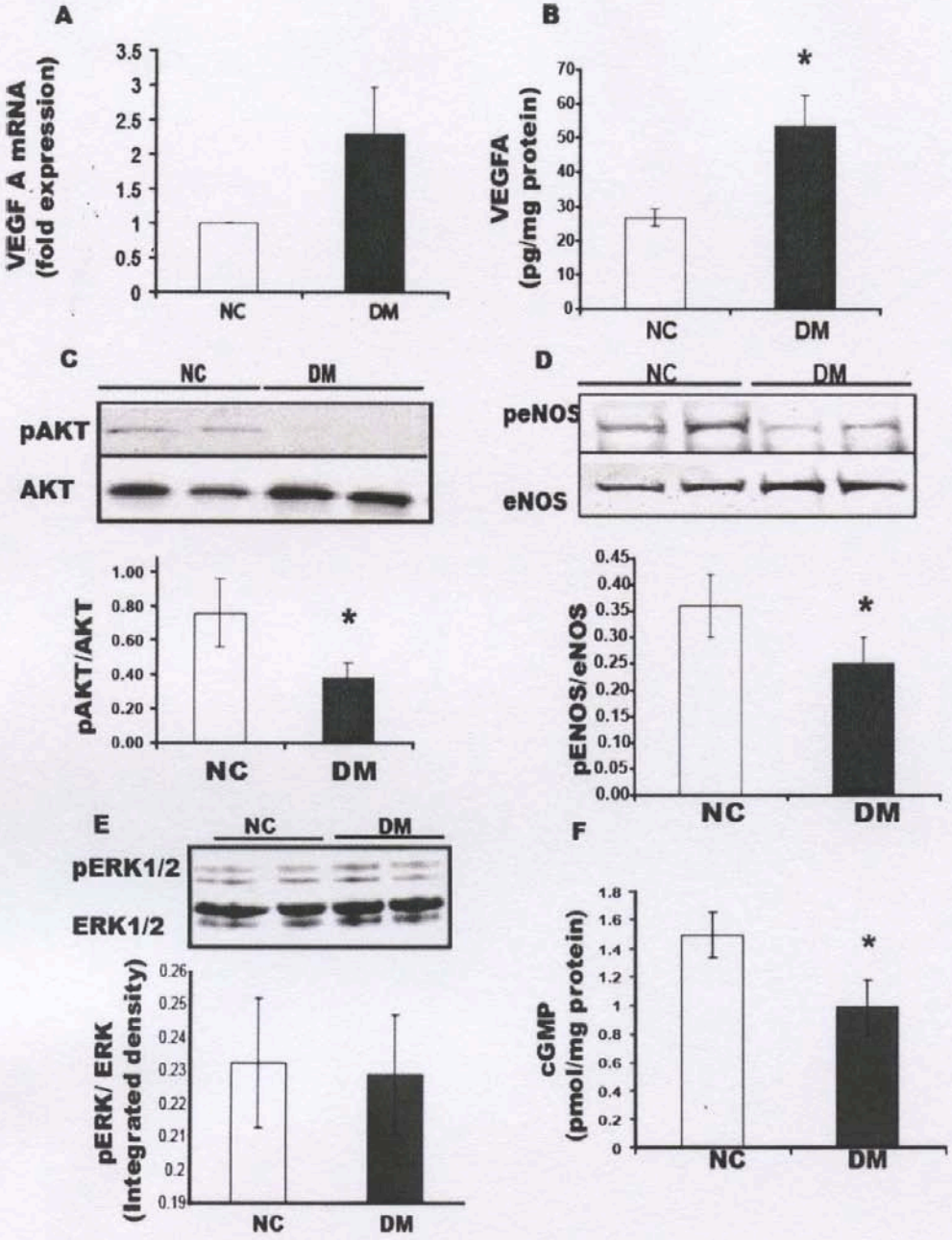


Figure 2. VEGF signaling is reduced in diabetic mice at baseline. Compared with muscle from NC-fed mice, nonischemic TA muscle from mice with diabetes (DM) displayed increased expression of VEGF-A mRNA (A) and protein (B). Nonischemic DM muscle also displayed evidence of reduced VEGF signaling, as indicated by decreased p-AKT/total AKT (C), reduced p-eNOS/total eNOS (D), and reduced cGMP concentration (E). However, there was no change in the expression or activation of ERK1/2 (F). *$P<0.05$ (n=5 to 8/group).

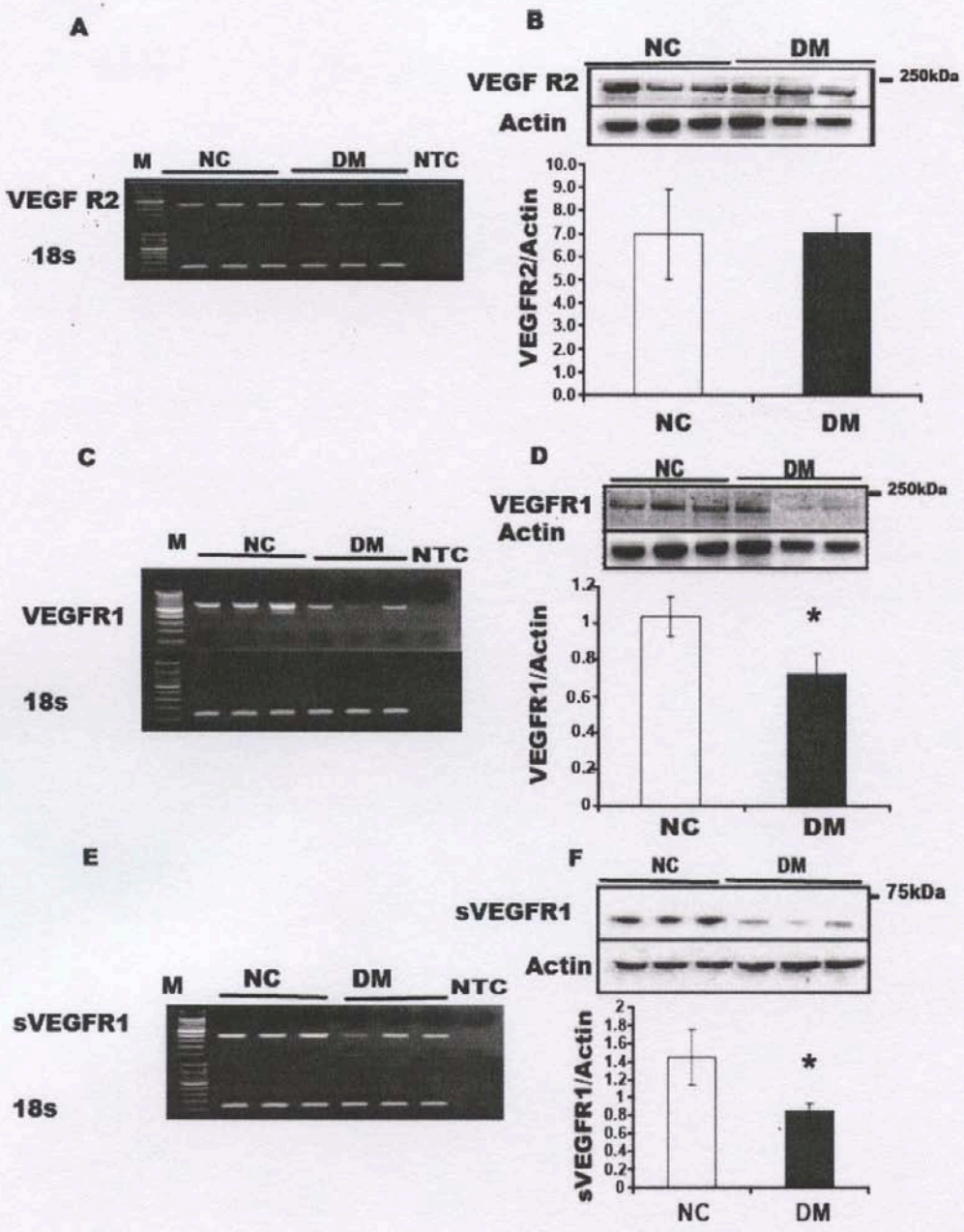
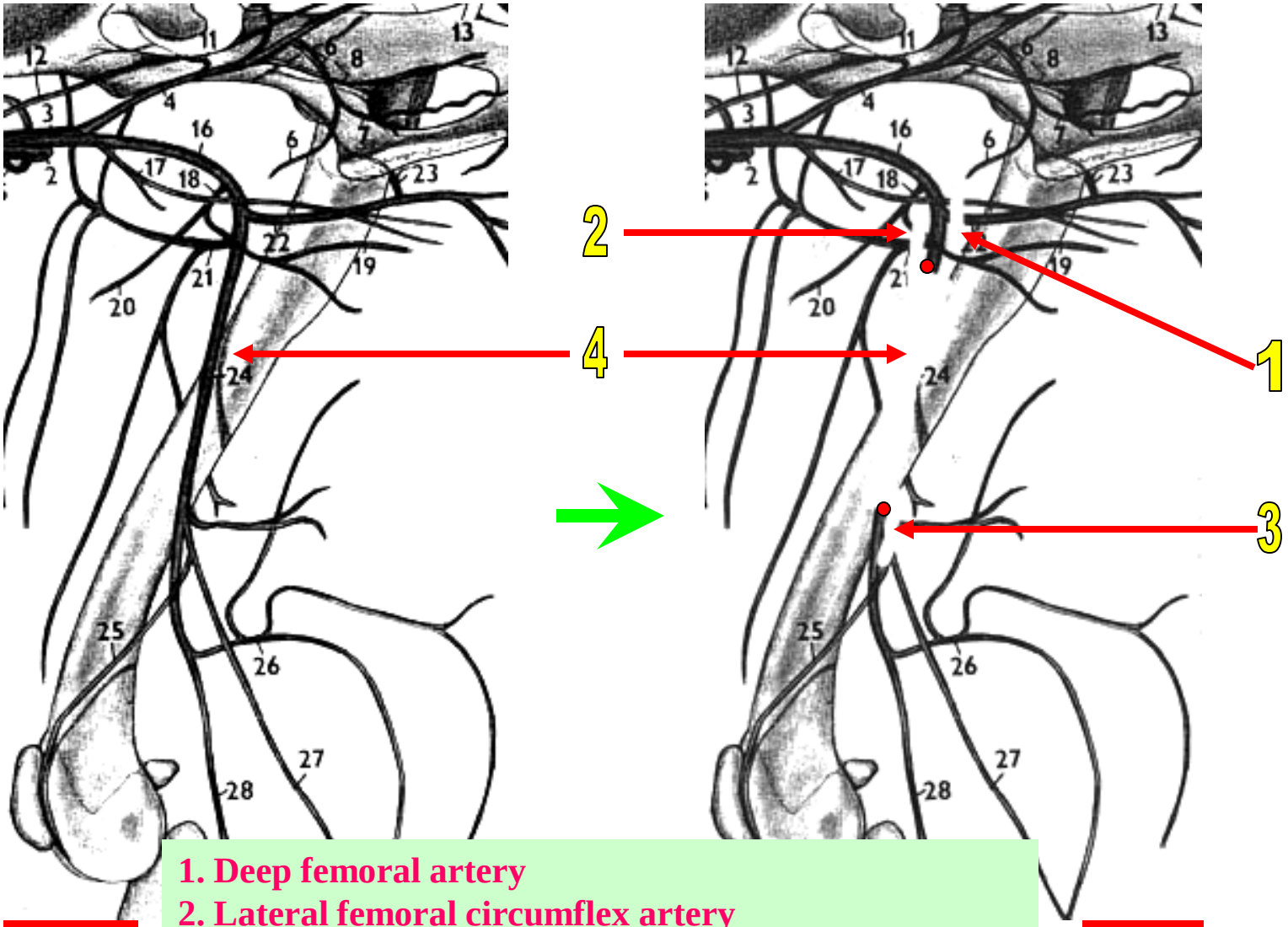
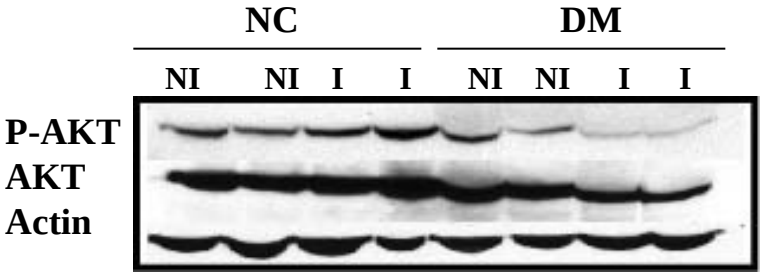


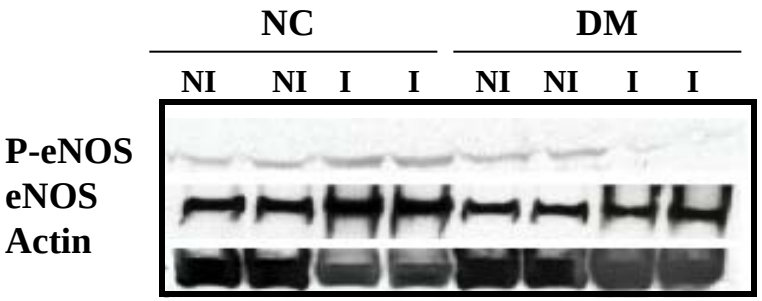
Figure 3. Expression of VEGFR-1 and sVEGFR-1 is reduced in diabetic mice at baseline. In nonischemic muscle, there was no difference in expression of VEGFR-2 mRNA (A) or protein (B) between diabetic (DM) and nondiabetic (NC) mice. However, DM mice had decreased expression of full-length VEGFR-1 mRNA (C) and protein (D). Similarly, sVEGFR-1 expression was downregulated in DM mice at both the mRNA (E) and protein (F) levels. *$P<0.05$ (n=8/group).

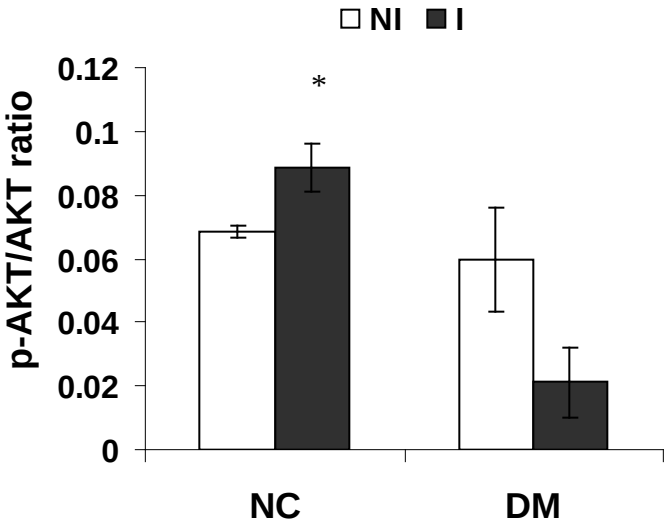


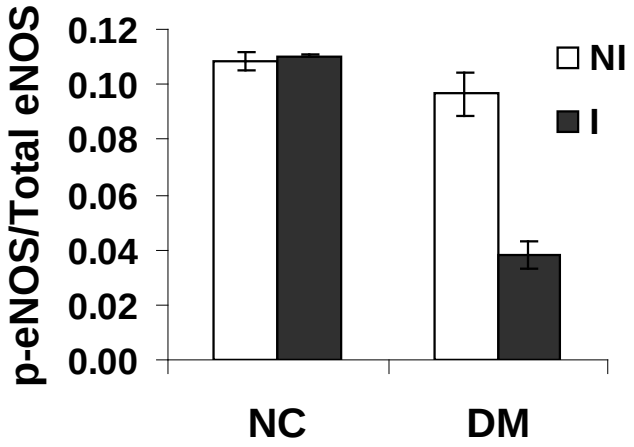
1. Deep femoral artery
2. Lateral femoral circumflex artery
3. The origin of saphenous artery and popliteal artery
4. Femoral artery

Post-Ligation Changes in pAkt/Akt and p-eNOS/eNOS Differ Between NC and DM Mice

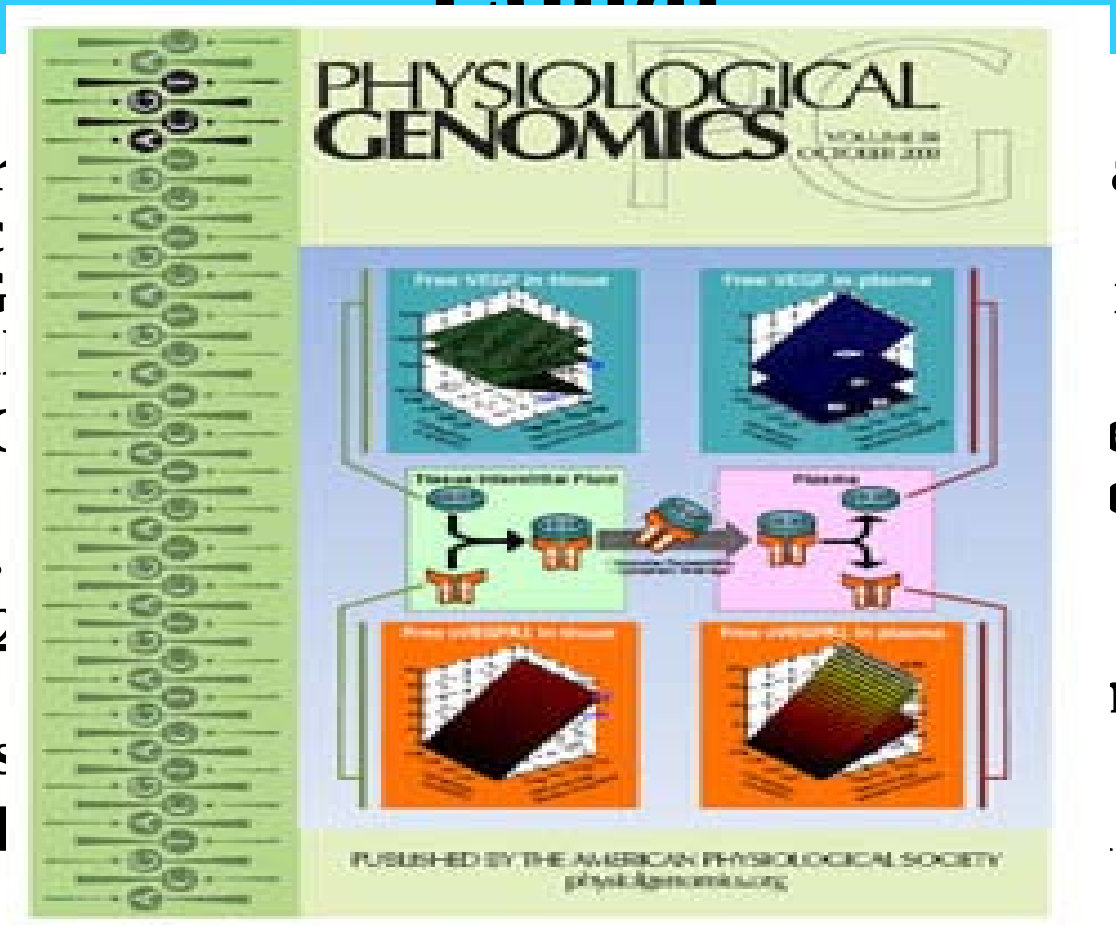








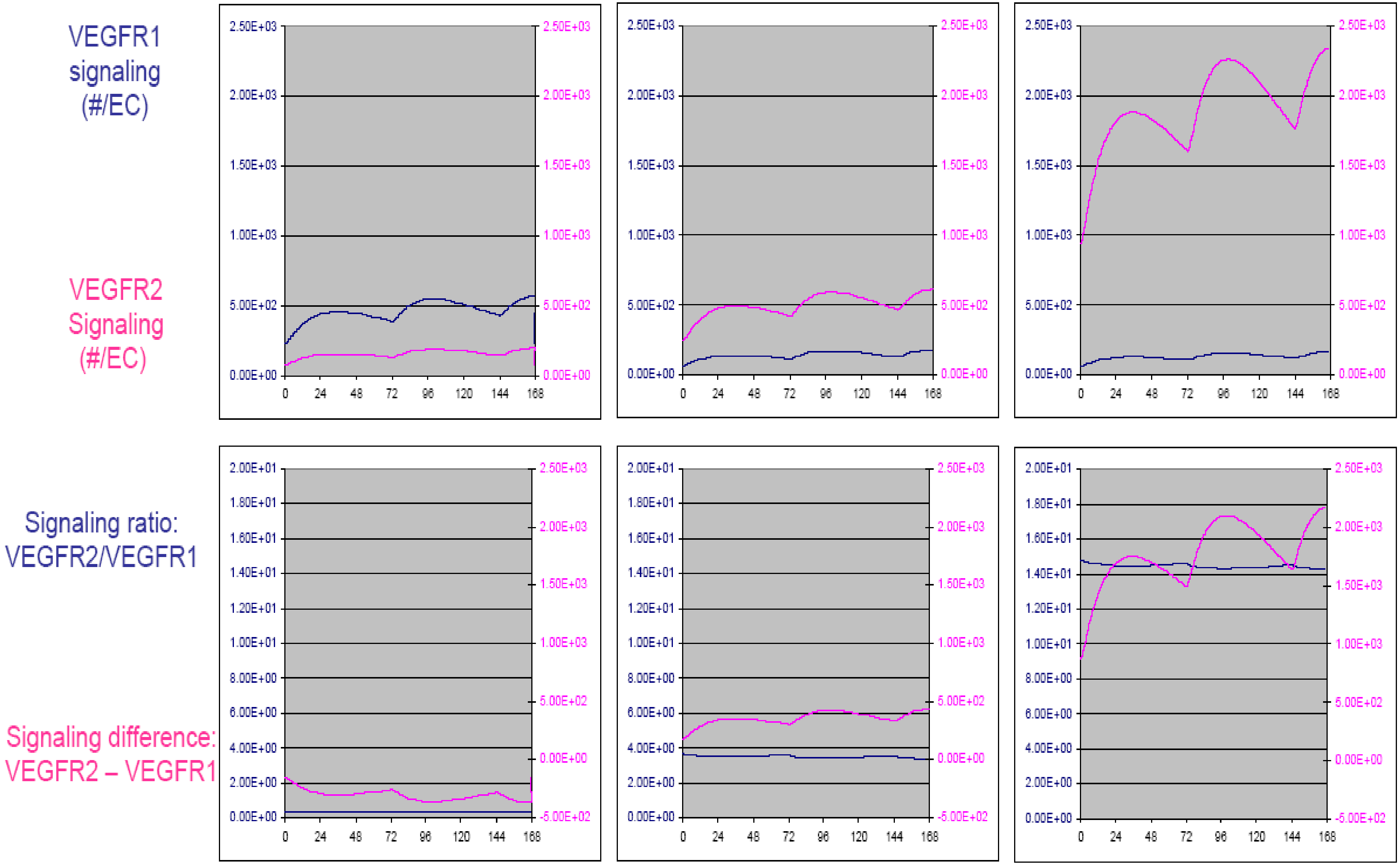
VEGFR1 and VEGFR2 Are Not Equal



- A role for … at follows pre-clinic … hypothesis that VEG … involved in post-nata…
- Both VE… …ent however VEGFR1 … …egative R1 knock-in.
- VEGFR-2… … of ligand mediated … …nance of vascular s…
- In cell cul… …1 activation results in …
- On a pure affinity basis, VEGF is (slightly) more likely to bind to VEGFR1 than VEGFR2.

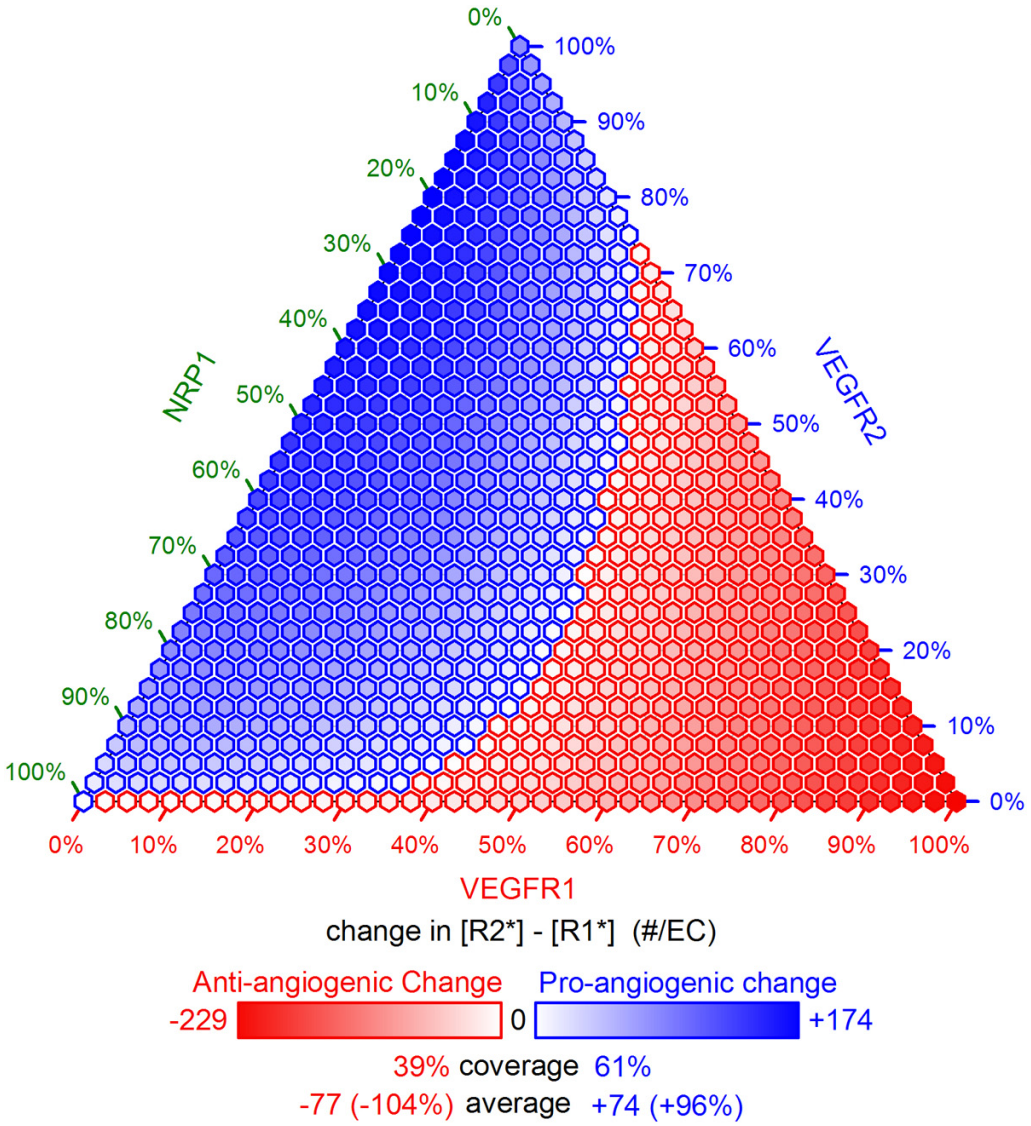
VEGF
Is an Anti-Angiogenic Agent

[redacted]

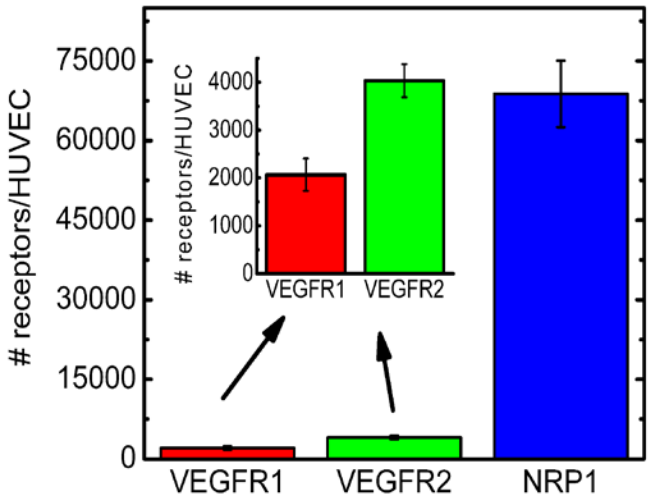


Thanks: Feilim Mac Gabhann, Alexandra Popel, Chris Kontos

Model predictions for pro-angiogenic therapies



VEGF165 has 2 binding sites for both VEGFR2 and NRP1. Thus when VEGF165 binds NRP1 it stays bound at cell surface where it can bind VEGFR



P. Imoukhuede 2009

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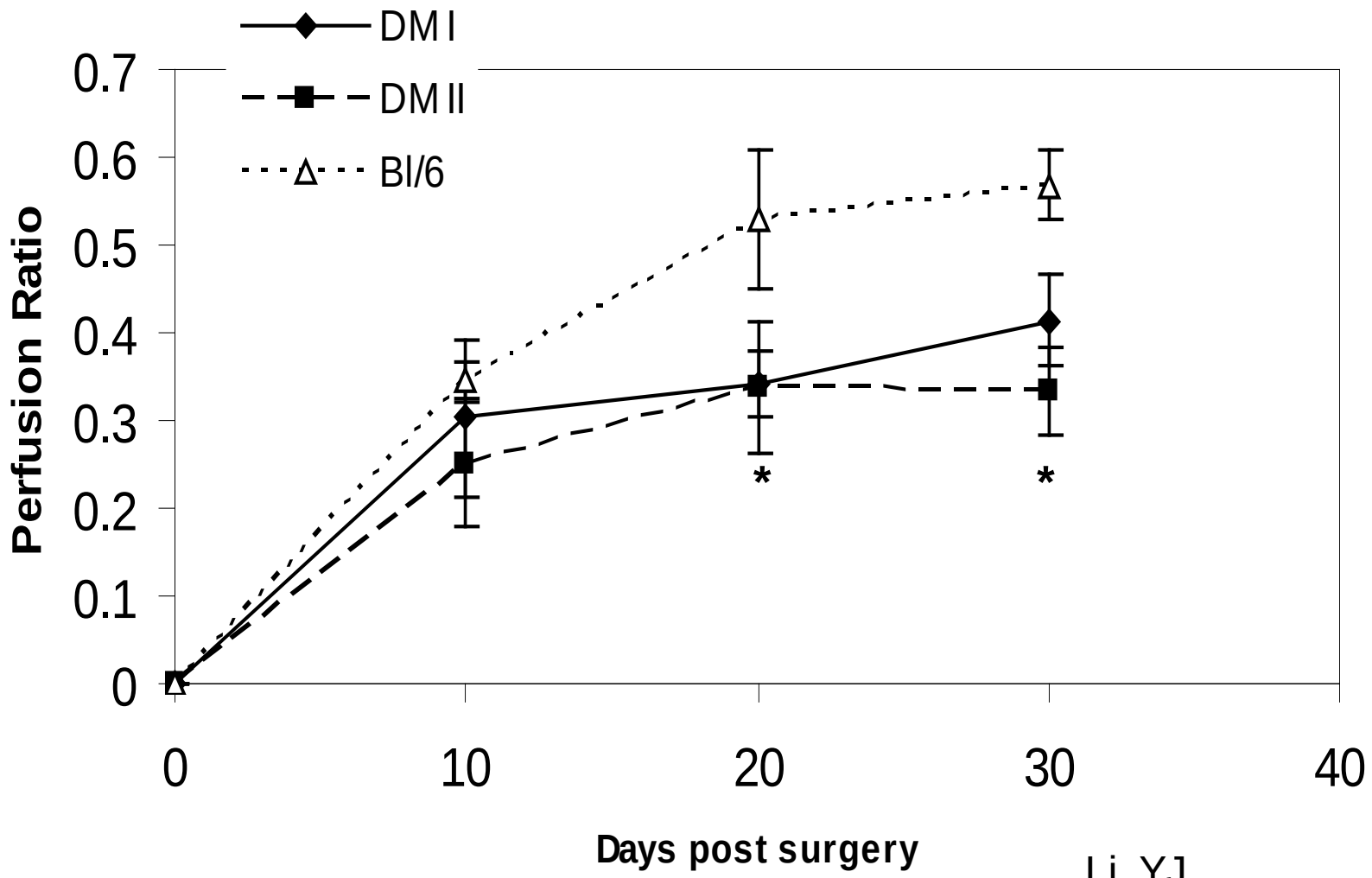
Perfusion Recovery



Li, YJ

MATERIALS AND METHODS

Mouse Model for Type 1 DM
Male C57Bl/6J (n=40)

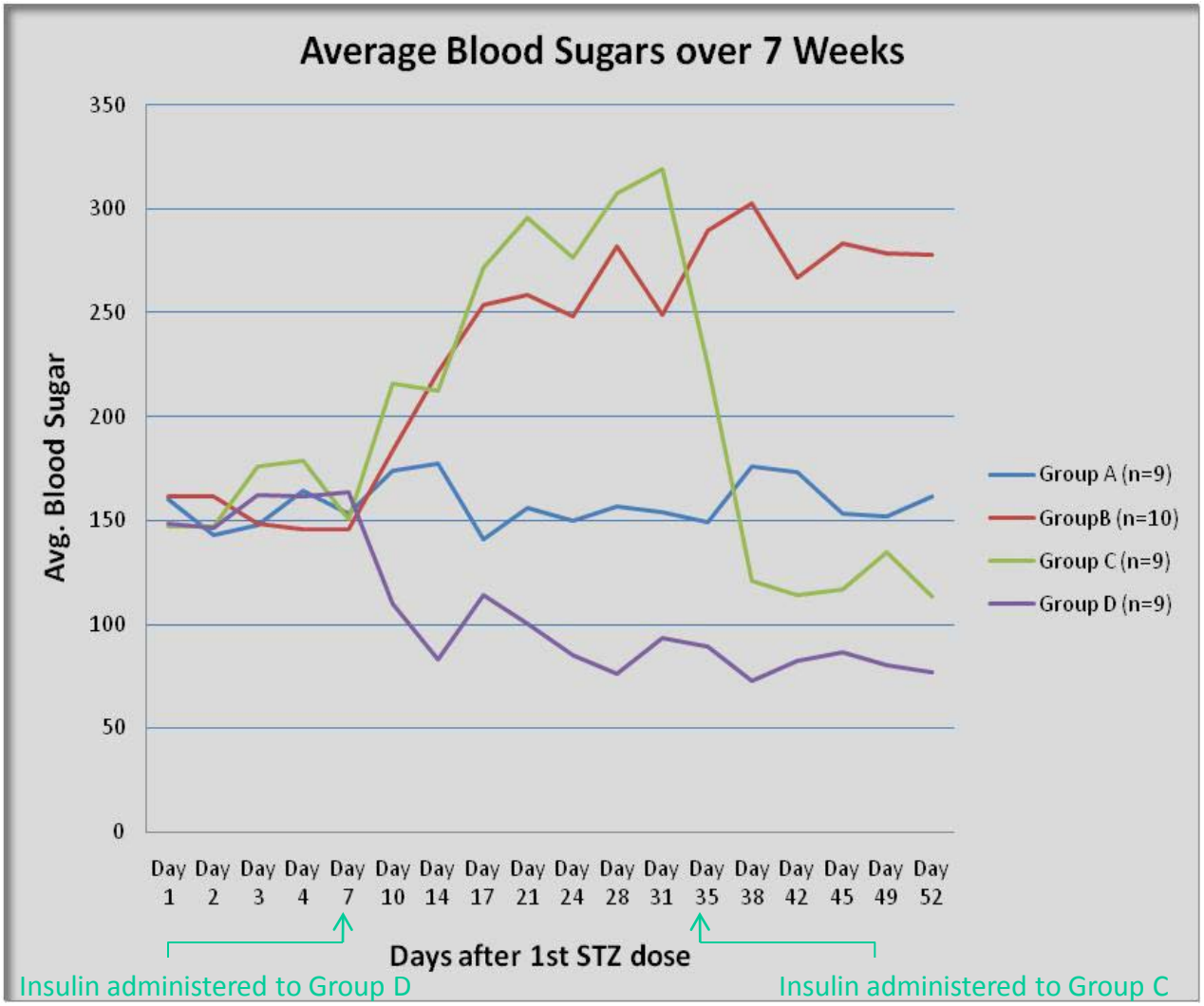
STZ (BIOMOL®) according to the AMDCC protocol:
Mice were fasted for 4 hrs, anesthetized, and injected IP with 50 mg/kg/mouse for 5 consecutive days

Mice were divided into 4 groups:
Group 1 (control) received no STZ
Group 2 received STZ only
Group 3 received STZ; then insulin for 4 wks beginning 4 weeks post-STZ
Group 4 received STZ; then insulin for 8 wks beginning 0 weeks post-STZ

Blood glucose was measured 2x weekly
Mice with glucose concentrations ≥ 250 mg/dl were considered to be diabetic
HbA1c was measured 4 and 8 weeks post-STZ

Eight weeks post- STZ penectomies were performed on all mice and the mice were euthanized. Corporal tissue, TA, GA, soleus, plantaris, and kidneys were harvested and stored at -80 ^{o}C

Subcutaneous insulin pellet administration reduced blood glucose to below normal levels in STZ-treated mice



4-hour fasting blood glucose measurements

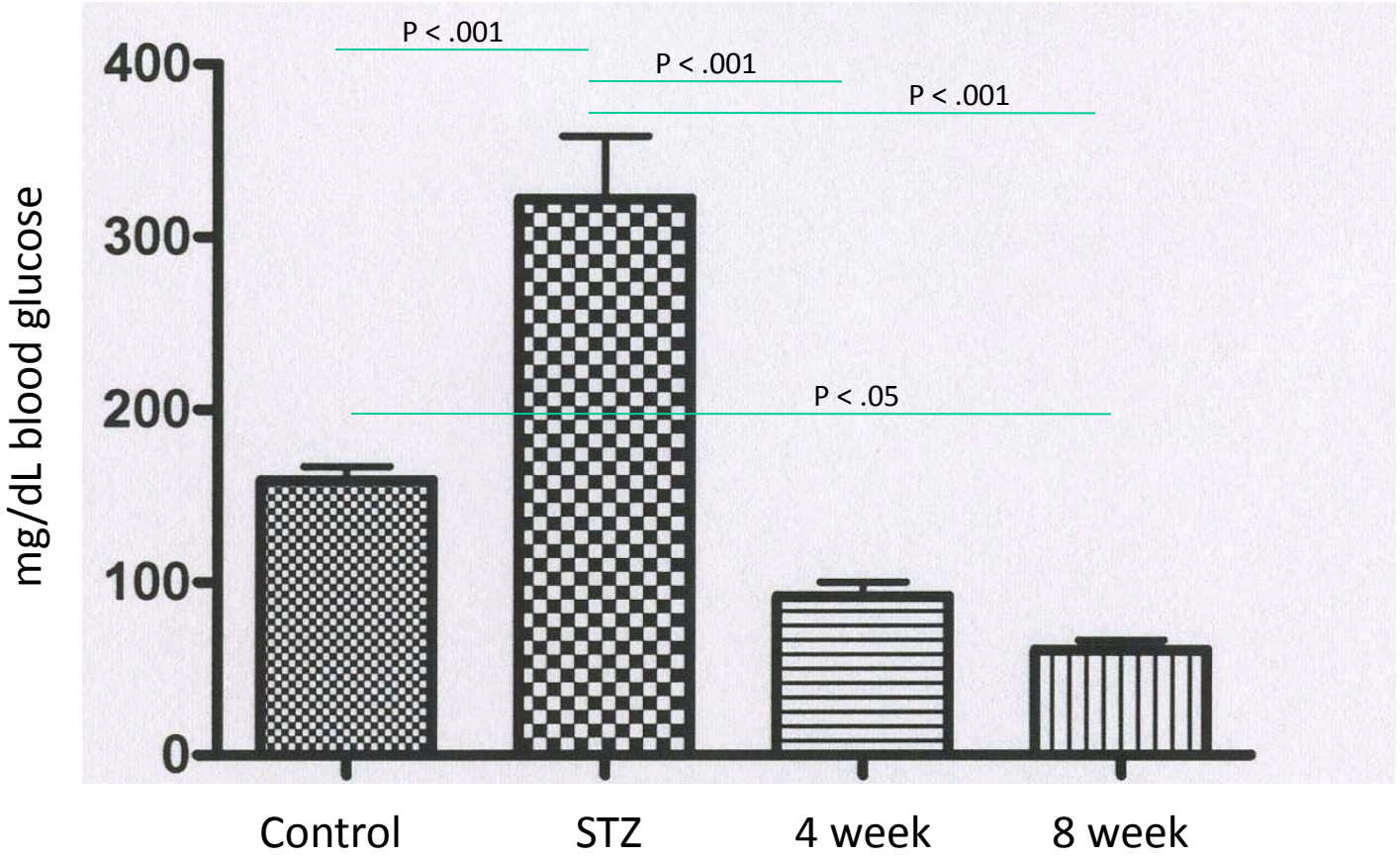
Group A: – STZ + Sham pellets

Group B : + STZ + Sham pellets

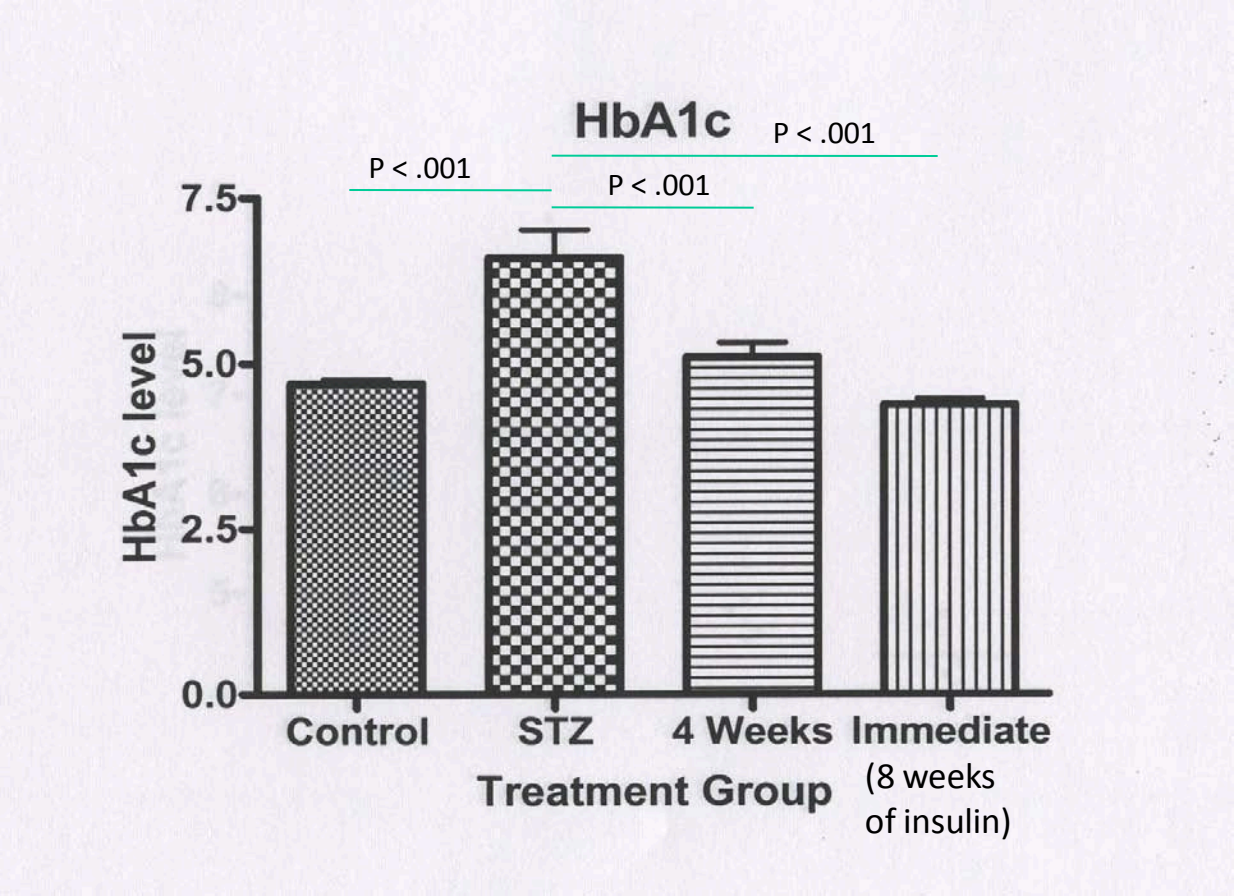
Group C: + STZ + Insulin pellets 4wks post-STZ

Group D: + STZ + Insulin pellets
0 wks post-STZ

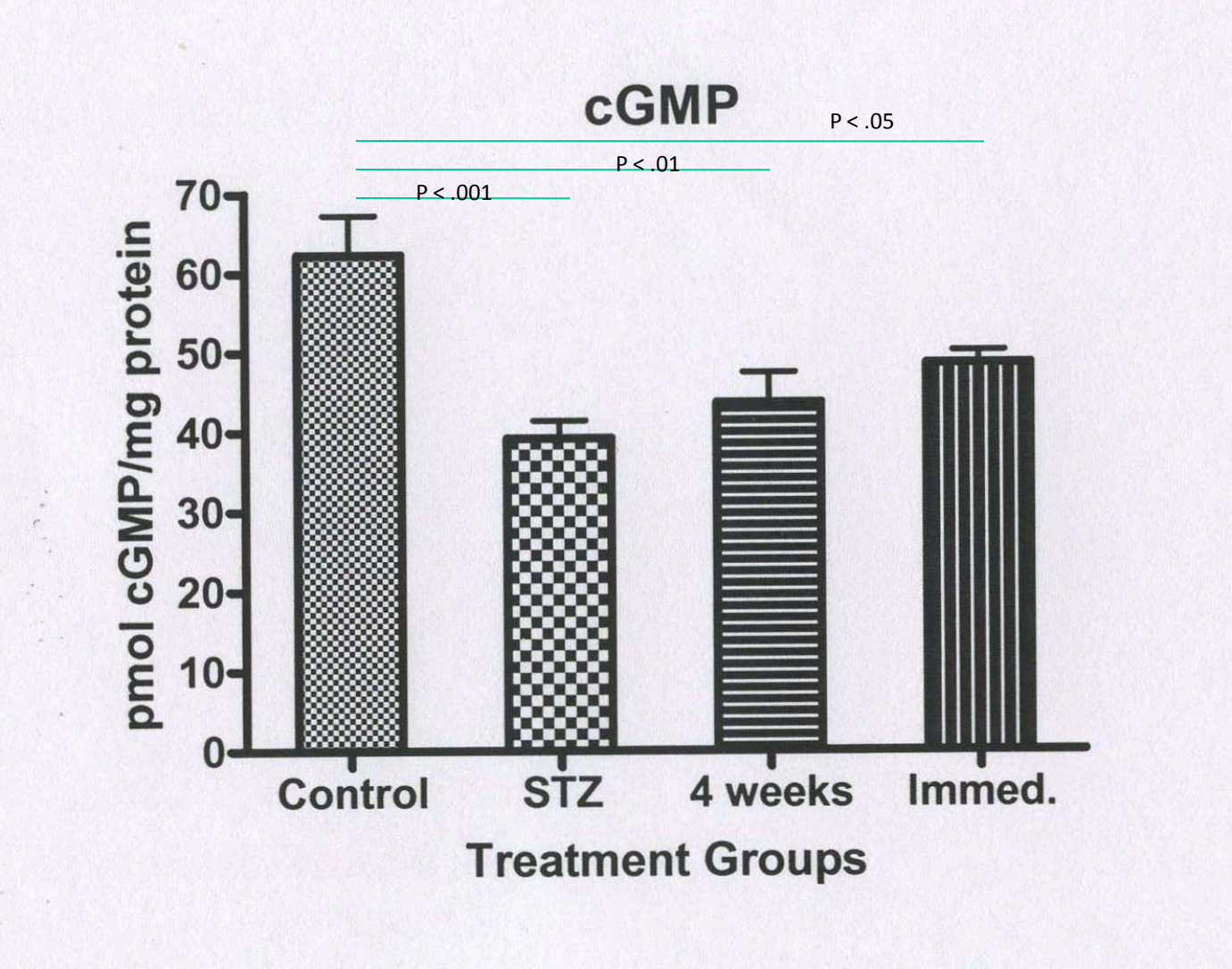
STZ treatment significantly increased blood glucose levels compared to control, and insulin given for 4 or 8 weeks post-STZ significantly reduced blood glucose levels compared to STZ alone



Continuous insulin treatment of STZ-treated (DM type 1) mice for 8 or 4 weeks beginning 0 or 4 weeks post-STZ restored HbA1c concentrations to normal levels

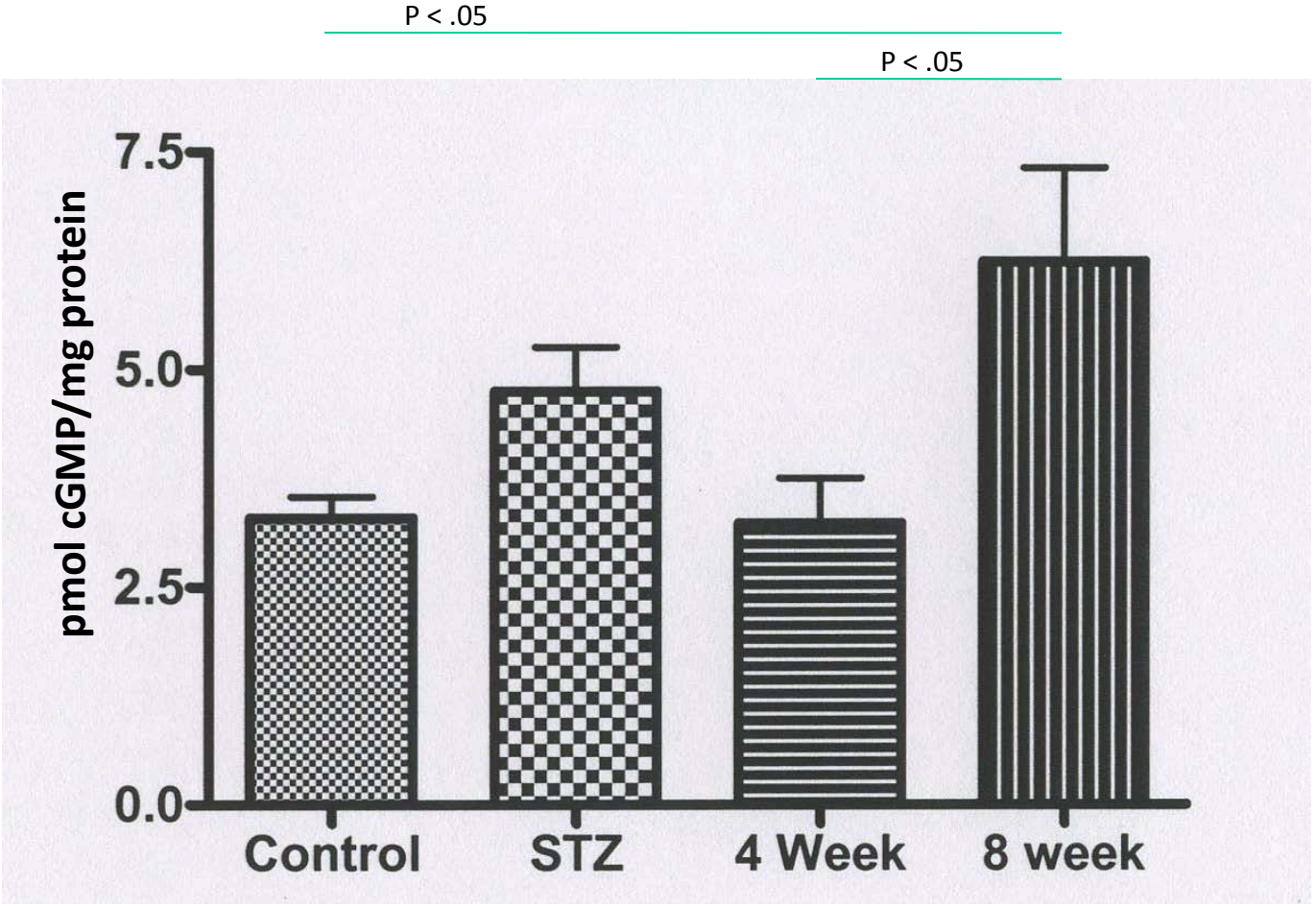


Cyclic GMP concentrations was significantly reduced in TA muscle of STZ-treated mice and concentrations were not significantly changed compared to STZ-treatment alone when normal blood glucose and HbA1c levels were restored by insulin



FINDINGS ARE GENERALIZABLE TO ALL TISSUE:

cGMP was not significantly changed in corporal tissue after STZ treatment compared to control; however, cGMP was significantly increased after 8 weeks of insulin treatment relative to control or 4 weeks of insulin treatment



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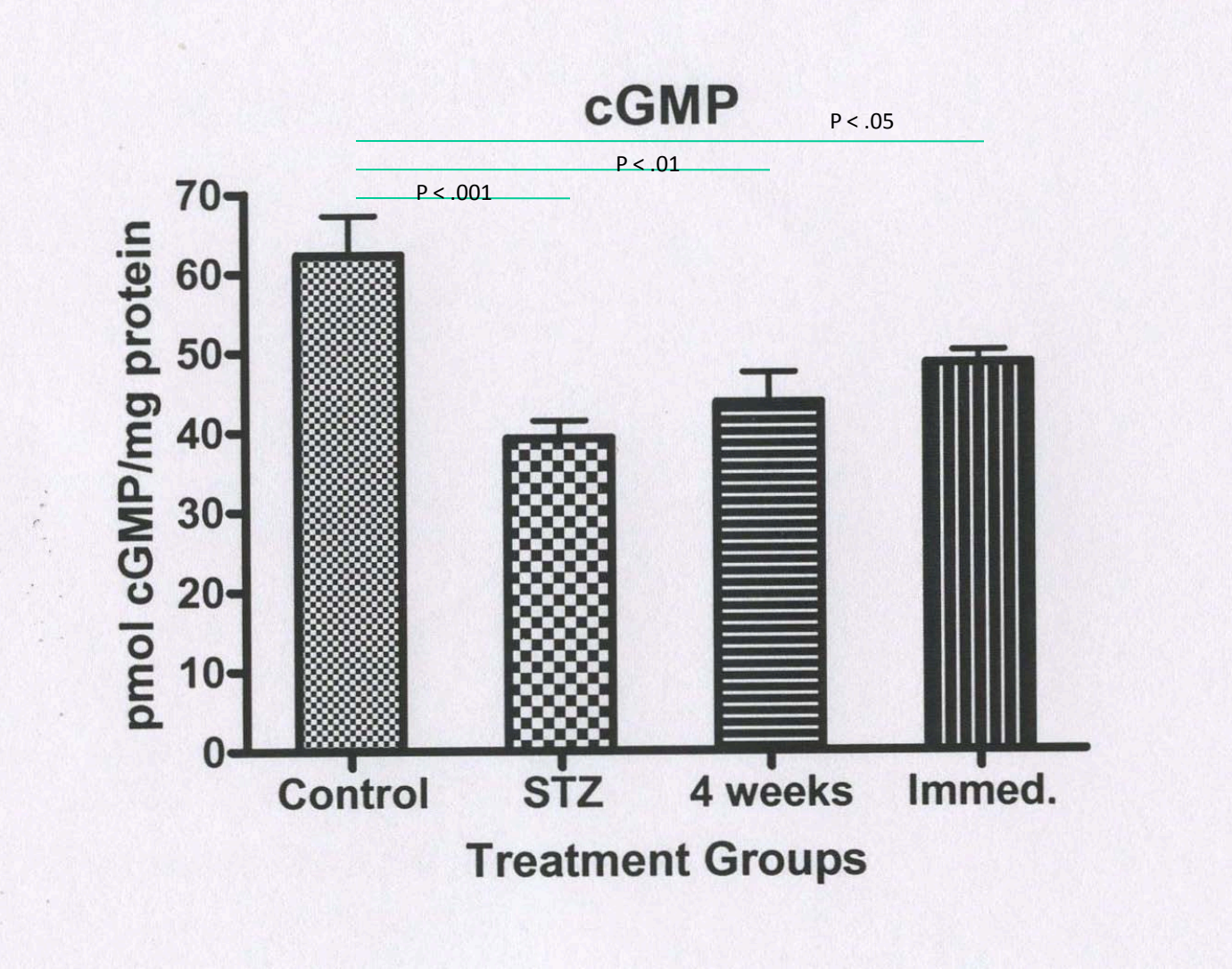
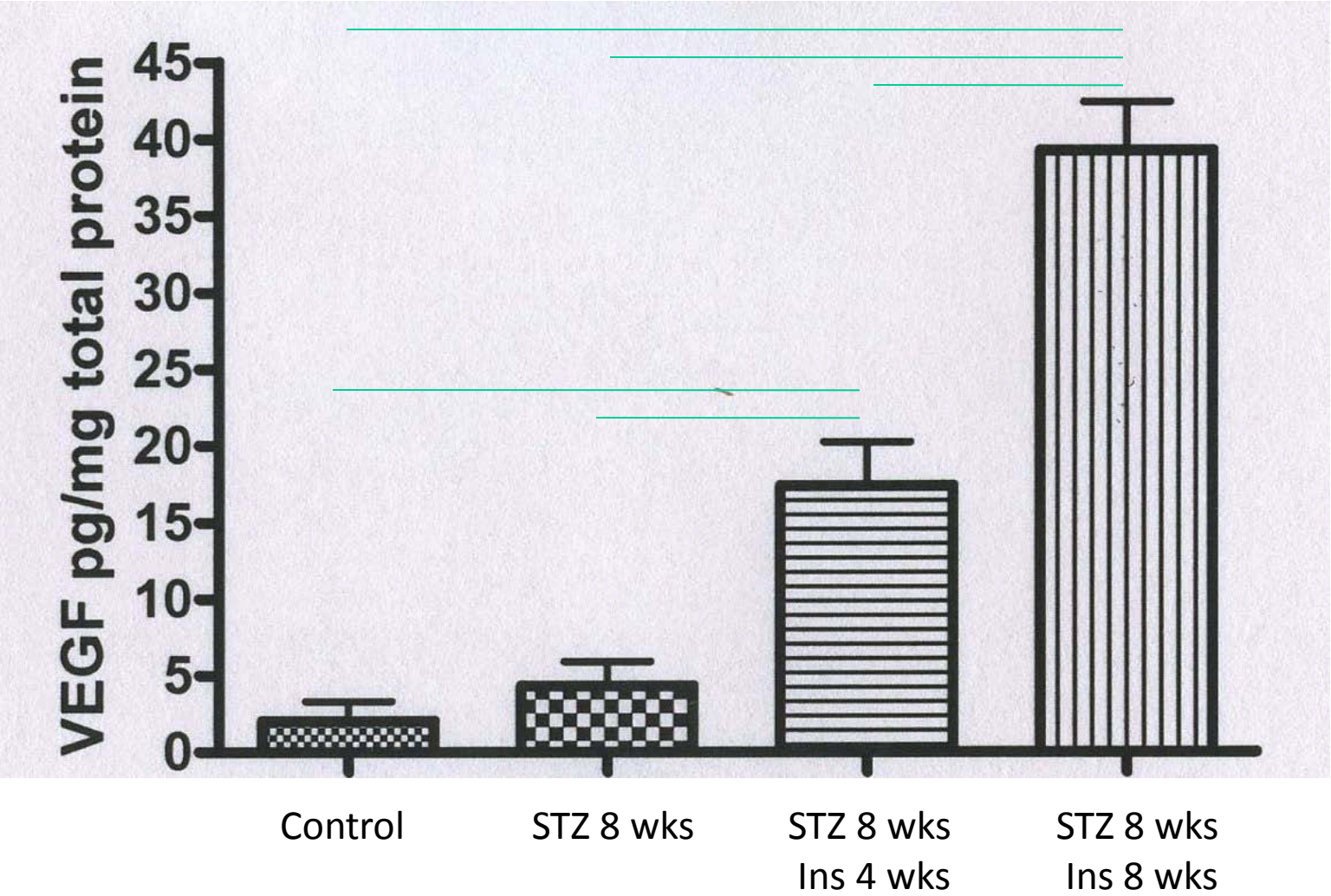
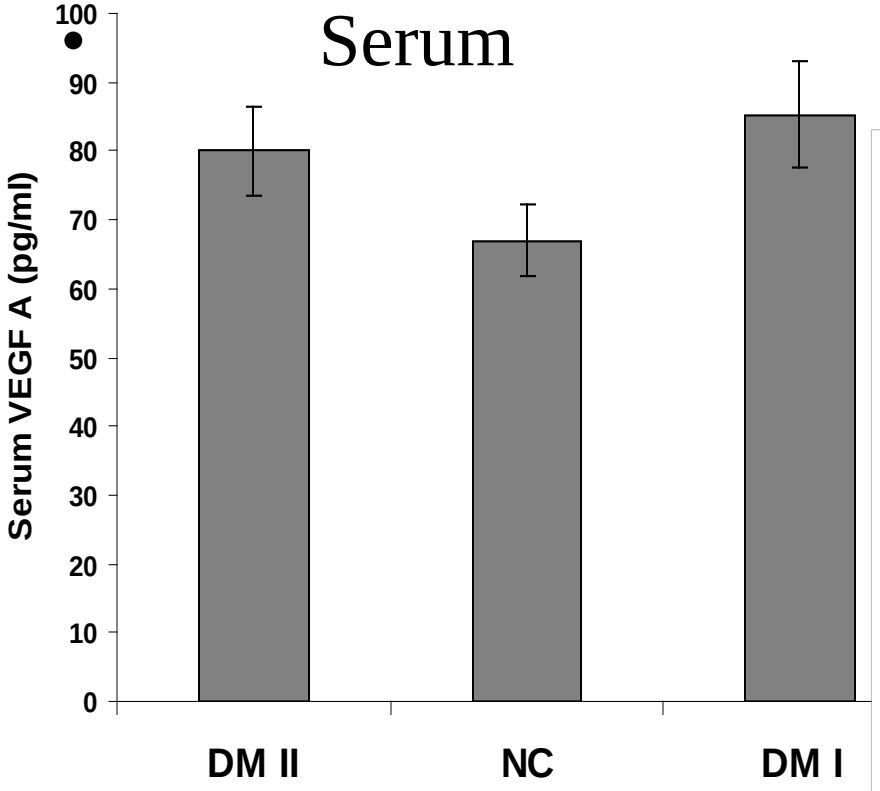


Insulin treatment significantly increased VEGF expression in TA muscle of STZ-treated mice

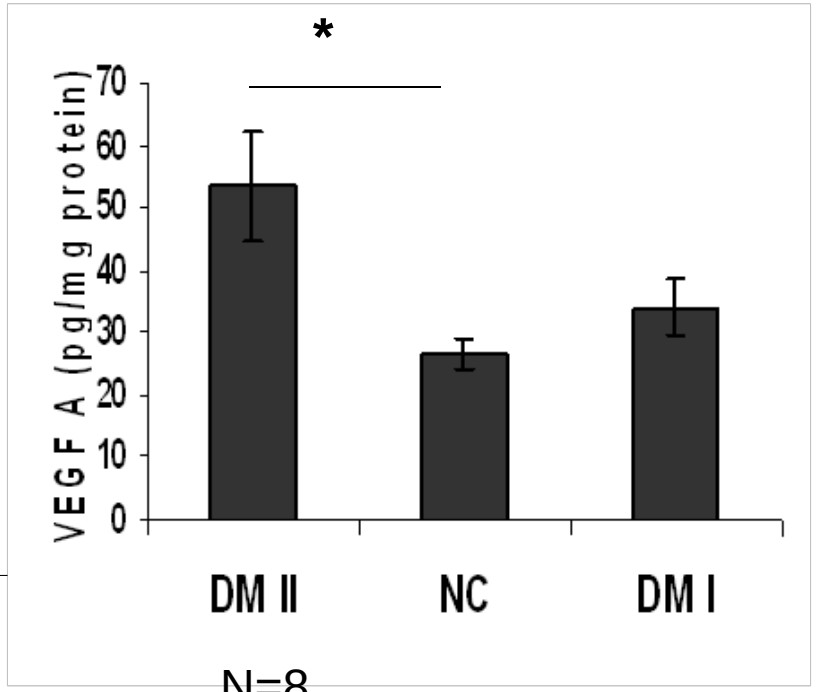


VEGF-A levels in Mice with Type 1 DM (8wks)

- Serum



- Muscle (TA)



N=8

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Hypothesis for Original Application

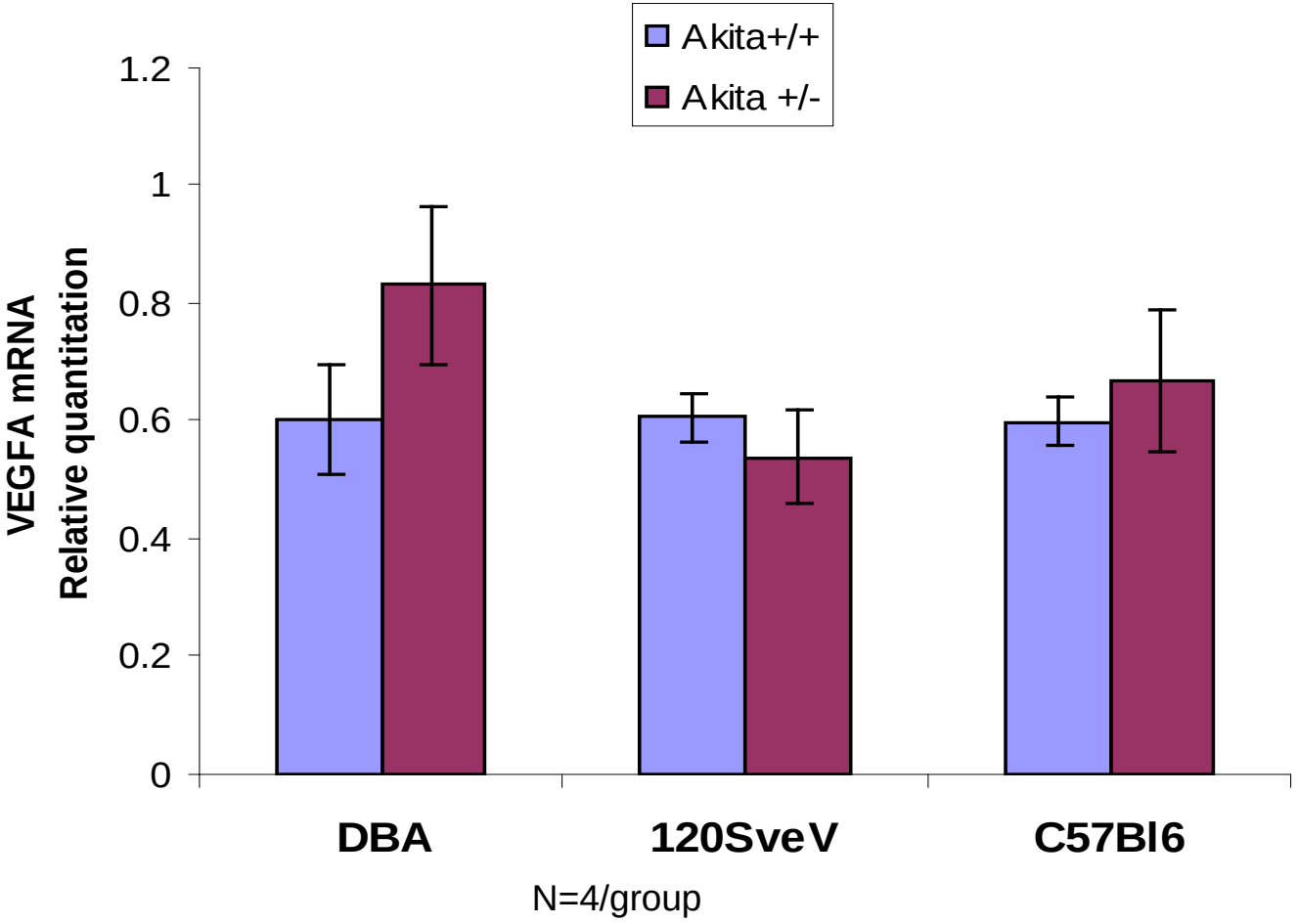
In humans with diabetes, abnormal angiogenesis contributes to the development of end-organ damage.

"Excess" angiogenesis = nephropathy

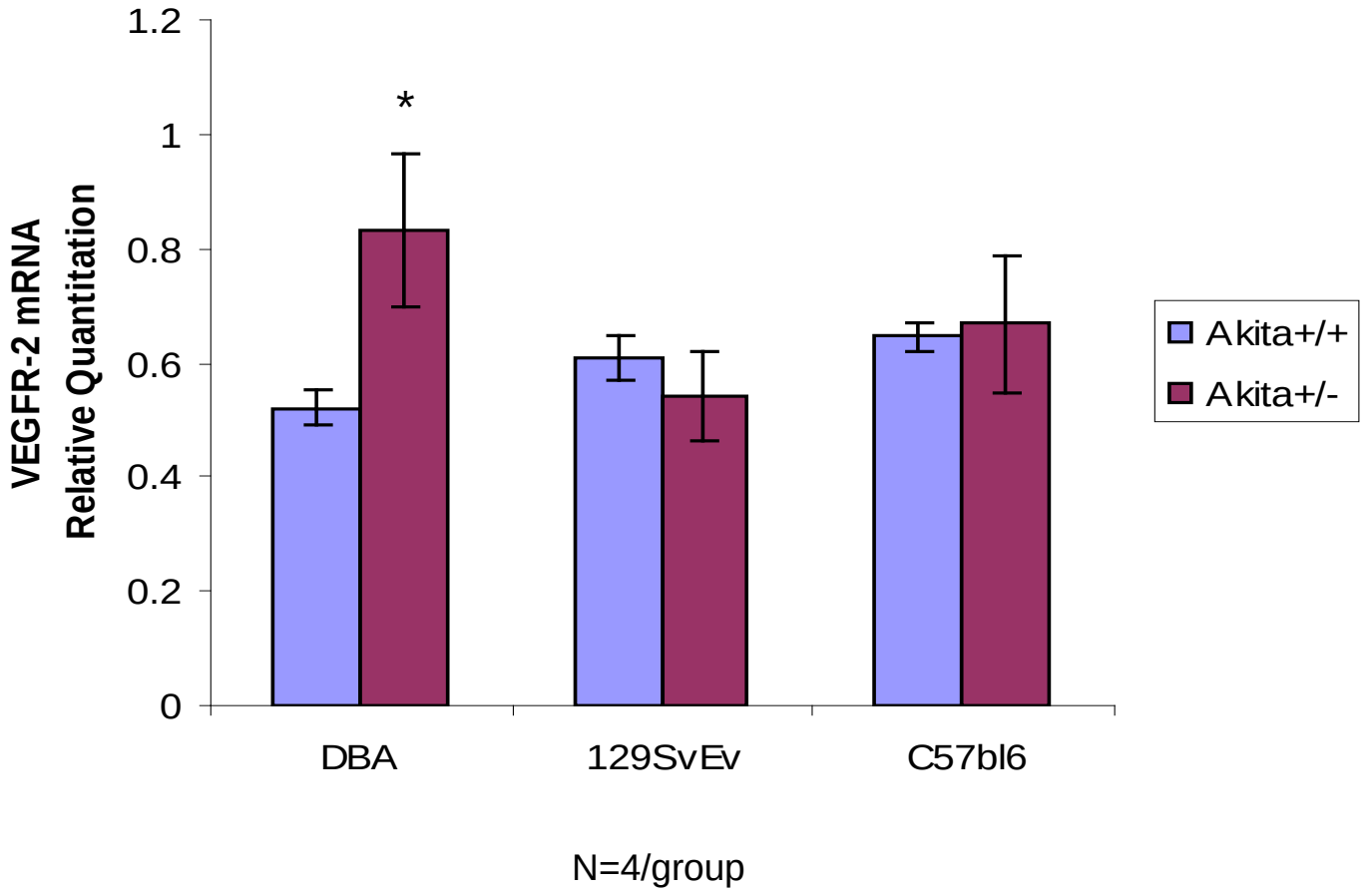
"Inadequate" angiogenesis = PAD

We hypothesize that abnormal signaling in VEGF-associated pathways is a critical factor in the pathogenesis of diabetic complications including nephropathy and peripheral artery disease (PAD).

Total VEGF-A Expression: Kidney

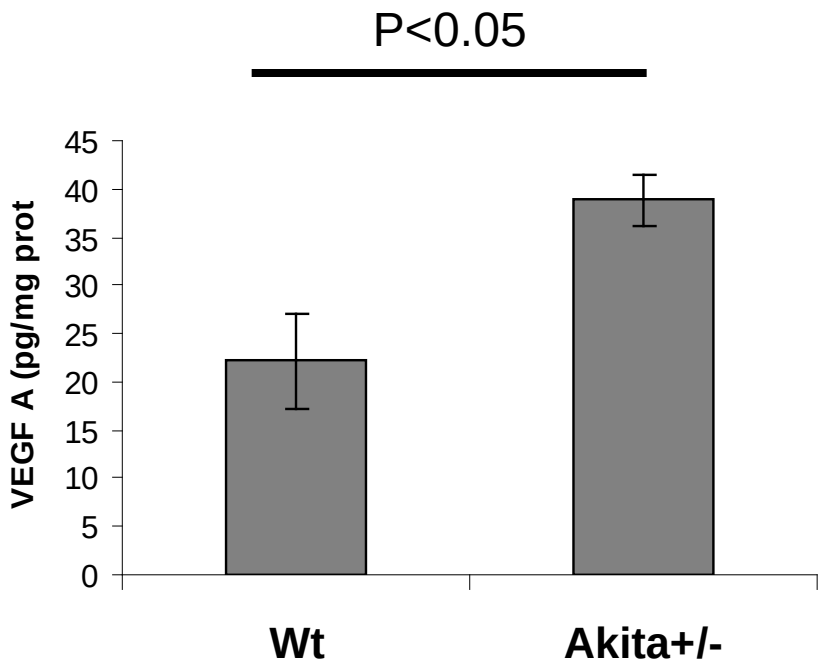


VEGFR-2 Expression: Kidney

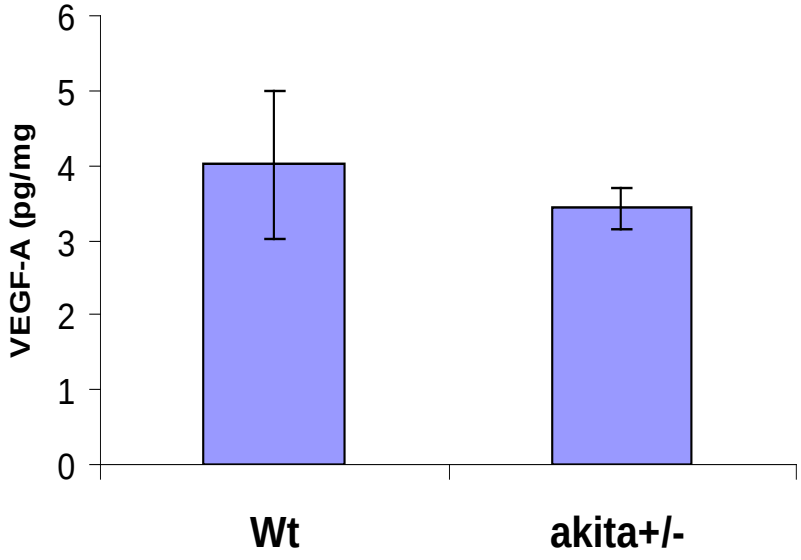


C57Bl6 Akita+/- and Wt mice Kidney cortex and TA: VEGF expression and signaling

VEGF-A (ELISA)

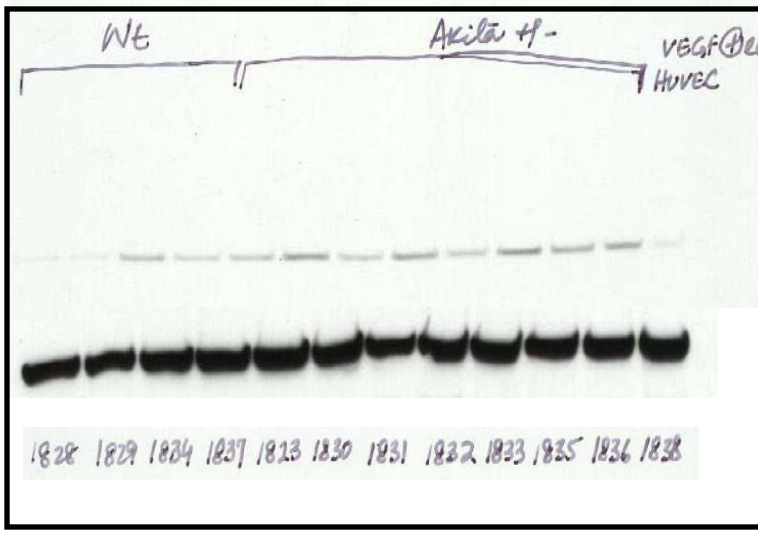


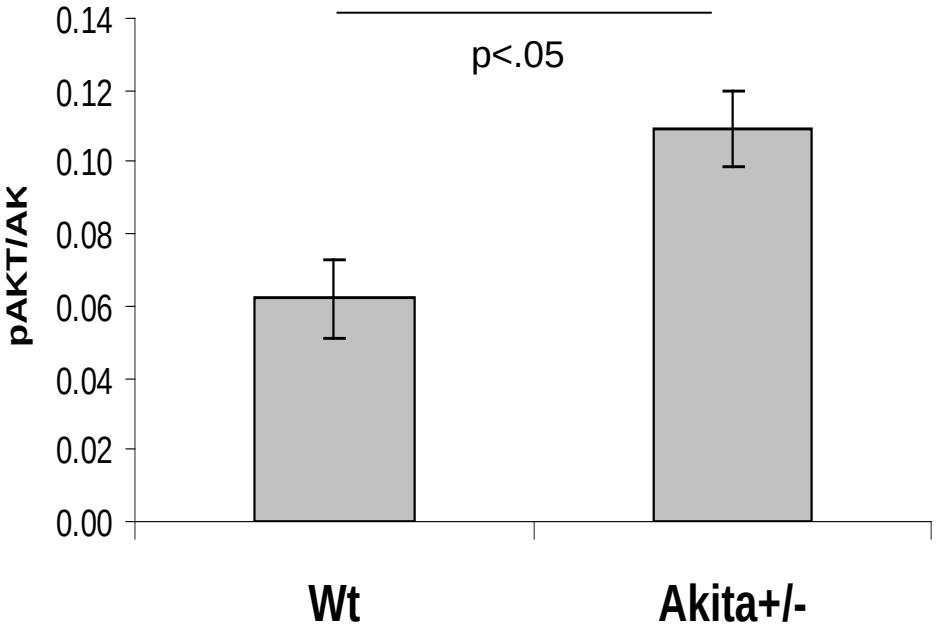
Kidney cortex



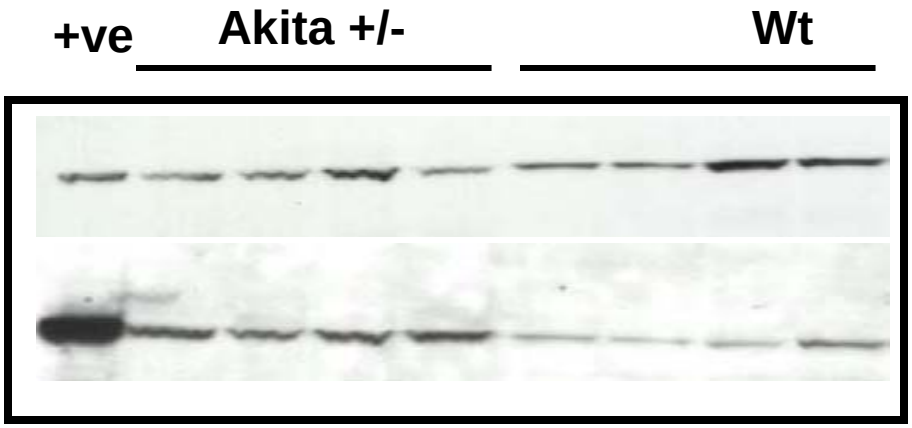
TA

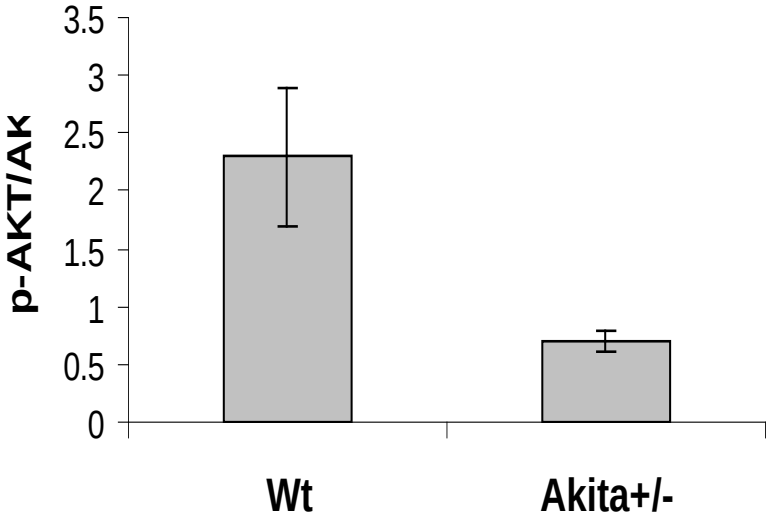
P-AKT/AKT: Kidney Cortex





pAKT/AKT: TA





"Angiogenic Signals in Diabetic Complications"

Thomas M. Coffman, M.D. P.I., Duke University complication = nephropathy
Co-PI's
Susan E. Quaggin (U of Toronto) complication = nephropathy
Brian H. Annex, M.D., University of Virginia as of 7/1 complication = PAD

Main Focus: Alterations in the VEGF receptor-ligand system and impaired angiogenesis in PAD in the setting of high fat feeding induced "DM."

Currently:

a) examining the same issue in models of type 1 DM;
b) continue to examine and compare skeletal muscle and kidney
c) establish the dependency of the findings on hyperglycemia;
d) use genetic models with altered VEGFR1 expression as well as skeletal muscle alterations in VEGF in PAD

Publications to Date:

1) Hazarika S, Dokun AO, Li Y, Popel AS, Kontos CD, Annex BH. Impaired angiogenesis after hindlimb ischemia in type 2 diabetes mellitus: differential regulation of vascular endothelial growth factor receptor 1 and soluble vascular endothelial growth factor receptor 1. ***Circ Res* 2007;101:948-56.**

2) Kappas NC, Zeng G, Chappell JC, Kearney JB, Hazarika S, Kallianos KG, Patterson C, Annex BH, Bautch VL. The VEGF receptor Flt-1 spatially modulates Flk-1 signaling and blood vessel branching. ***J Cell Biol* 2008 May 26. [Epub ahead of print].**

3) Xie D, Hazarika S, Andrich AJ, Padgett ME, Kontos CD, Donatucci CF, Annex BH. High cholesterol feeding in C57/Blc6 mice alters expression within the VEGF receptor-ligand family in corporal tissue. ***J Sex Med* 2008;5:1137-48.**

4) Wu FT, Stefanini MO, Gabhann FM, Kontos CD, Annex BH, Popel AS. Computational kinetic model of VEGF trapping by soluble VEGF receptor-1, effects of transendothelial and lymphatic macromolecular transport. Physiol Genomics. 2009 Jun 10;38(1):29-41