DIACOMP PILOT PROJECT

Wnt/G3BP1 Signaling In Arterial Calcification: Mitigation by PADI Inhibition with Cl-Amidine

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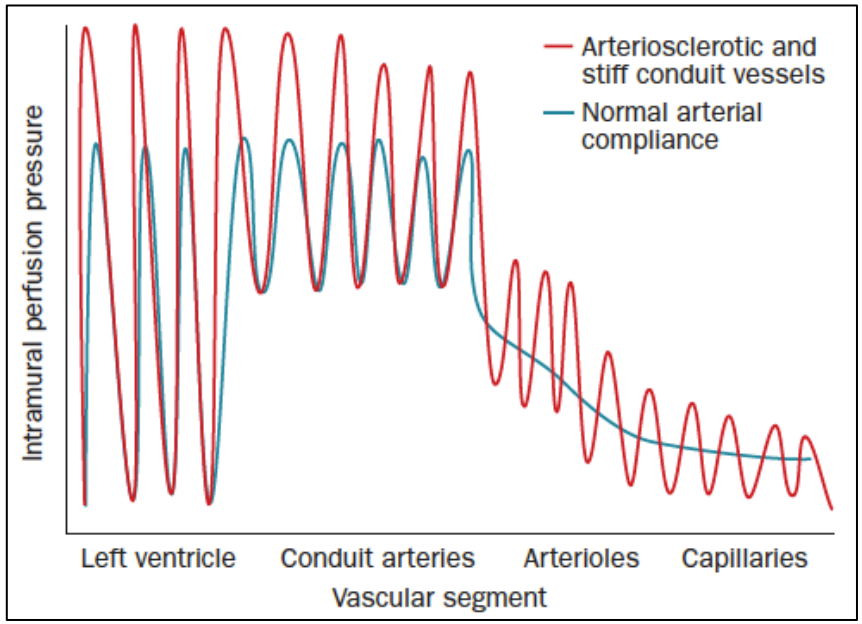
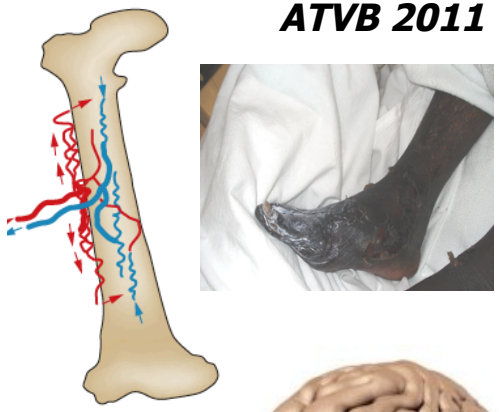
Arteriosclerotic Calcification, Windkessel Physiology, & End-Organ Disease

Thompson and Towler, Nat Rev Endo 2012



Towler lab, ATVB 2011



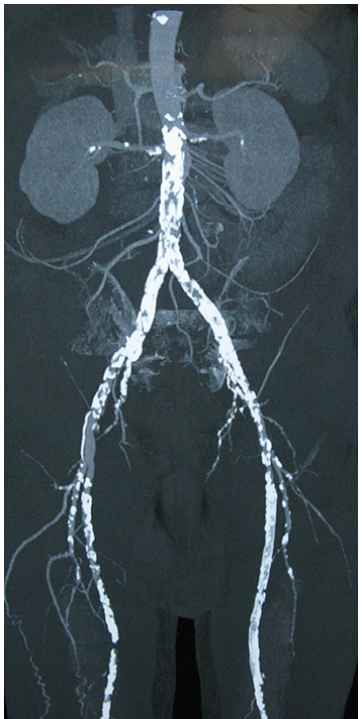
Peshock, Dallas Heart Radiol 2013

- Diabetes increases arterial stiffness
- Increased arterial stiffness impairs Windkessel physiology, the rubbery elasticity of conduit vessels that ensures smooth distal tissue perfusion throughout the cardiac cycle
- Increased arterial stiffness increases risk for stroke, dementia, heart failure, declining renal function and lower extremity amputation
- Arterial pulse wave velocity (PWV) can provide a non-invasiveness measure of arterial stiffness → Moens-Korteweg relationship

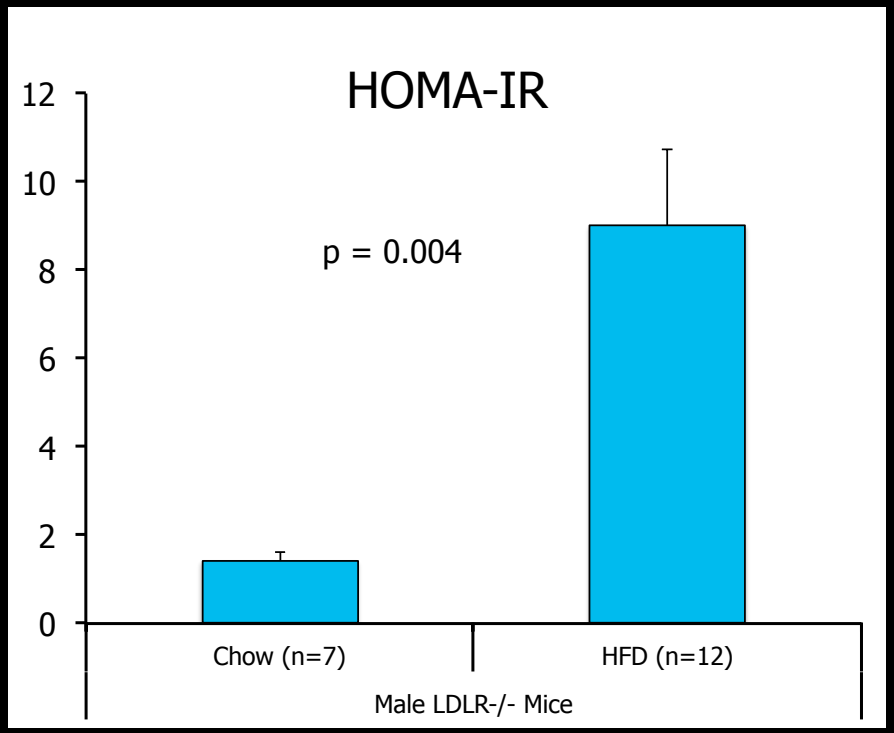
$$PWV = \sqrt{\frac{E \times \text{Wall thickness}}{2 \times \text{radius} \times \text{blood density}}}$$

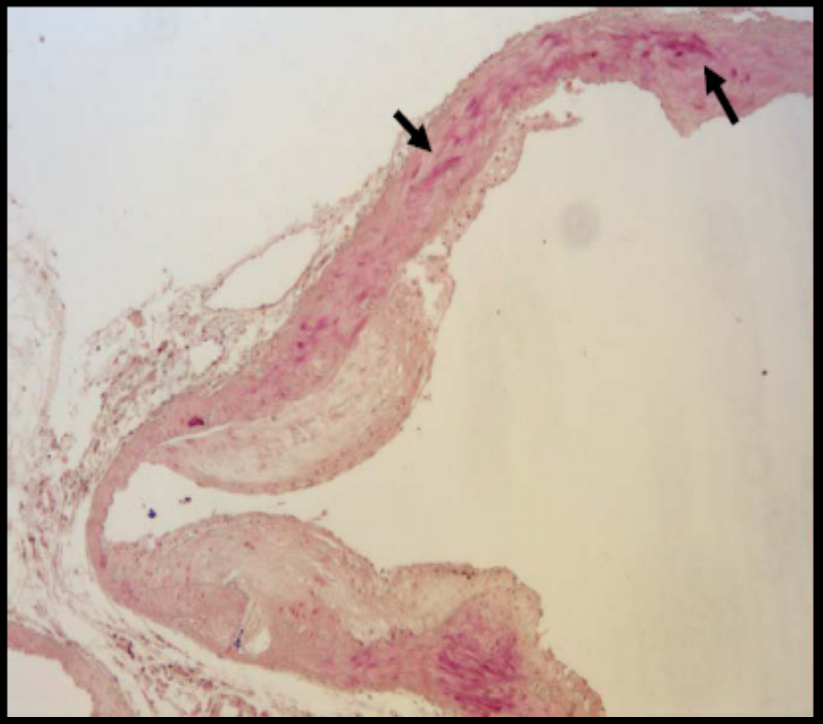
E = Young's Modulus → stiffness index of a material

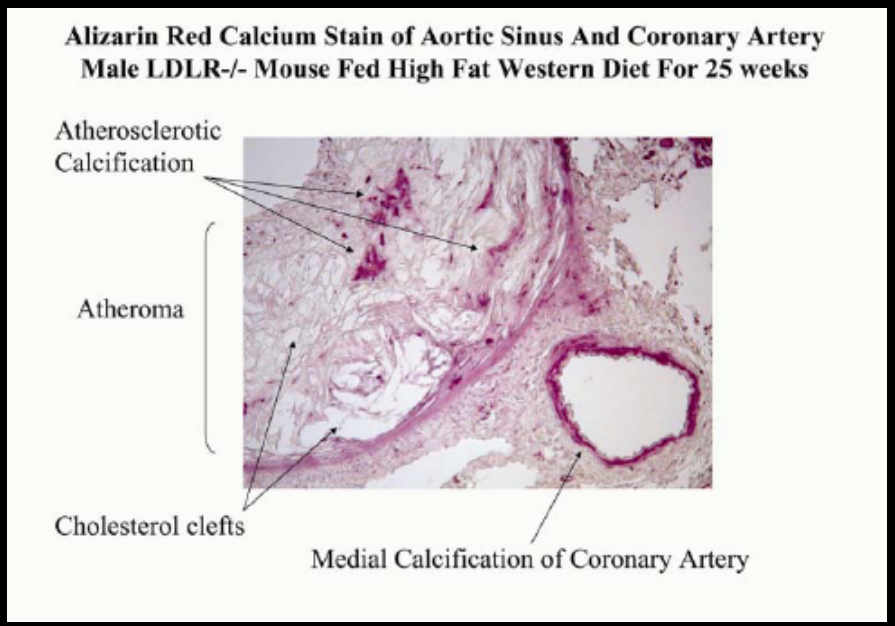
Sarnak, Health ABC CJASN 2013

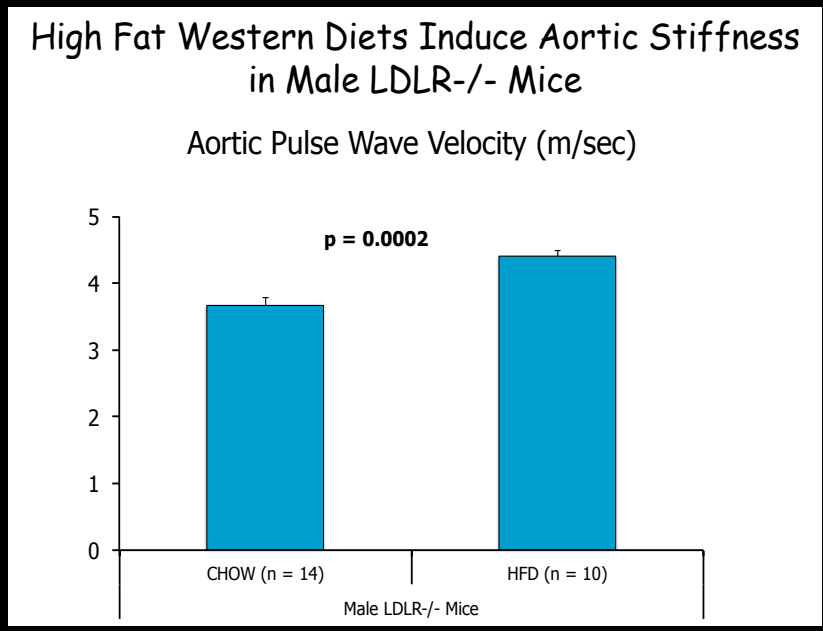


High Fat Western Diets Induce Insulin-Resistant Diabetes, Dyslipidemia, Arterial Calcification, and Aortic Stiffness in LDLR-/- Mice

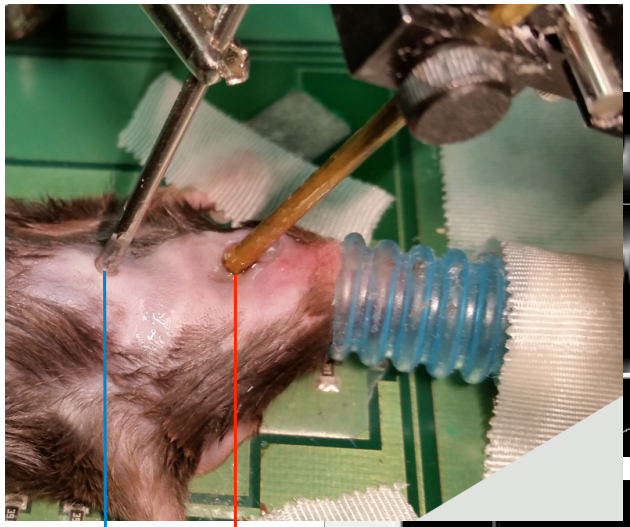


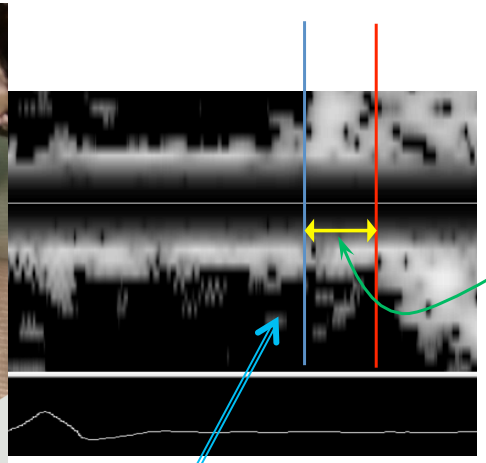






DUAL TRANSDUCER –MEDIATED DIRECT ASSESSMENT OF ARTERIAL PULSE WAVE VELOCITY: CAROTID, AORTIC, ETC.





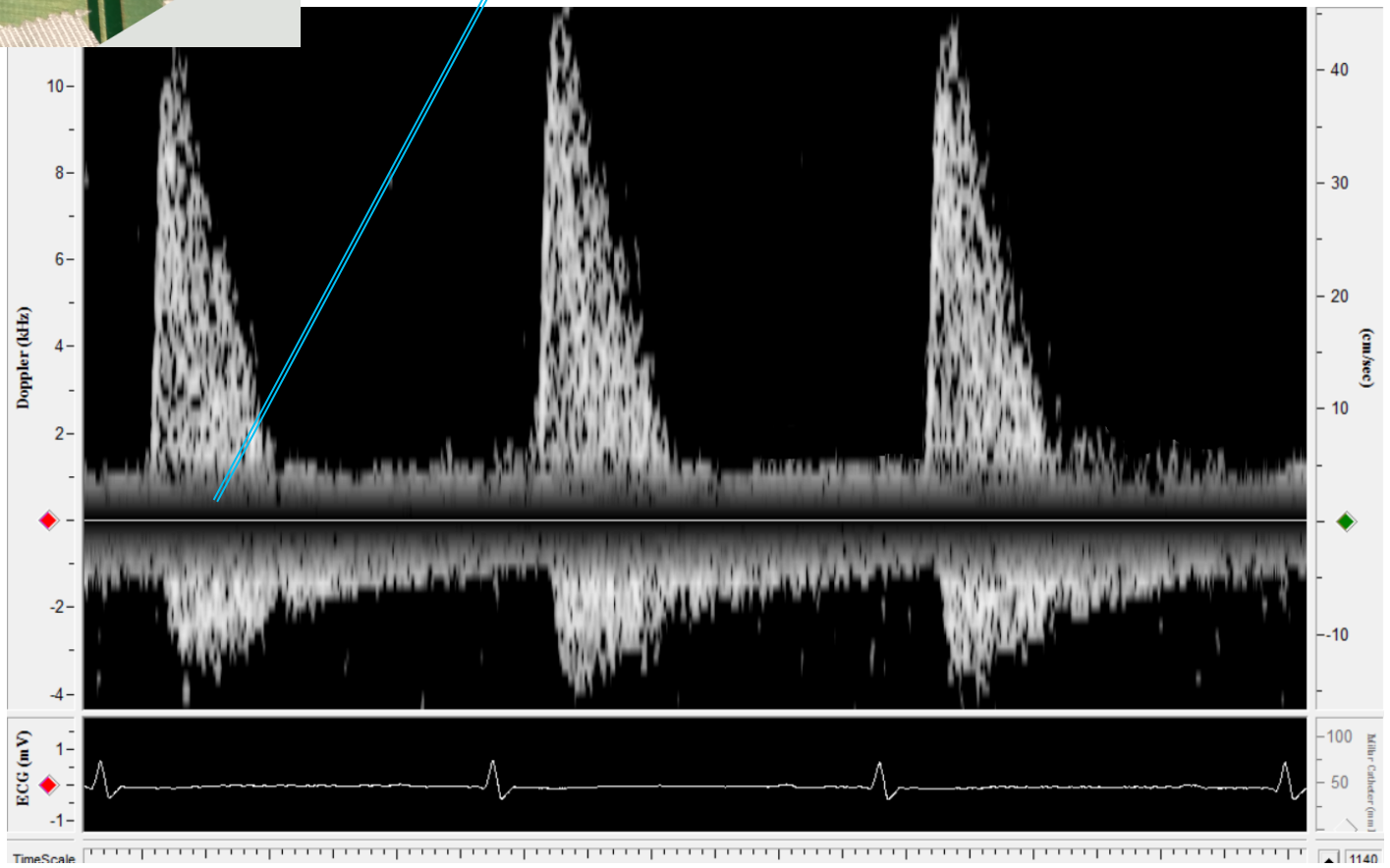
5.2ms

PWV= 13mm/5.2ms
= 2.5 mm/ms
or = 2.5 m/s

13mm

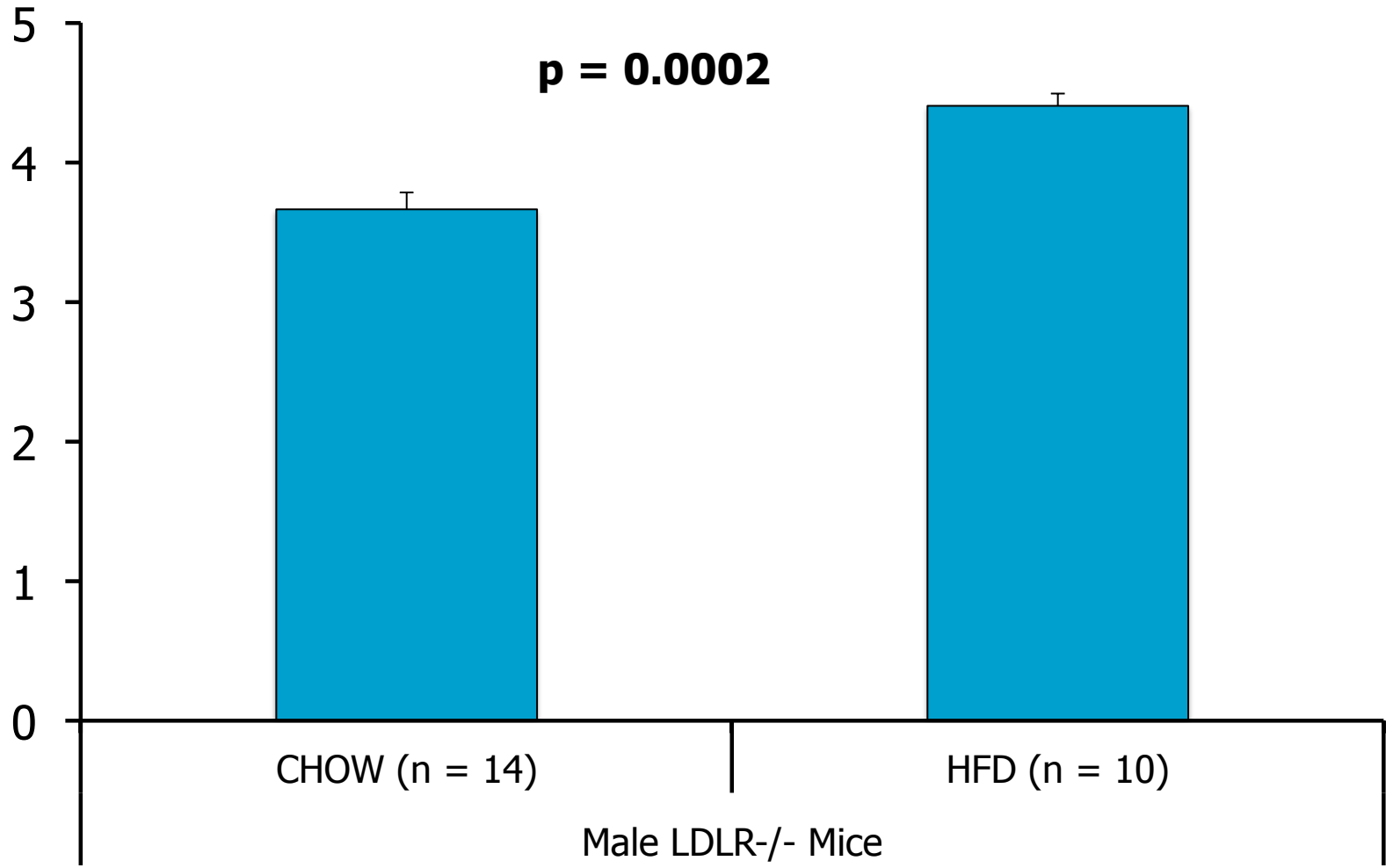
Aortic Sinus

R. Carotid Artery



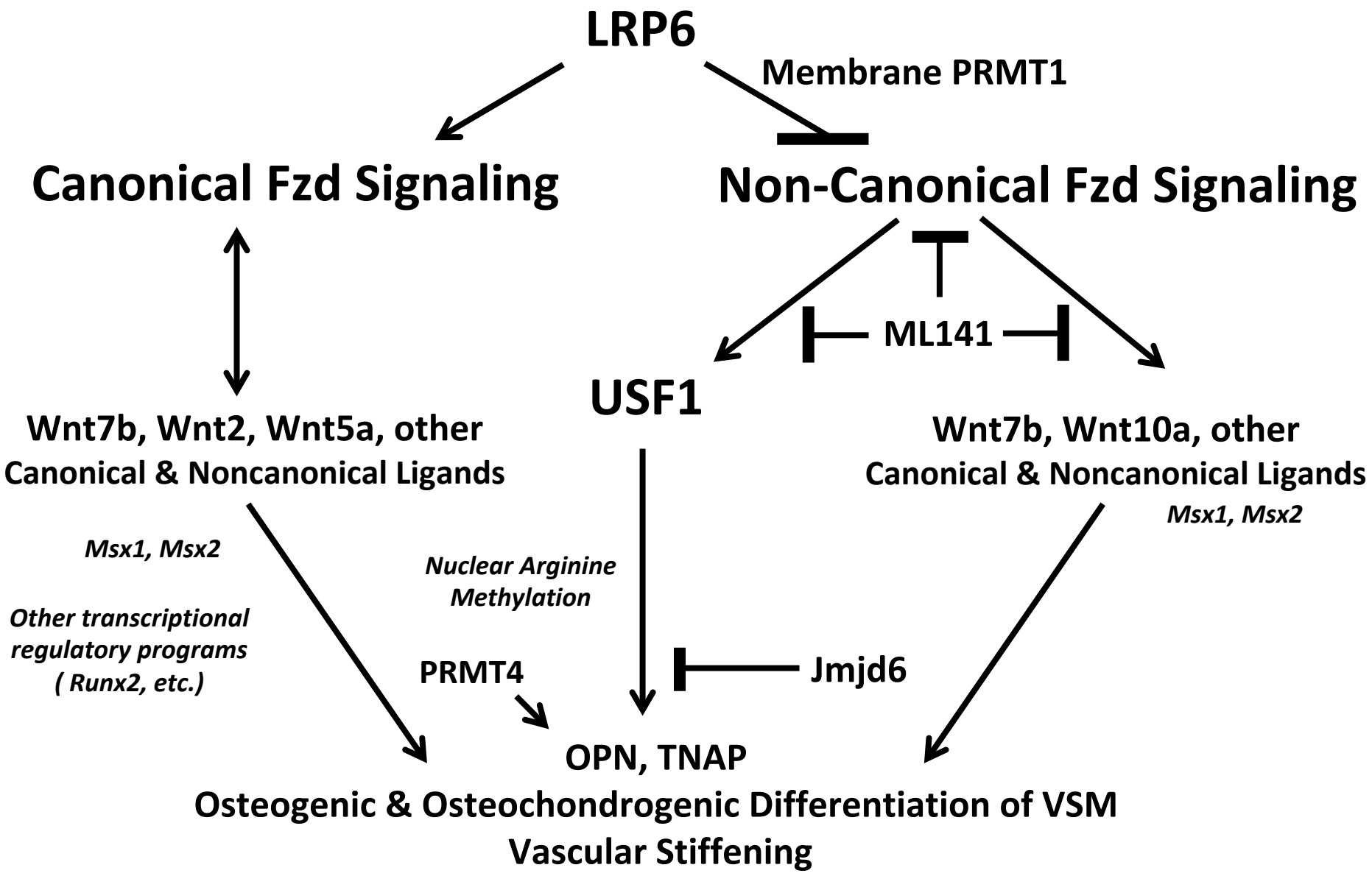
High Fat Western Diets Induce Aortic Stiffness in Male LDLR-/- Mice

Aortic Pulse Wave Velocity (m/sec)



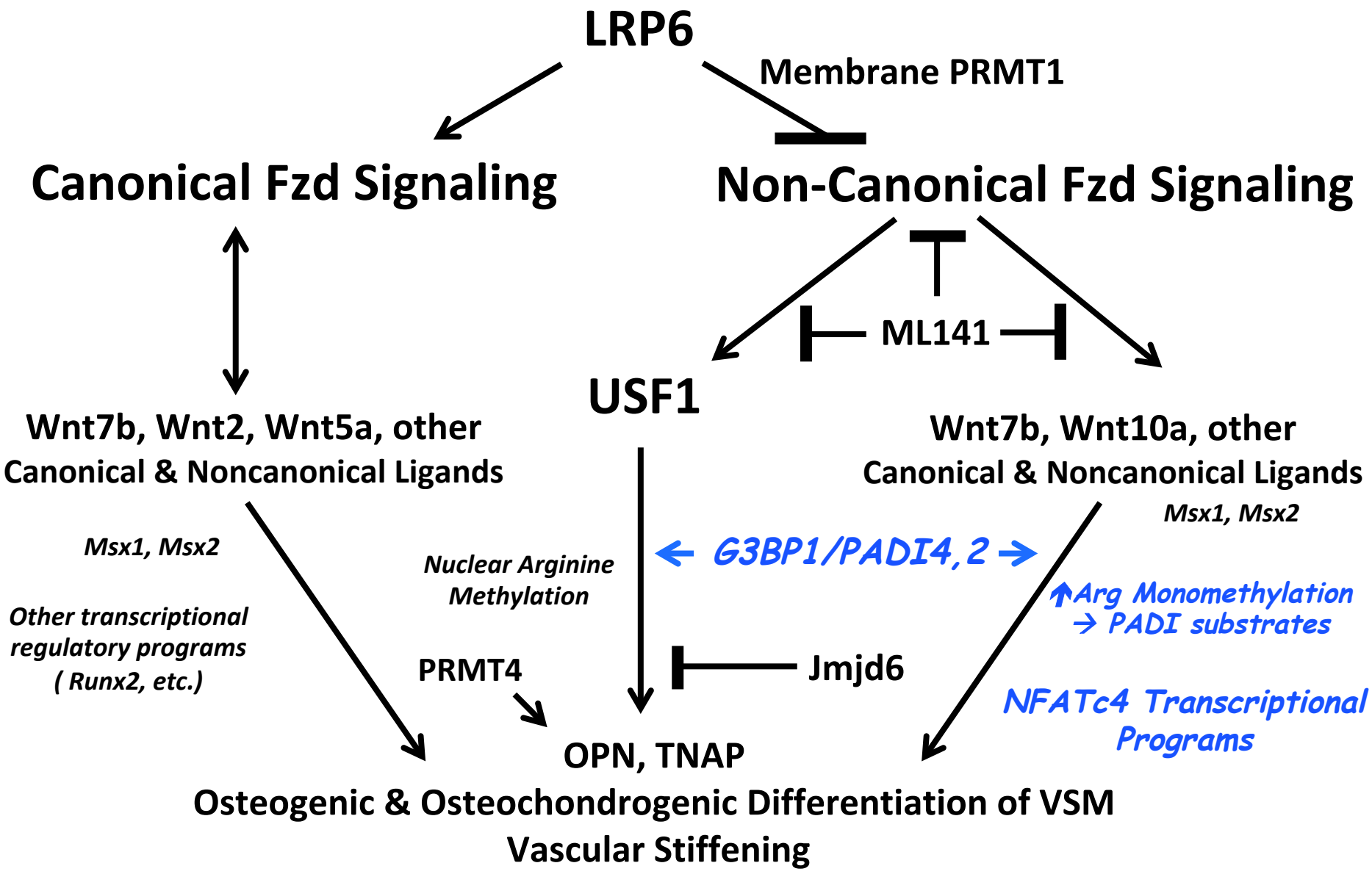
Towler lab, unpublished

LRP6 Signal Relays in Diabetic Arteriosclerosis



Circ Res. 2015 Jul 3;117(2):142-56. doi: 10.1161/CIRCRESAHA.117.306712.
Arterioscler Thromb Vasc Biol. 2017 Feb;37(2):205-217. doi: 10.1161/ATVBAHA.116.306258.
J Biol Chem. 2018 Apr 6. pii: jbc.RA118.002046. doi: 10.1074/jbc.RA118.002046. [Epub ahead of print]

UPDATED WORKING MODEL: MONOMETHYLARGININE AND G3BP1/PADI CONTRIBUTIONS

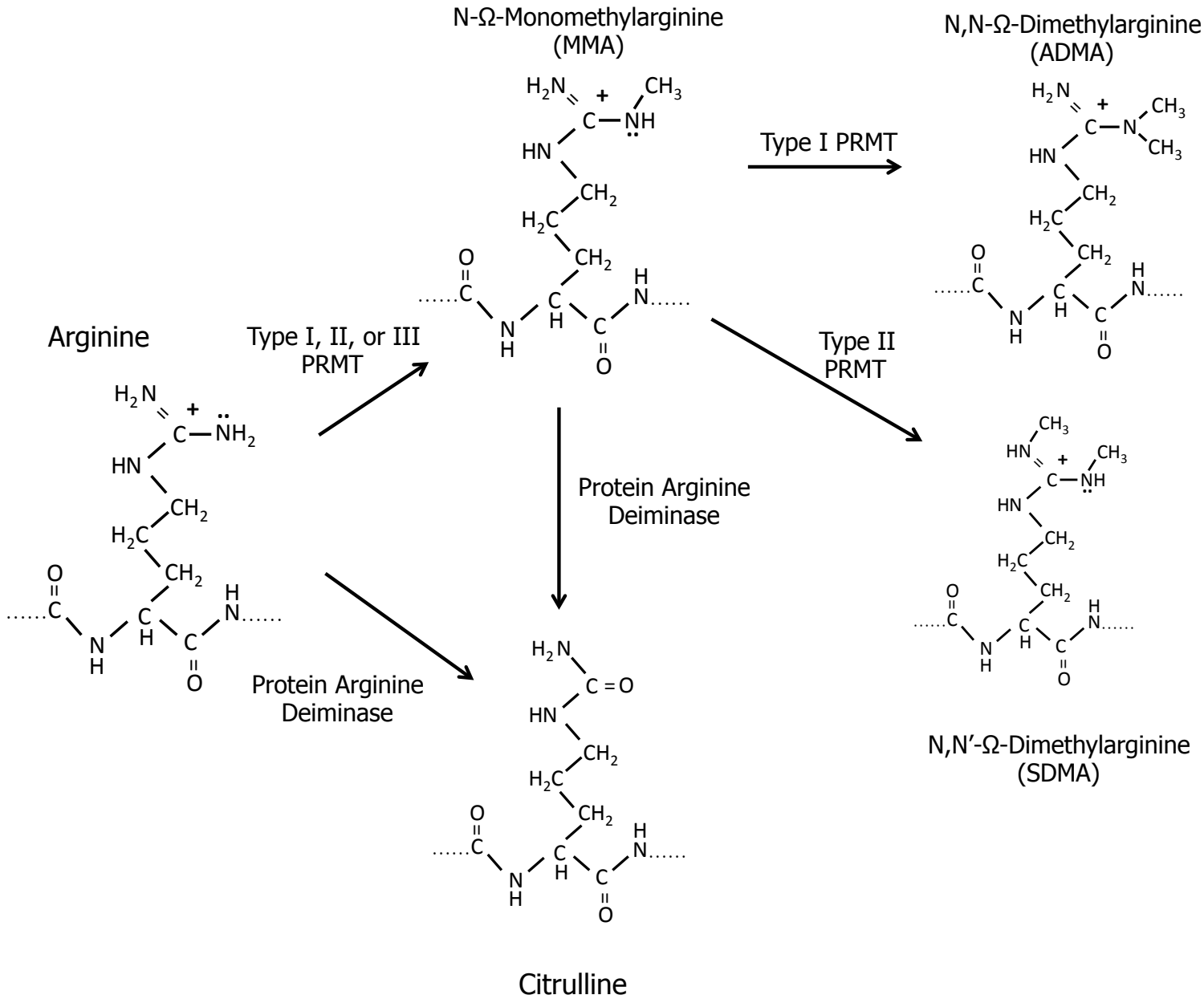


Circ Res. 2015 Jul 3;117(2):142-56. doi: 10.1161/CIRCRESAHA.117.306712.
Arterioscler Thromb Vasc Biol. 2017 Feb;37(2):205-217. doi: 10.1161/ATVBAHA.116.306258.
J Biol Chem. 2018 Apr 6. pii: jbc.RA118.002046. doi: 10.1074/jbc.RA118.002046. [Epub ahead of print]

PADI4

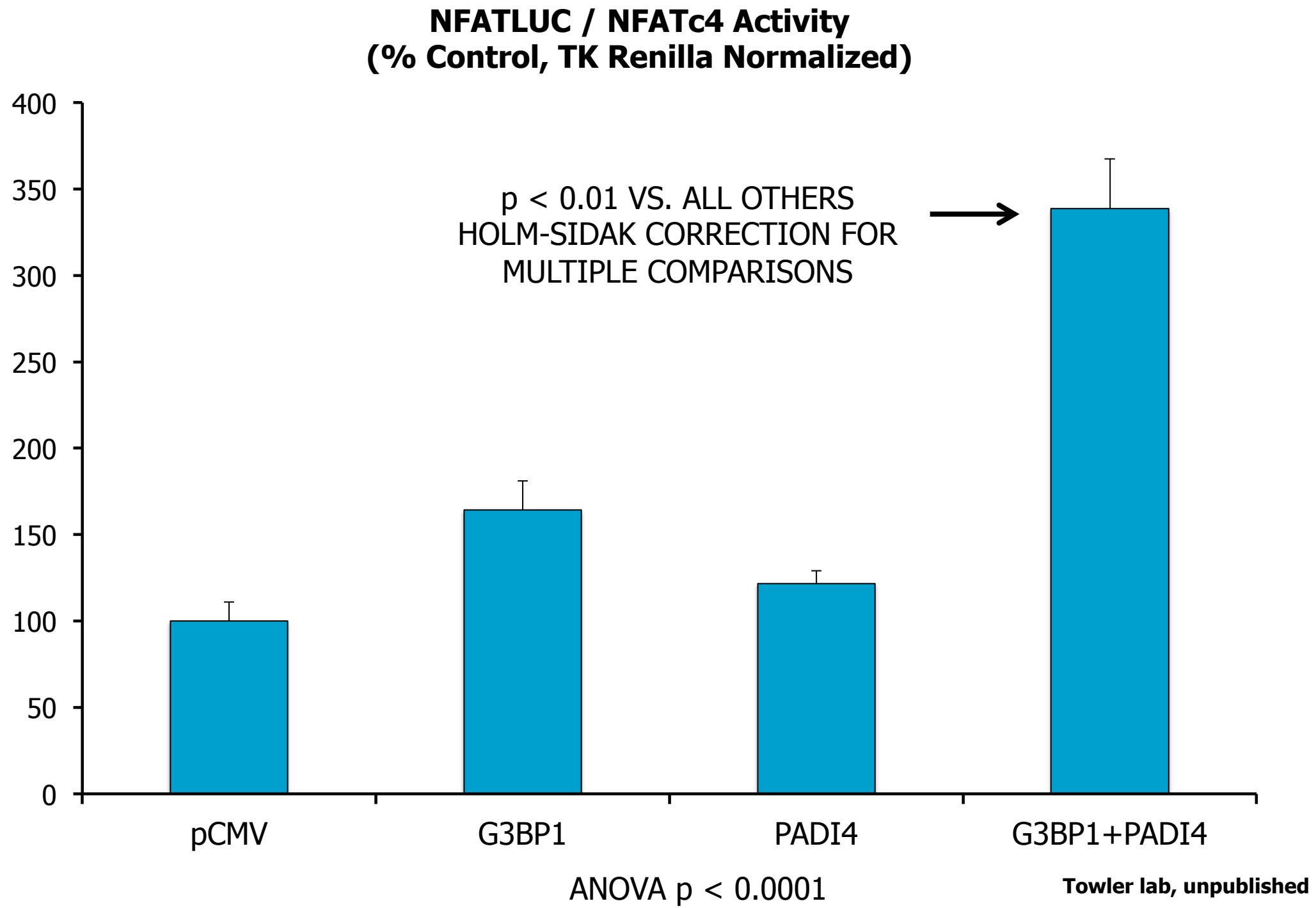
- Protein Arginine Deiminase 4
- Member of PADI family
  - PADI1, PADI2, PADI3, PADI4, PADI6
- Originally identified as key regulator of NETosis
  - Neutrophil extracellular traps
    - Nat Chem Biol. 2015 Mar;11(3):189-91. doi: 10.1038/nchembio.1735.
- Expressed in many other cells and tissues including conduit arteries
  - Towler lab, unpublished
- Supports pro-sclerotic noncanonical Wnt signaling
  - Towler lab, unpublished

Protein Arginine Deiminases Generate Citrullinated Proteins That Are No Longer Subject to N-Ω-Arginine Methylation, A Key Modification of G3BP1 Regulated By Wnt Signaling



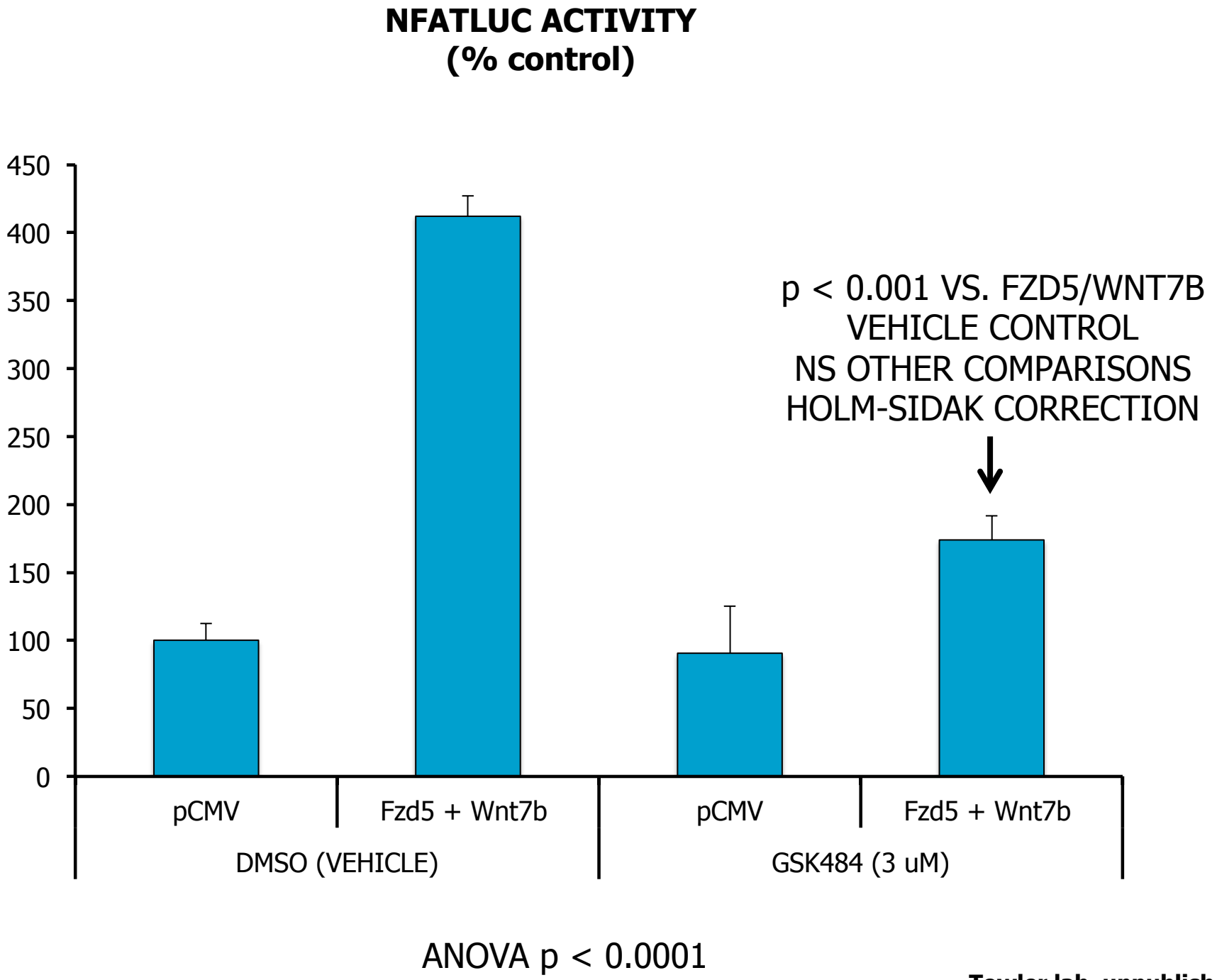
J Biol Chem. 2018 Apr 6. pii: jbc.RA118.002046. doi: 10.1074/jbc.RA118.002046. [Epub ahead of print]
Towler lab, unpublished

PADI4 Synergistically Enhances G3BP1 Activation of NFATc4 Transcription In Transient Transfection Assays



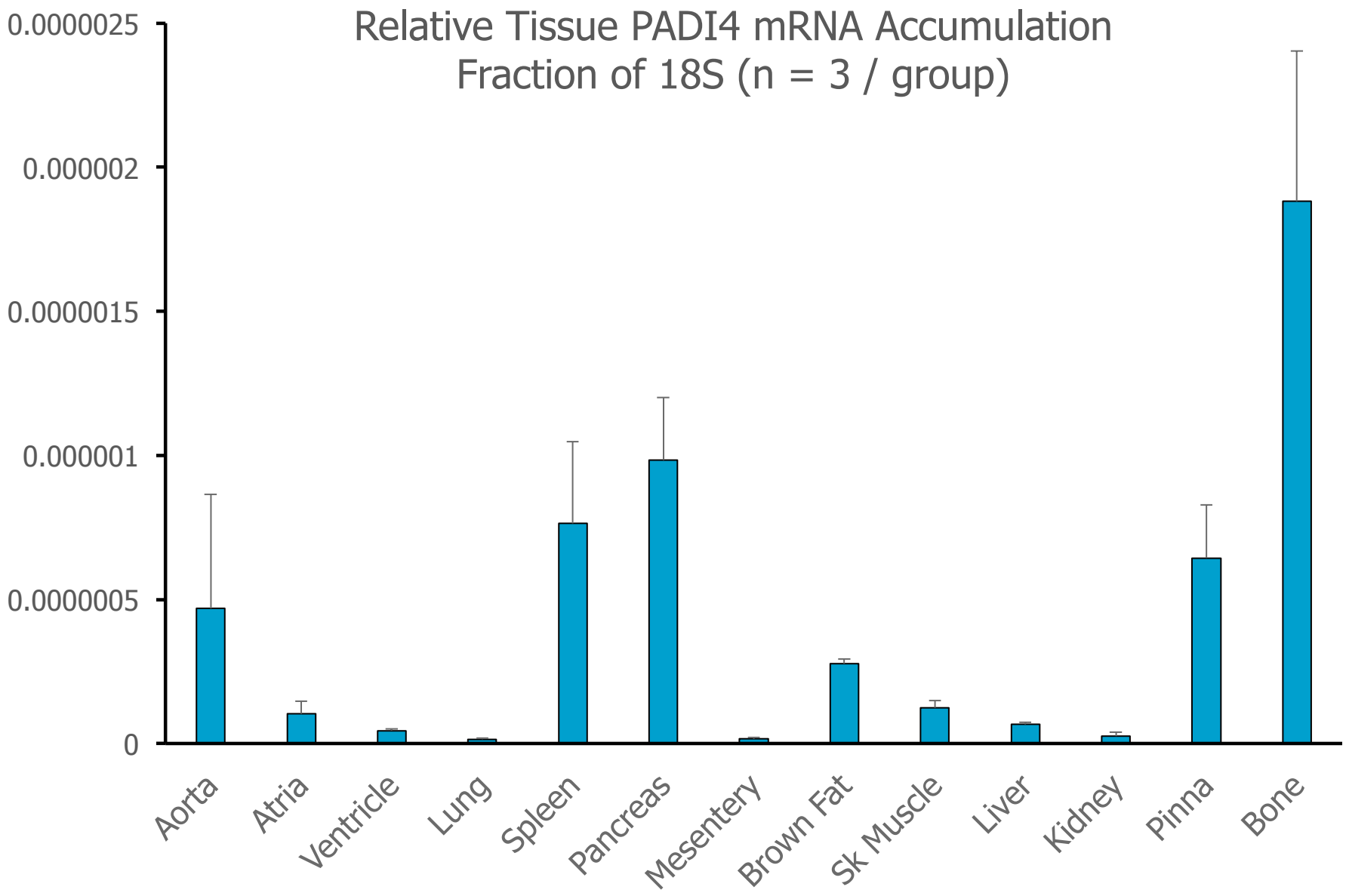
Towler lab, unpublished

The PADI4 ANTAGONIST GSK484 INHIBITS WNT/Ca++/NFAT SIGNALING



Towler lab, unpublished

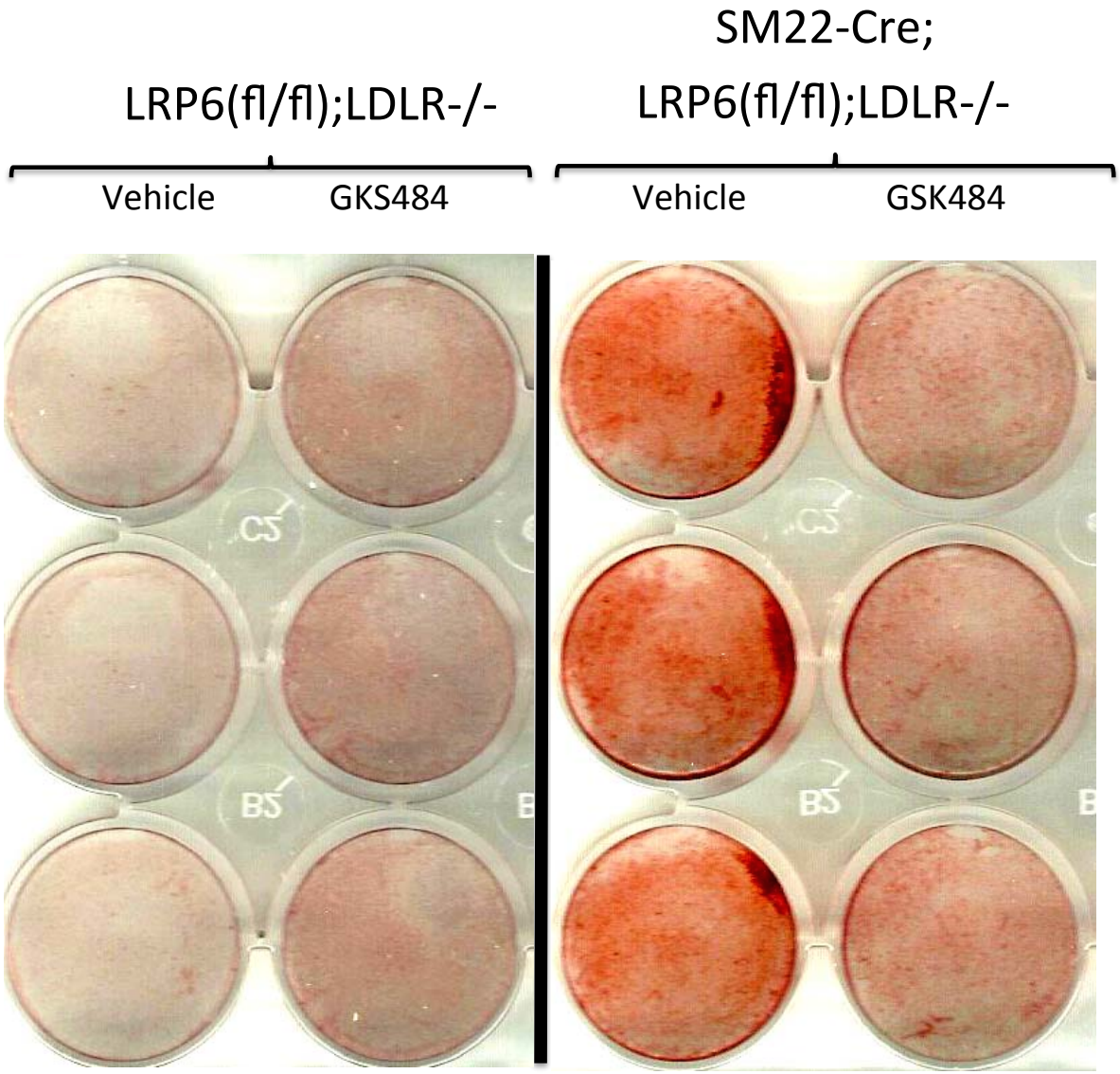
PADI4 mRNA is Expressed in a Variety of Murine Tissues, Including Bone, Aorta, Spleen, Pancreas, Brown Fat, and Pinna



Towler lab, unpublished

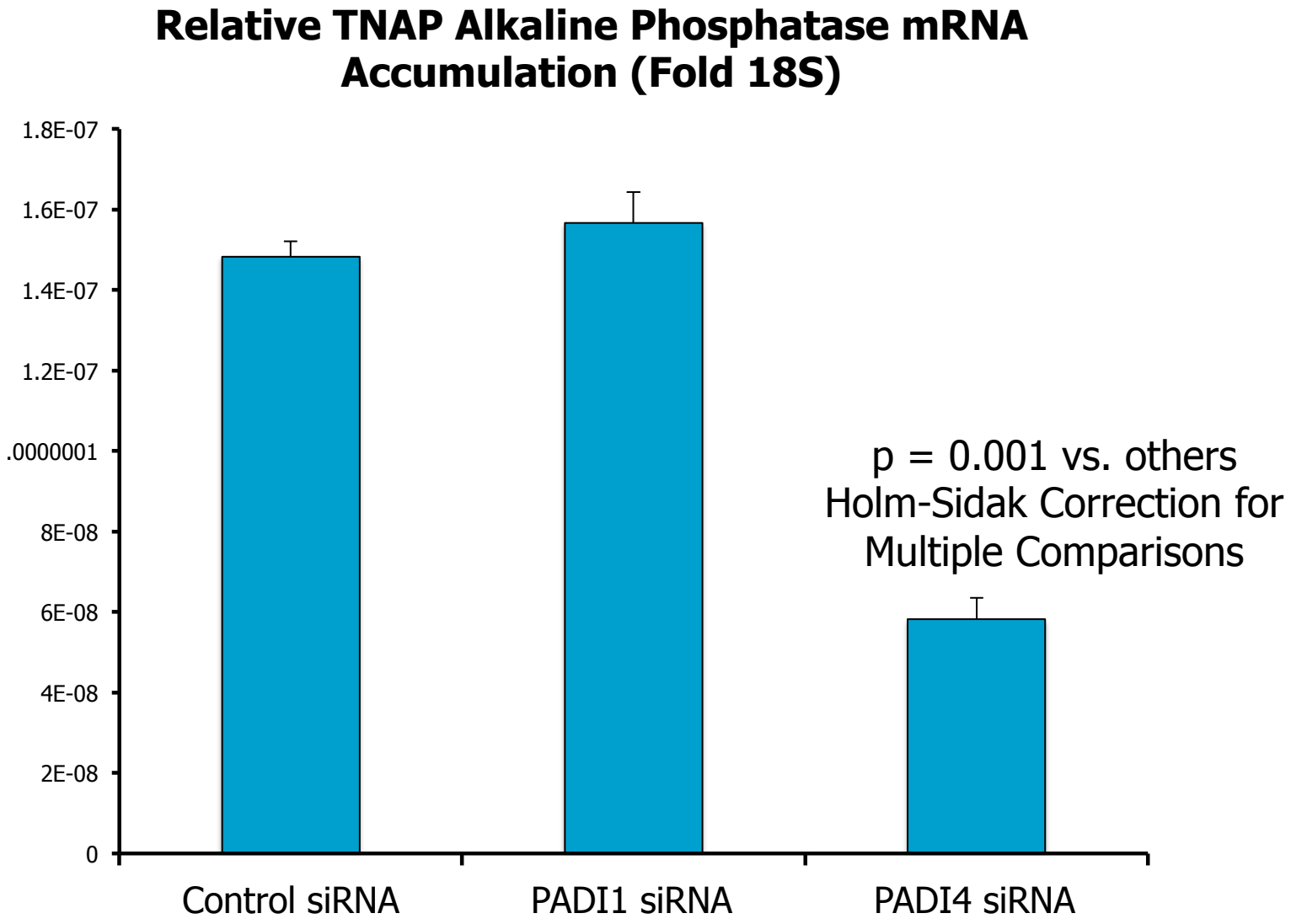
The PADI2/4 Inhibitor GSK484 Reduces Mineralization in LRP6-Deficient VSM

Alizarin Red Calcium Stain



Towler Lab, unpublished

RNAi Targeting PADI4, but not PADI1, Reduces Pro-Osteogenic Alkaline Phosphatase mRNA Accumulation In Primary Aortic VSMCs



ANOVA $p < 0.0001$

Towler lab, unpublished

In Vivo Study

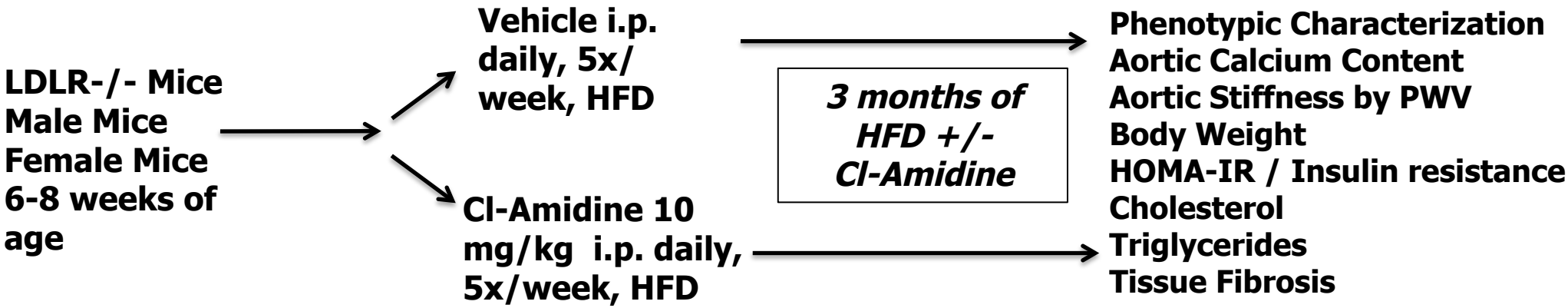
- Assess whether PADI4 Inhibition Alters the Pathobiology of Arteriosclerosis in LDLR-/- Mice Fed High Fat Diabetogenic Diets
  - Arterial calcification
  - Arterial stiffness
  - Insulin resistance / HOMA-IR
  - Basic lipid profiles
  - Male and female mice

Cl-Amidine

- Broad specificity PADI inhibitor
- Very well characterized in vivo and in vitro
  - IC50 ~ 6 uM
- Known efficacy in vivo at 10 mg/kg intraperitoneal dosing
  - Sepsis models
  - Arthritis models
- No overt toxicity to doses as high as 100 mg/kg

- Biochemistry 45: 11727–11736, 2006
- Am J Physiol Gastrointest Liver Physiol. 2011 Jun; 300(6): G929–G938.

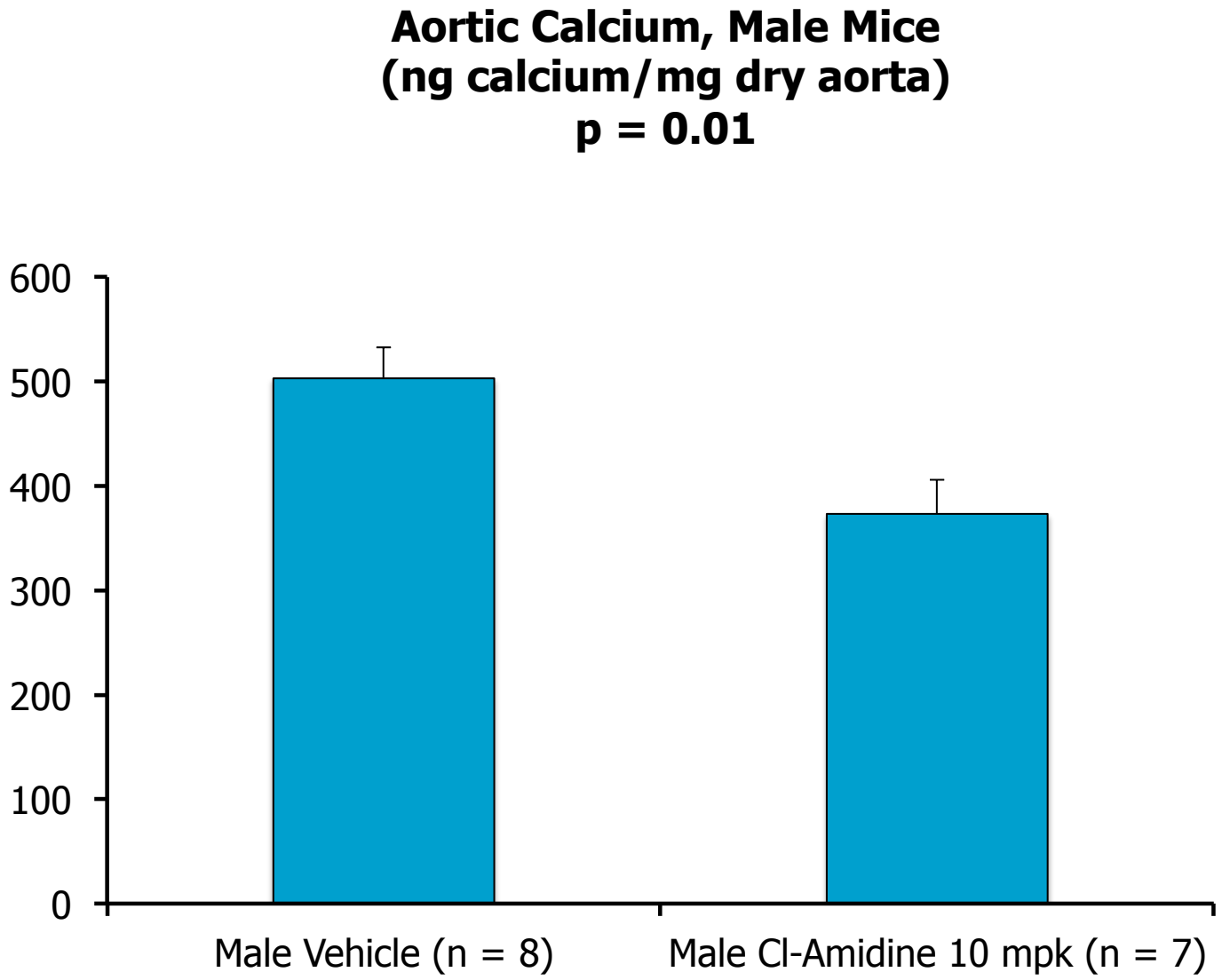
PROTOCOL: IMPACT OF THE PADI INHIBITOR CL-AMIDINE ON DIET-INDUCED ARTERIOSCLEROSIS IN LDLR-/- MICE FED HIGH FAT DIABETOGENIC DIETS



HFD = High Fat Diabetogenic Western Diet
EnvigoTD.88137
42% of calories from fat

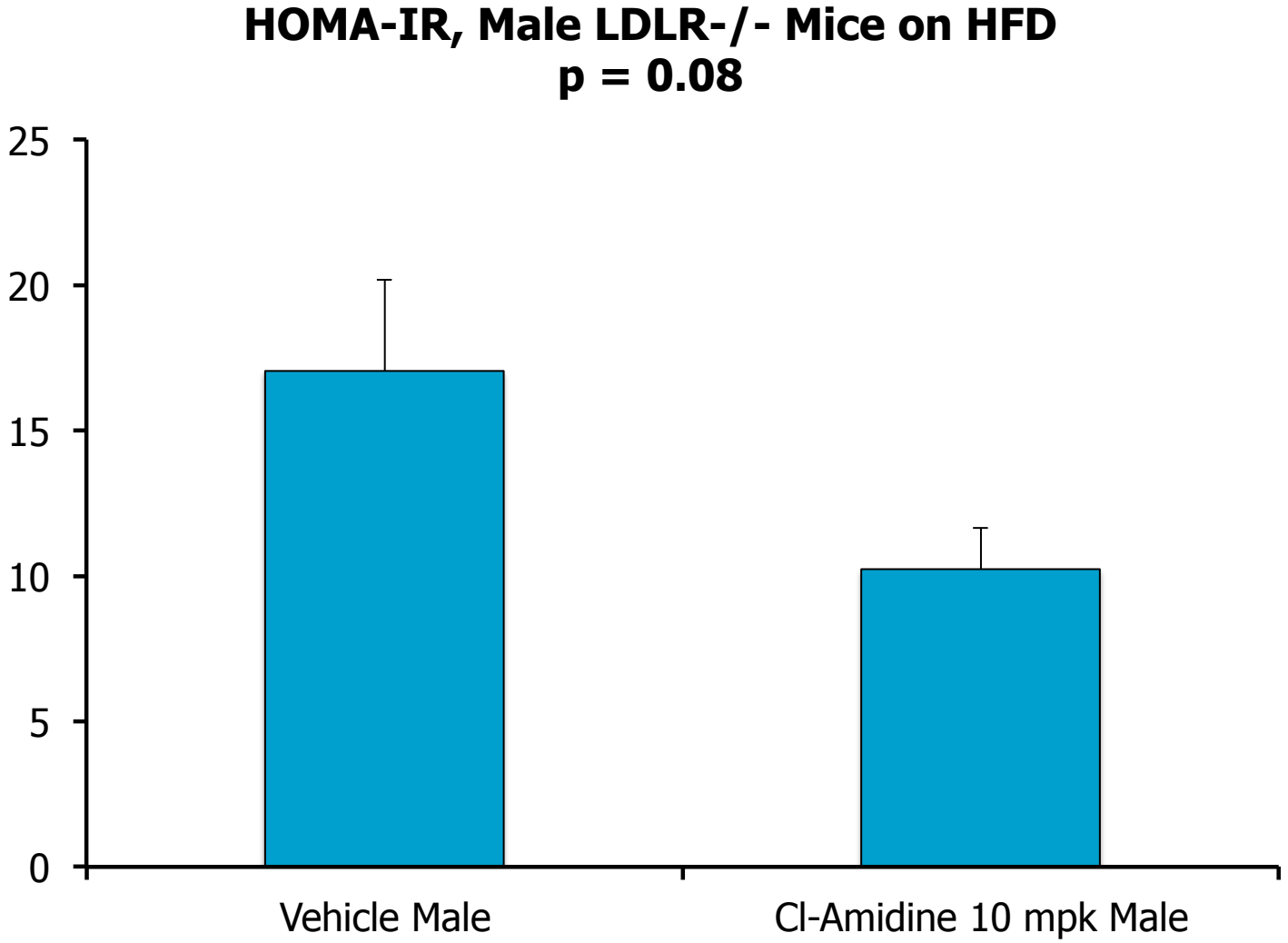
Towler lab, unpublished

The PADI4 Inhibitor Cl-Amidine Significantly Reduces Aortic Calcium In Male LDLR-/- Mice Fed High Fat Diabetogenic Diets



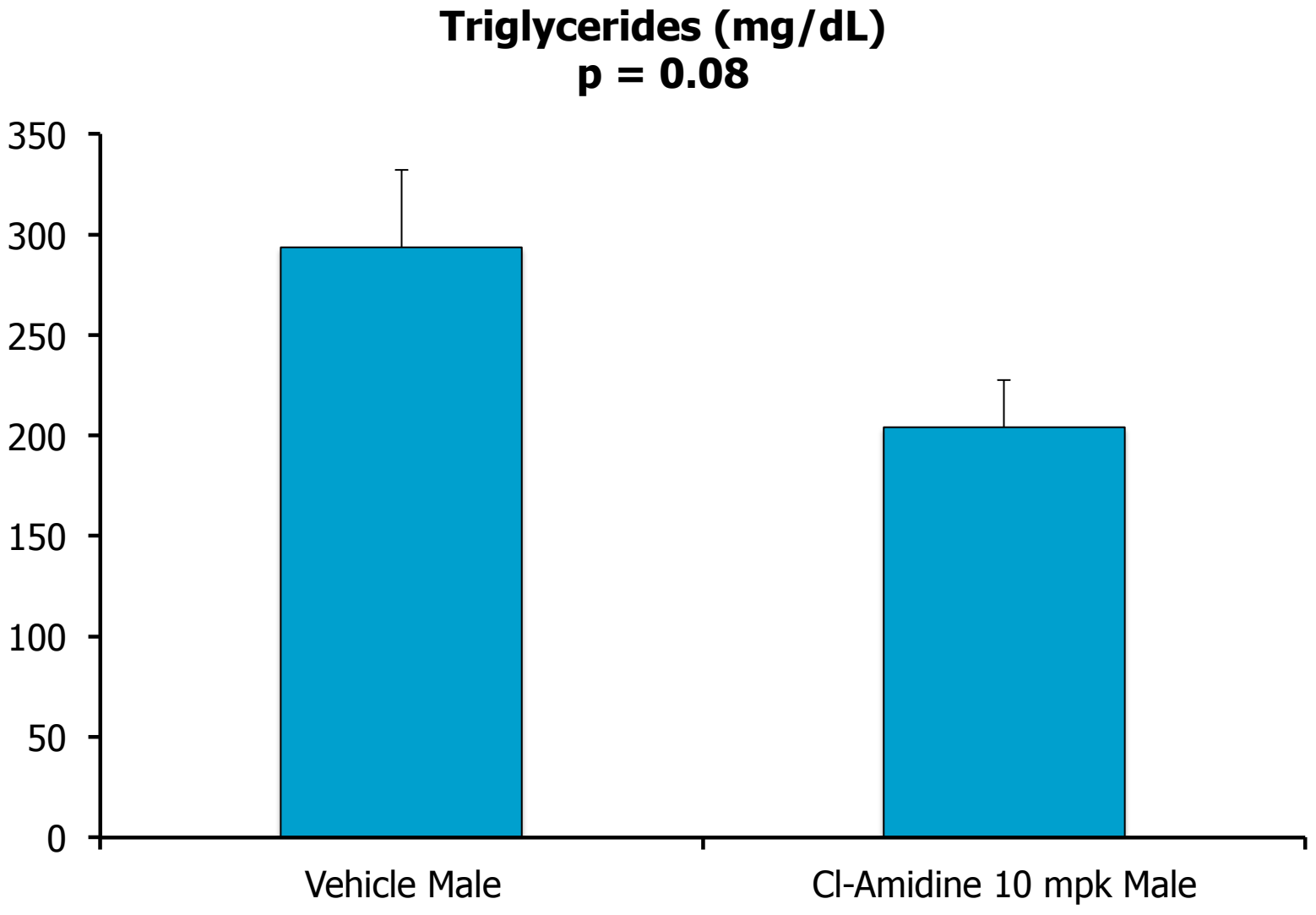
Towler lab, unpublished

The PADI4 Inhibitor Cl-Amidine Exerts a Non-Significant Trend For A 40% Reduction In HOMA-IR (Index of Insulin Resistance) in Male LDLR-/- Mice Fed High Fat Diabetogenic Diets



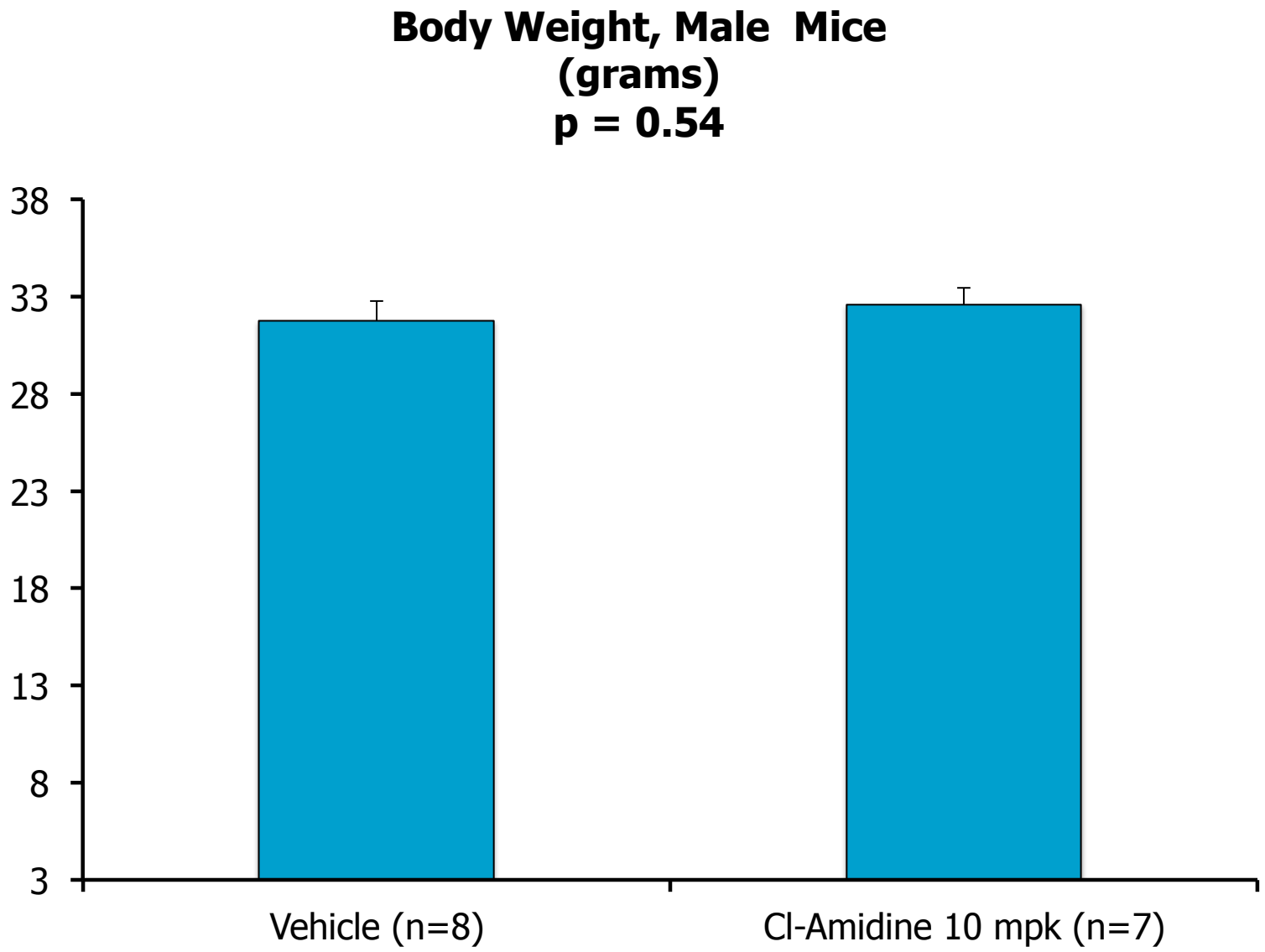
Towler lab, unpublished

The PADI4 Inhibitor Cl-Amidine Exerts a Non-Significant Trend To Reduce Fasting Plasam Triglyceride Levels in Male LDLR-/- Mice Fed High Fat Diabetogenic Diets – Paralleling the Reductions in Insulin Resistance



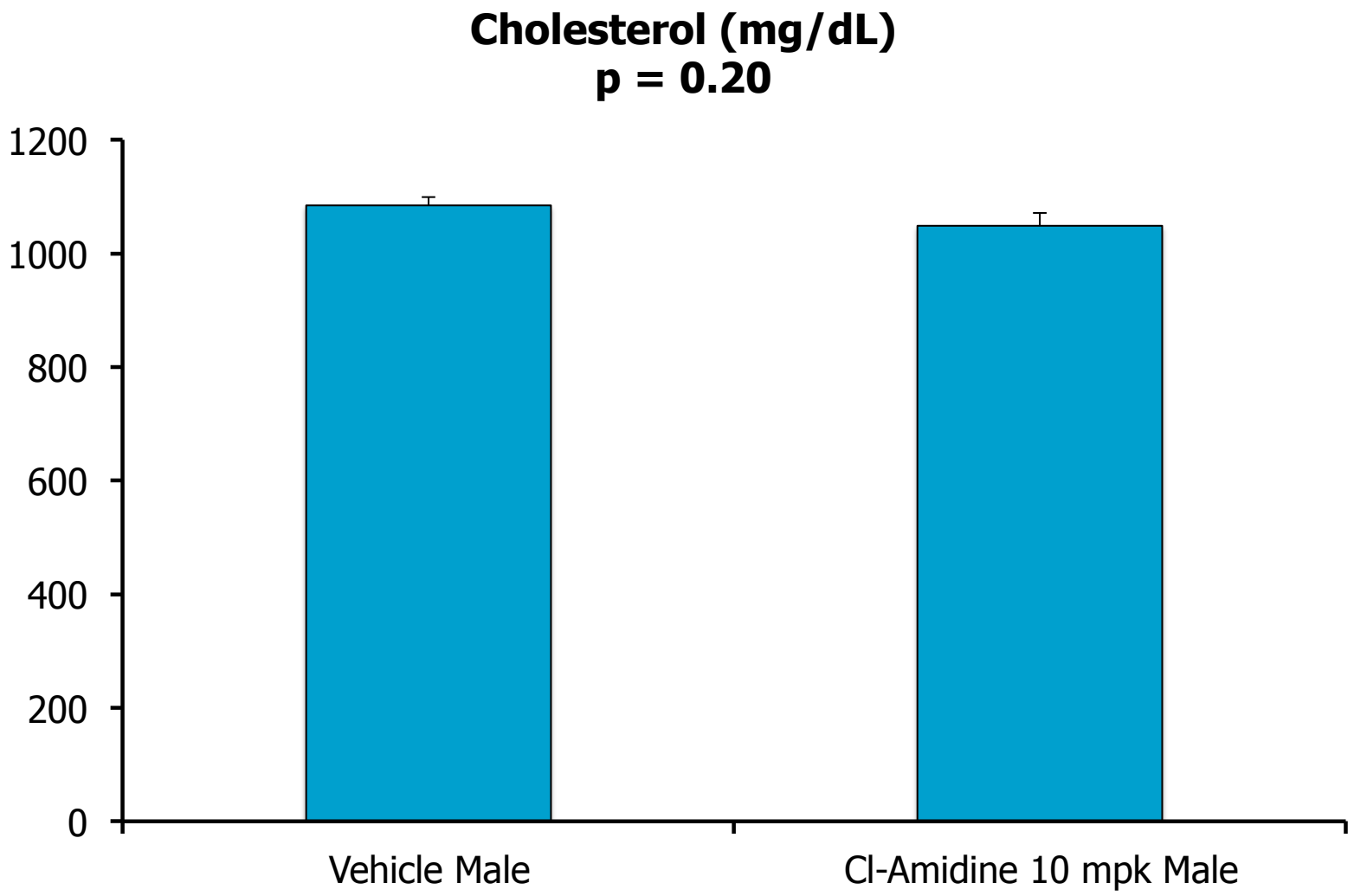
Towler lab, unpublished

The PADI4 Inhibitor Cl-Amidine Has No Impact On Body Weight In Male LDLR-/- Mice Fed High Fat Diabetogenic Diets



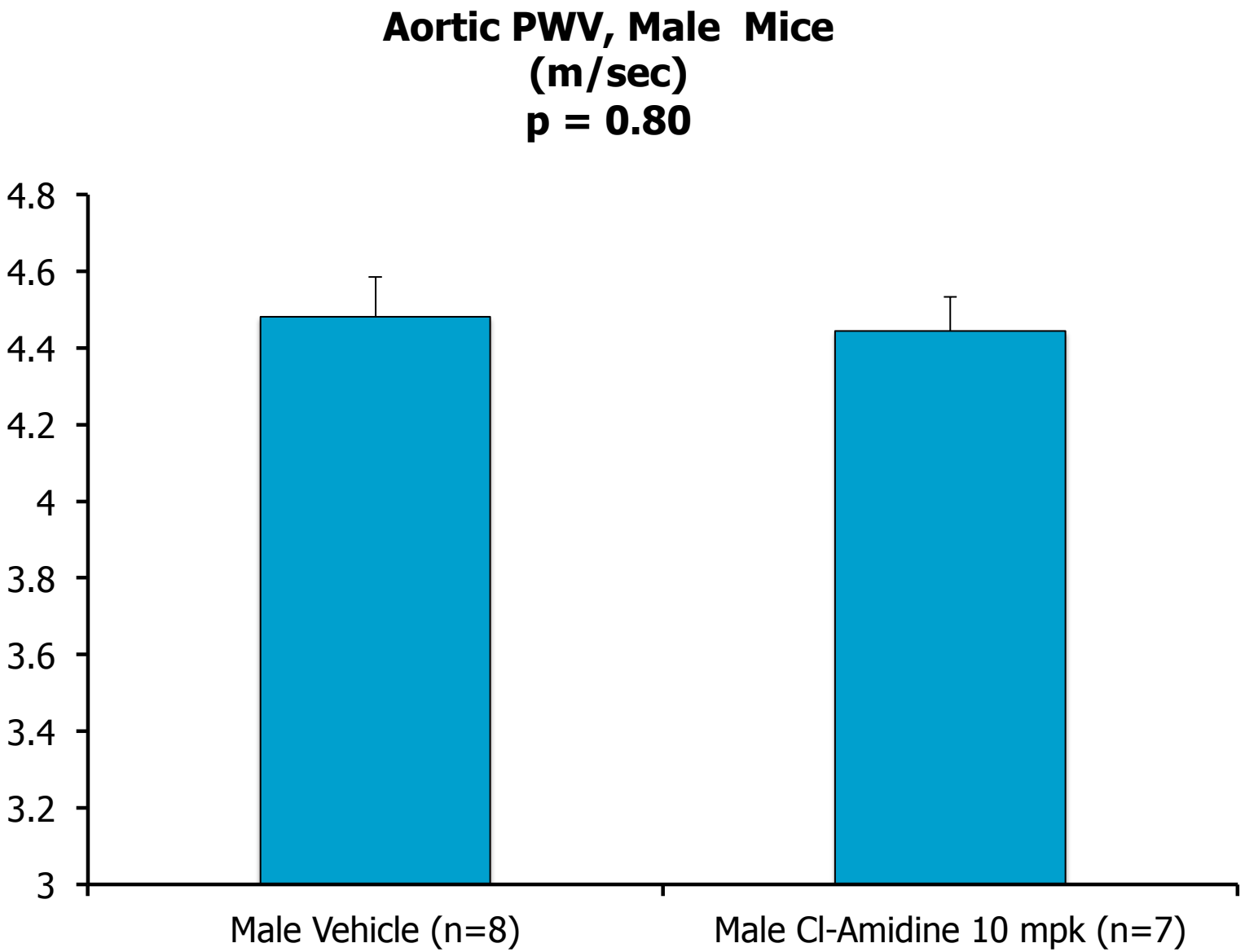
Towler lab, unpublished

The PADI4 Inhibitor Cl-Amidine Did Not Significantly Reduce Fasting Plasma Cholesterol Levels In Male LDLR-/- Mice Fed High Fat Diabetogenic Diets



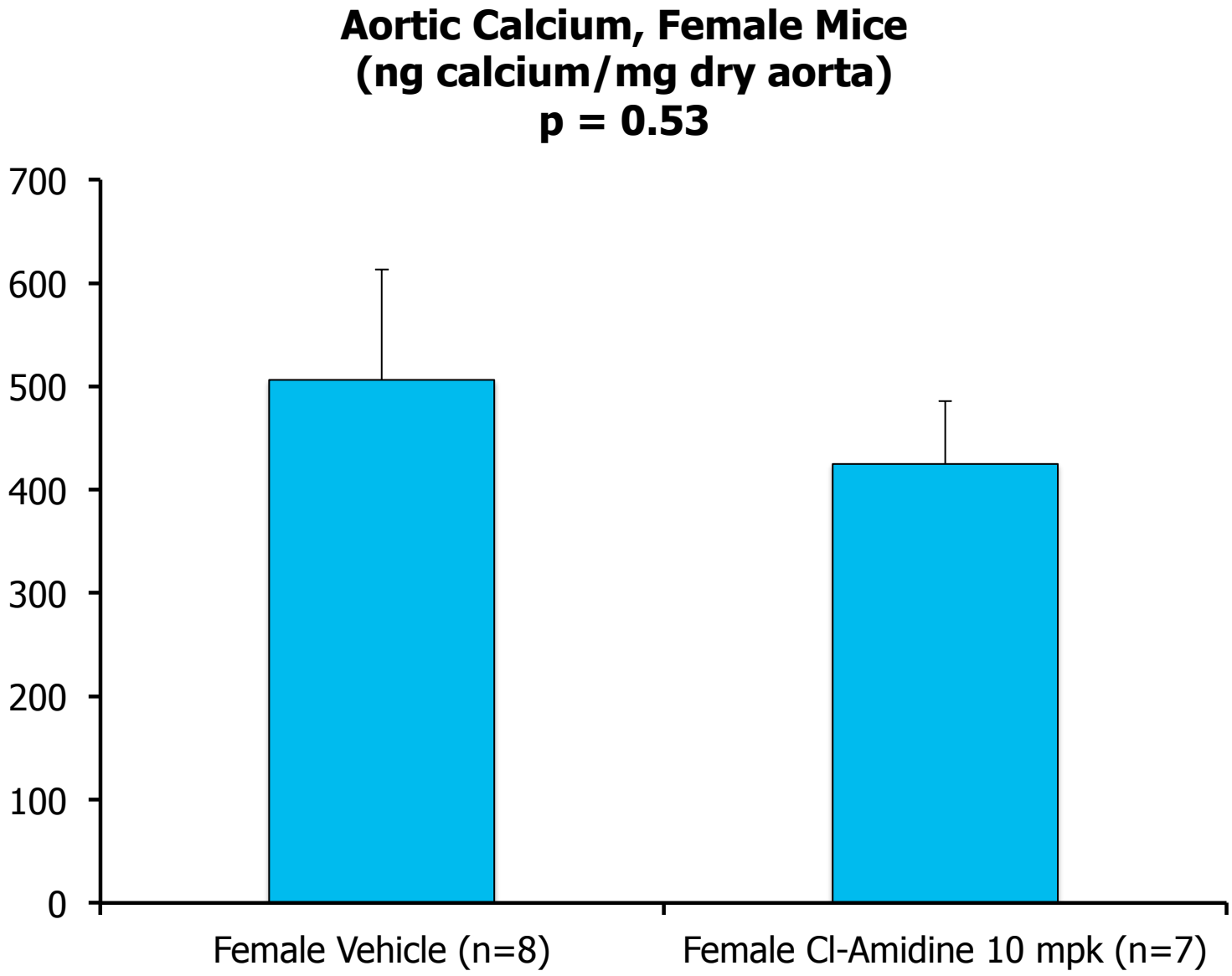
Towler lab, unpublished

The PADI4 Inhibitor Cl-Amidine Did Not Significantly Reduce Aortic PWV In Male LDLR-/- Mice Fed High Fat Diabetogenic Diets



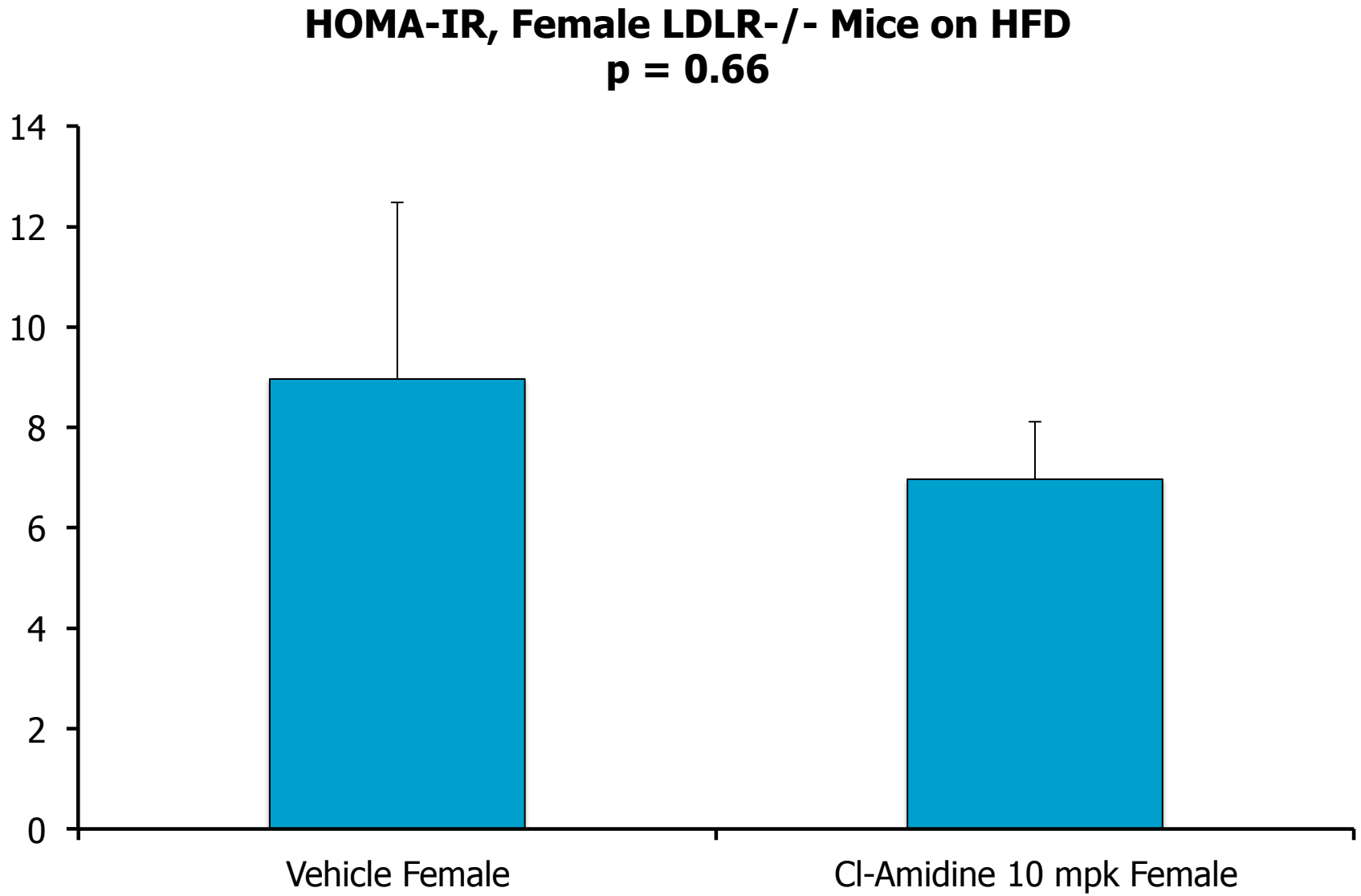
Towler lab, unpublished

The PADI4 Cl-Amidine Does Not Significantly Reduce Aortic Calcium In Female LDLR-/- Mice Fed High Fat Diabetogenic Diets



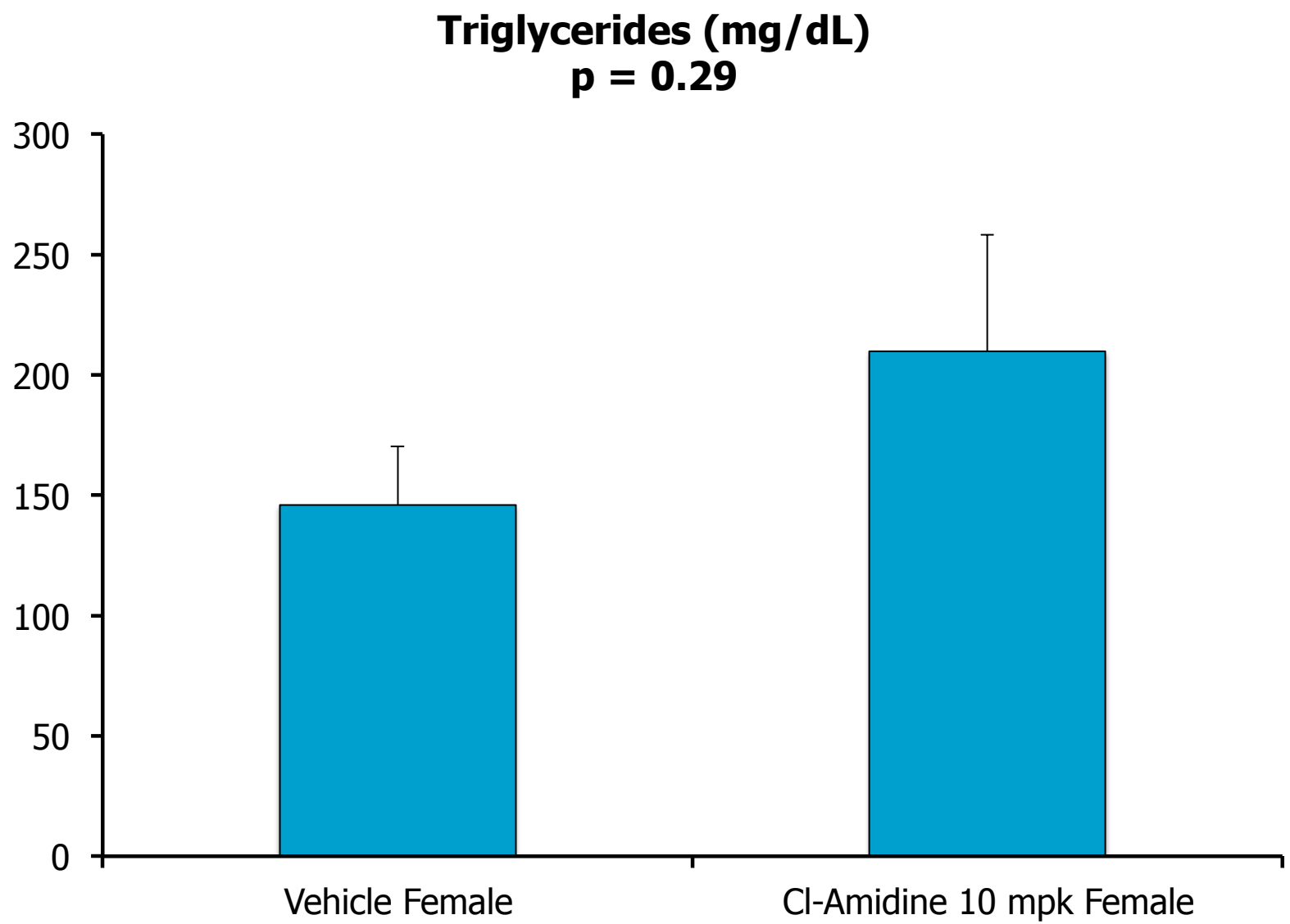
Towler lab, unpublished

The PADI4 Inhibitor Cl-Amidine Does Not Reduce the HOMA-IR Index of Insulin Resistance in Female LDLR-/- Mice Fed High Fat Diabetogenic Diets



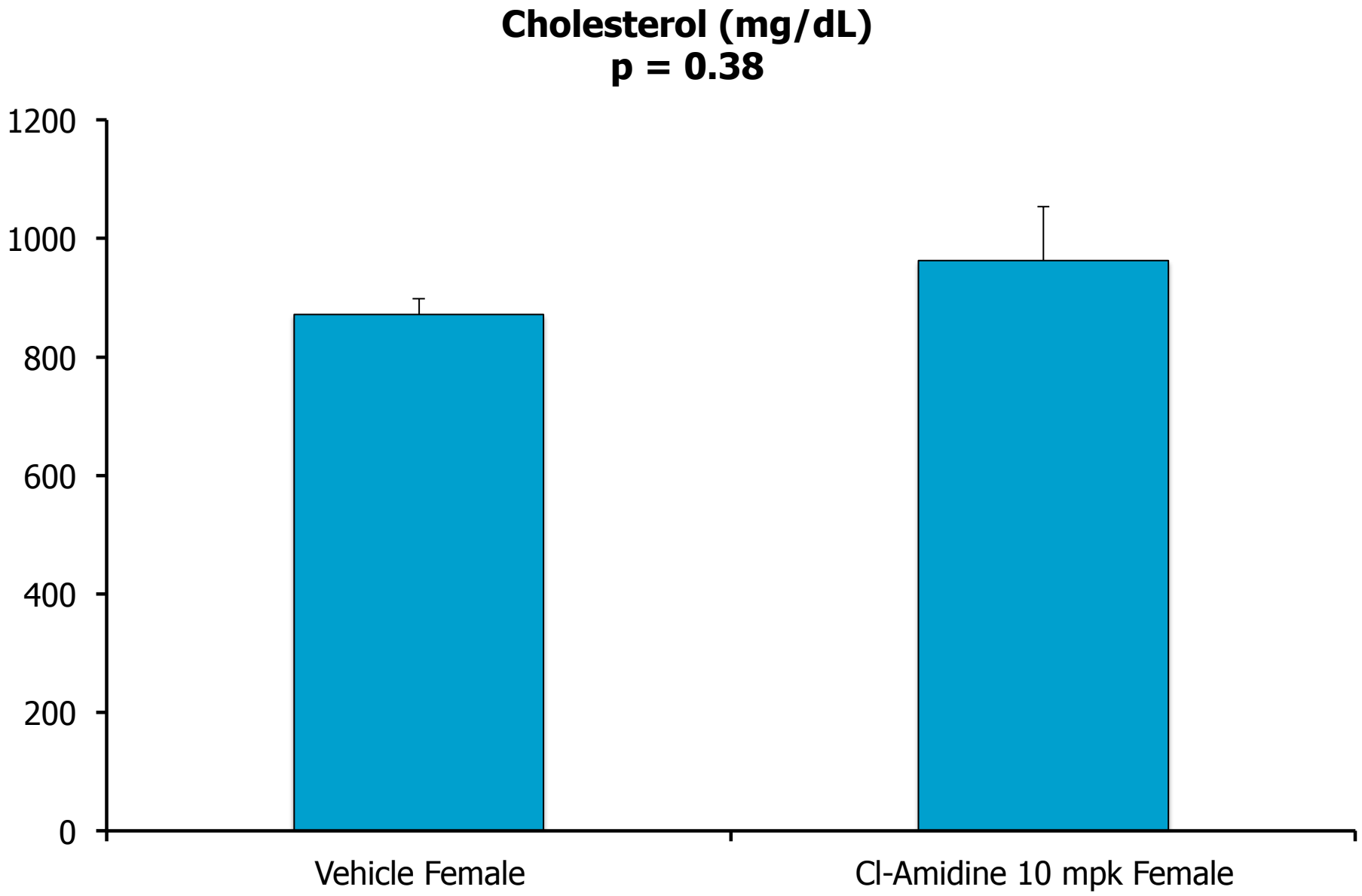
Towler lab, unpublished

The PADI4 Inhibitor Cl-Amidine Does Not Reduce Fasting Plasma Triglyceride Levels Female LDLR-/- Mice Fed High Fat Diabetogenic Diets



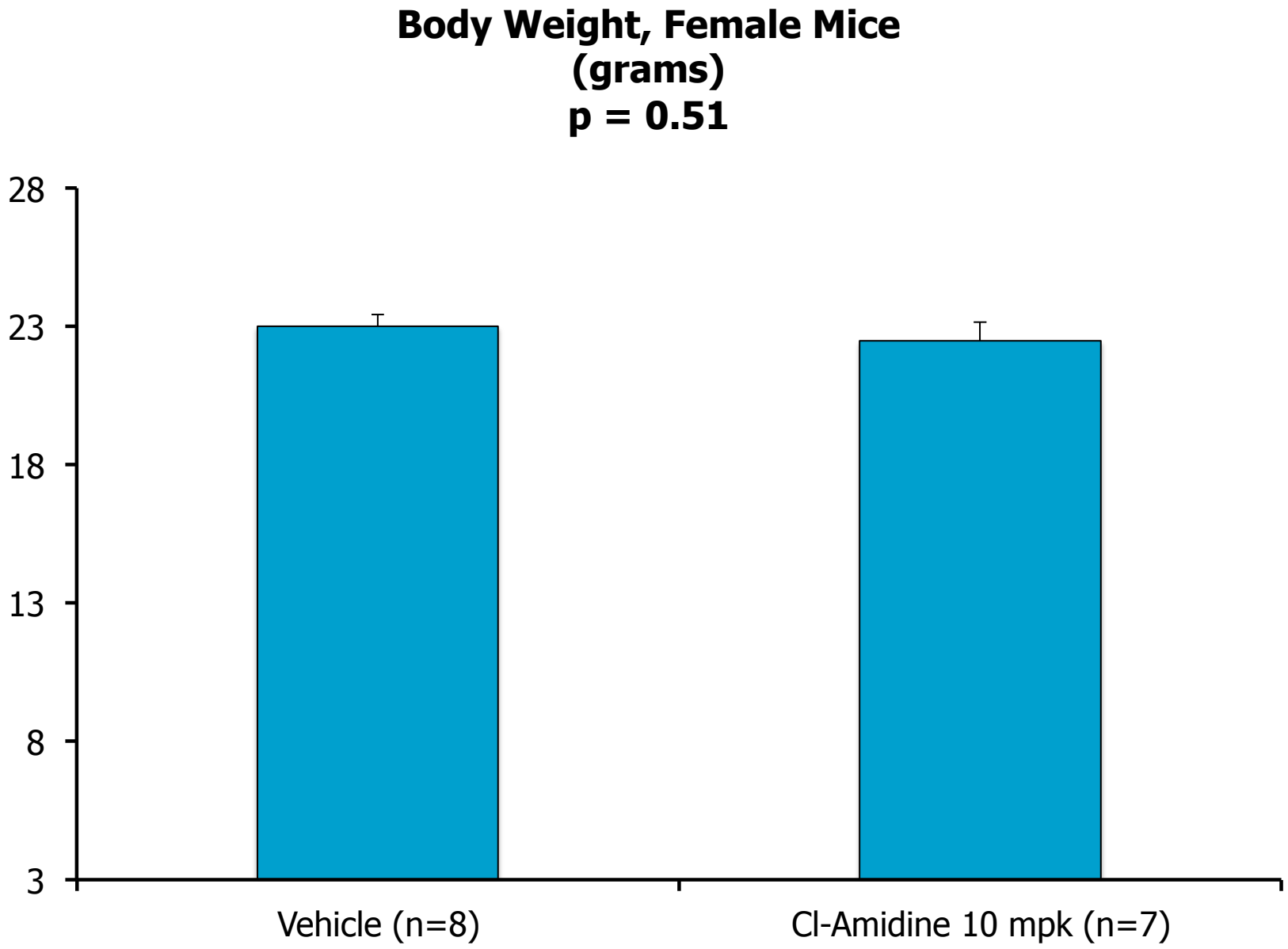
Towler lab, unpublished

The PADI4 Inhibitor Cl-Amidine Does Not Reduce Fasting Plasma Cholesterol Levels Female LDLR-/- Mice Fed High Fat Diabetogenic Diets



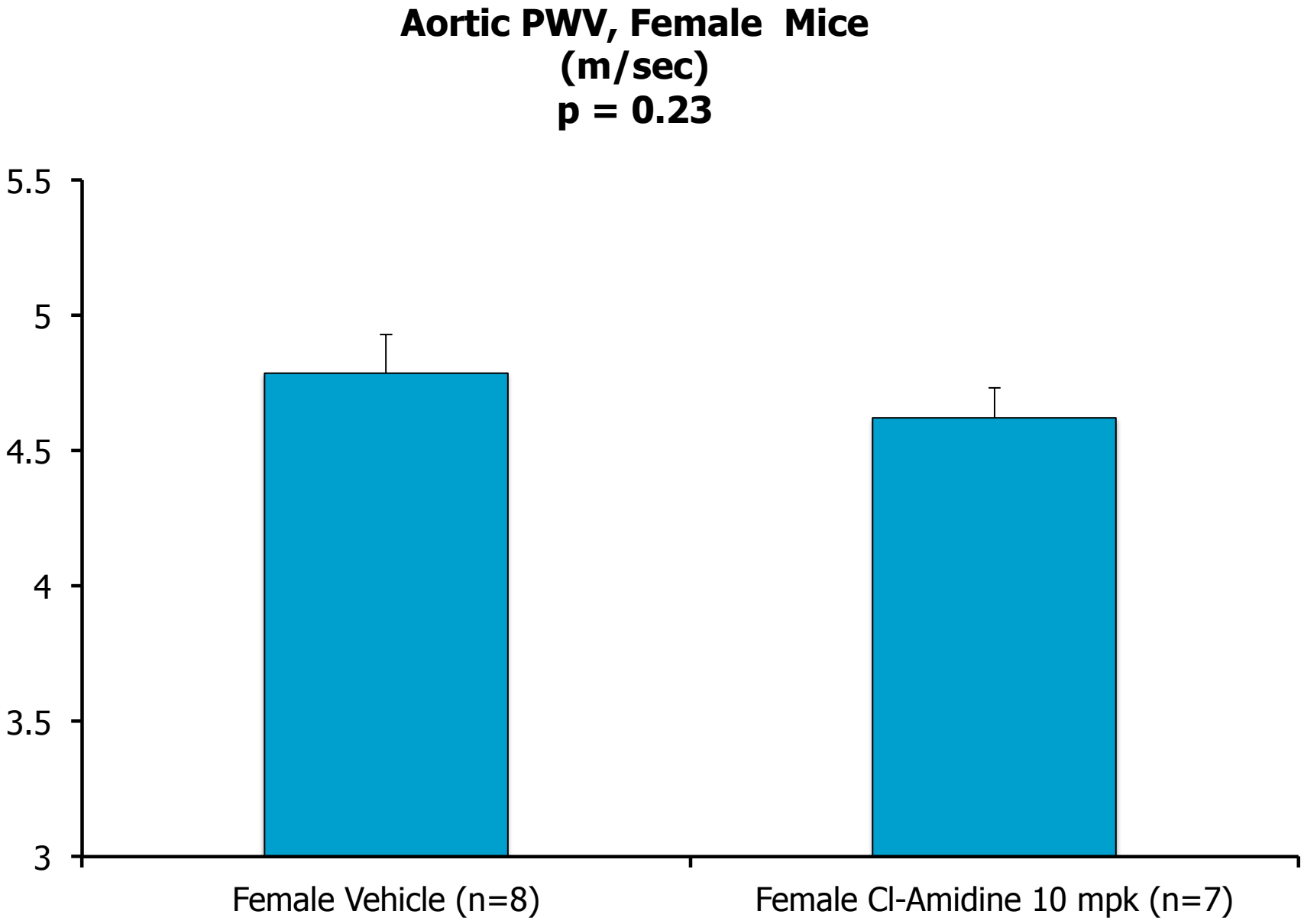
Towler lab, unpublished

The PADI4 Inhibitor Cl-Amidine Has No Impact On Body Weight In Female LDLR-/- Mice Fed High Fat Diabetogenic Diets



Towler lab, unpublished

The PADI4 Inhibitor Cl-Amidine Did Not Significantly Reduce Aortic PWV In Female LDLR-/- Mice Fed High Fat Diabetogenic Diets



Towler lab, unpublished

Conclusions

- The broad specificity PADI inhibitor Cl-Amidine significantly reduces arterial calcification in male LDLR-/- fed high fat diabetogenic diets
- This parallels non-significant trends for reduction in insulin resistance and fasting serum triglyceride levels
- These responses were not observed in female LDLR-/- mice
- Cl-Amidine treatment of female LDLR-/- mice fed high fat diabetogenic diets did not improve arterial calcification or metabolic profiles
- Moreover, in both sexes, arterial pulse wave velocity (PWV) was unaltered by Cl-Amidine treatment
- Consistent with the expression of PADI4 in the pancreas, PADI inhibition with Cl-Amidine exerted a non-significant trend for reduced insulin resistance and fasting triglyceride levels in male LDLR-/- mice fed high fat diabetogenic diets
- The cardiometabolic benefits of PADI inhibition is very likely to differ between male and female mice

Towler lab, unpublished

Ongoing Studies

- Assessment of arterial geometry or fibrosis
  - Does Cl-Amidine impact wall thickness/ radius ratio?

$$PWV = \sqrt{\frac{E \times \text{Wall thickness}}{2 \times \text{radius} \times \text{blood density}}}$$

- More selective Inhibition Of PADI4 Signaling In Diet-Induced Diabetes and Arteriosclerosis
  - Pharmacology
    - GSK484, PADI4-directed antisense oligos
  - Molecular genetics
    - Conditional knockout of PADI4
      - Vascular smooth muscle lineage
        - ADA 1-18-IBS-224
      - Myeloid lineage
      - Pancreatic islet cell lineage