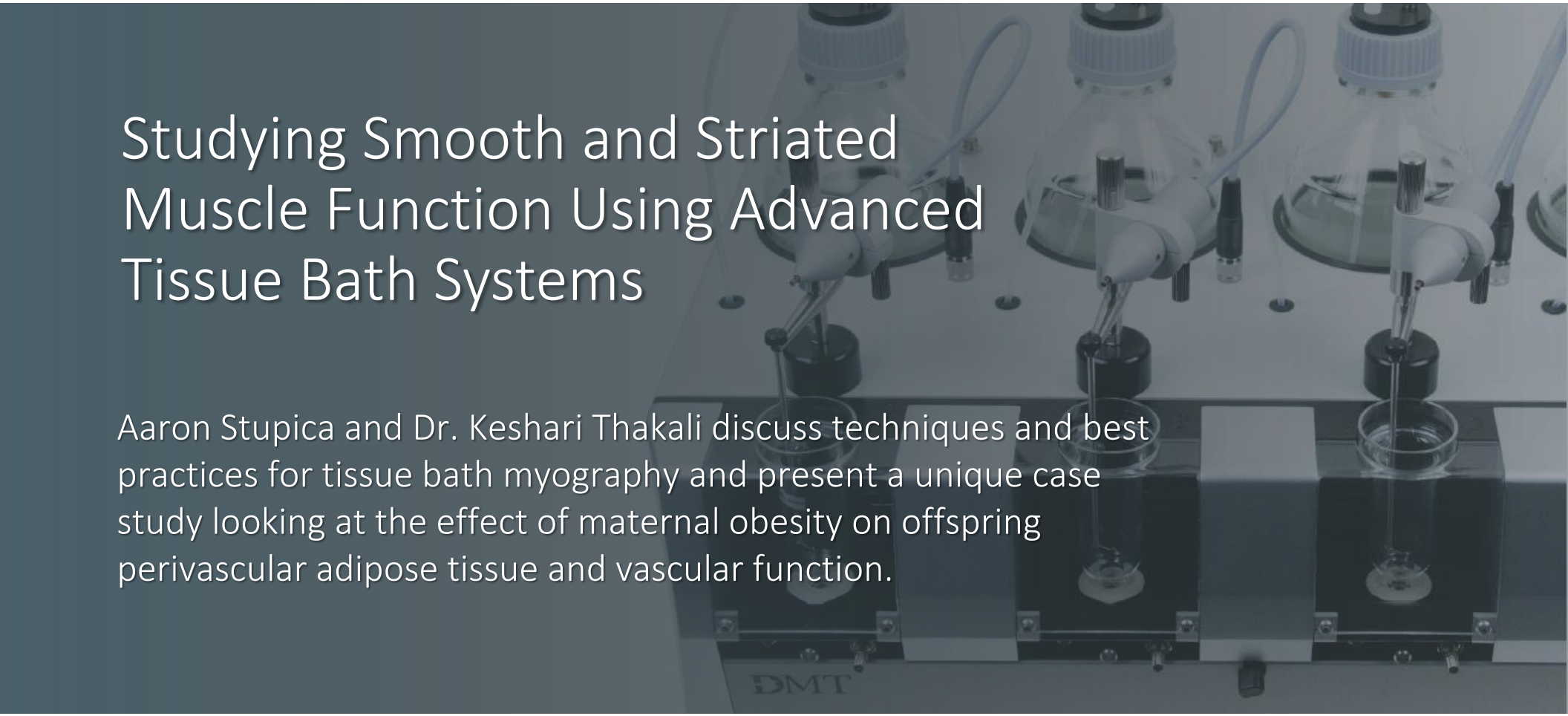
Studying Smooth and Striated Muscle Function Using Advanced Tissue Bath Systems



Aaron Stupica and Dr. Keshari Thakali discuss techniques and best practices for tissue bath myography and present a unique case study looking at the effect of maternal obesity on offspring perivascular adipose tissue and vascular function.

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Studying Smooth and Striated Muscle Function Using Advanced Tissue Bath Systems

Keshari Thakali, PhD
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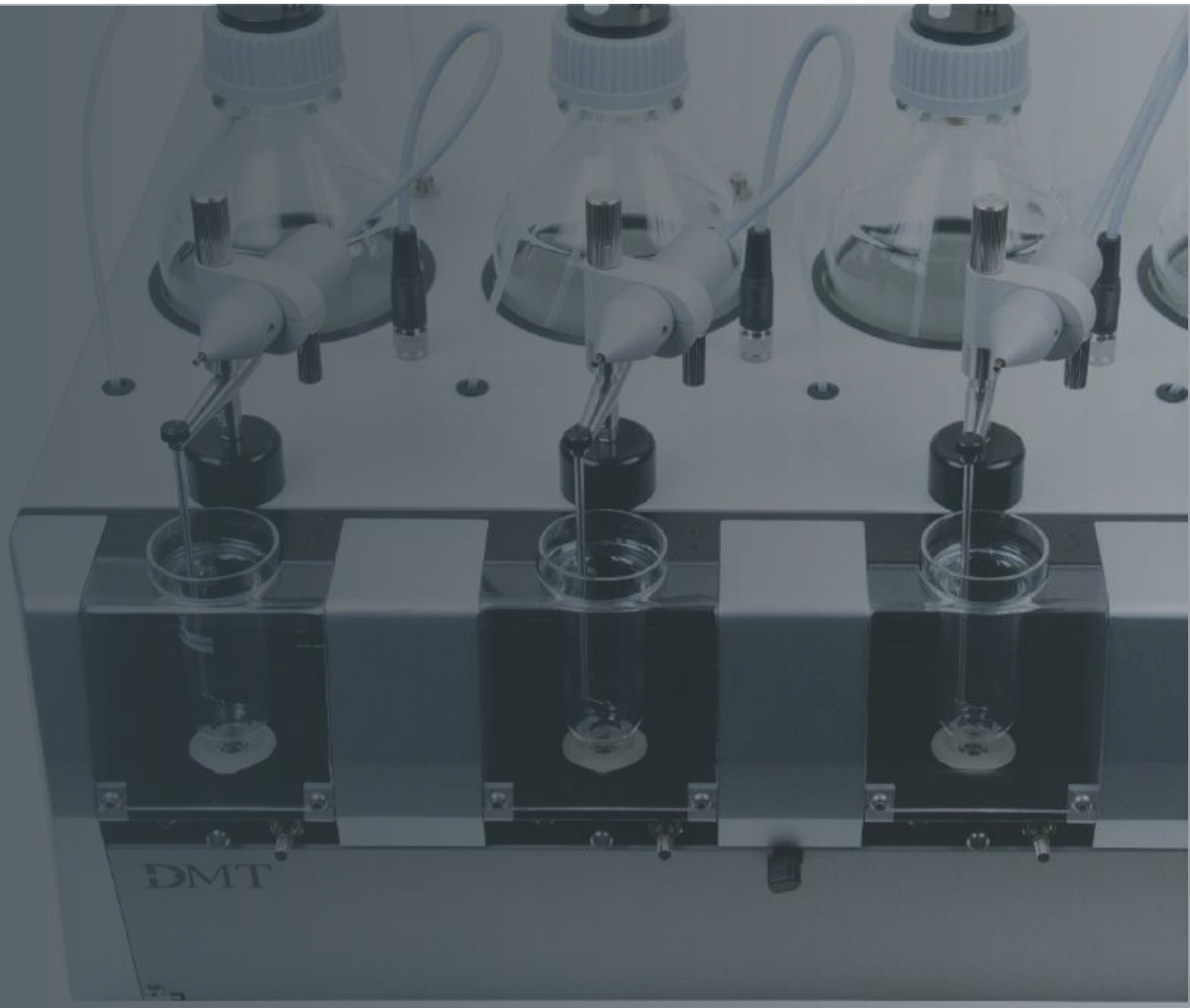

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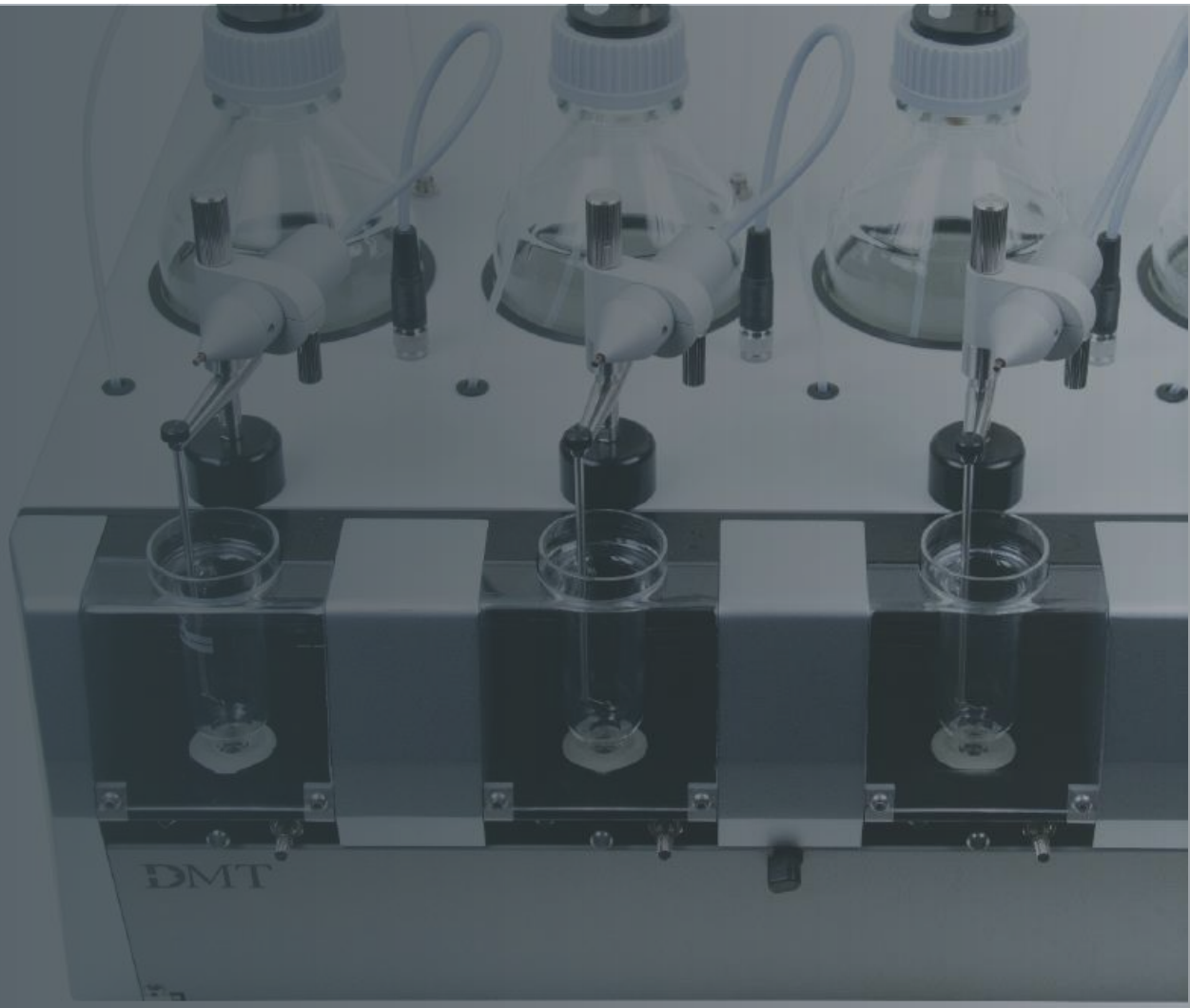

An Overview of Organ Bath Studies and Systems

Aaron J. Stupica

Scientific Product Specialist,
DMT-USA, Inc.
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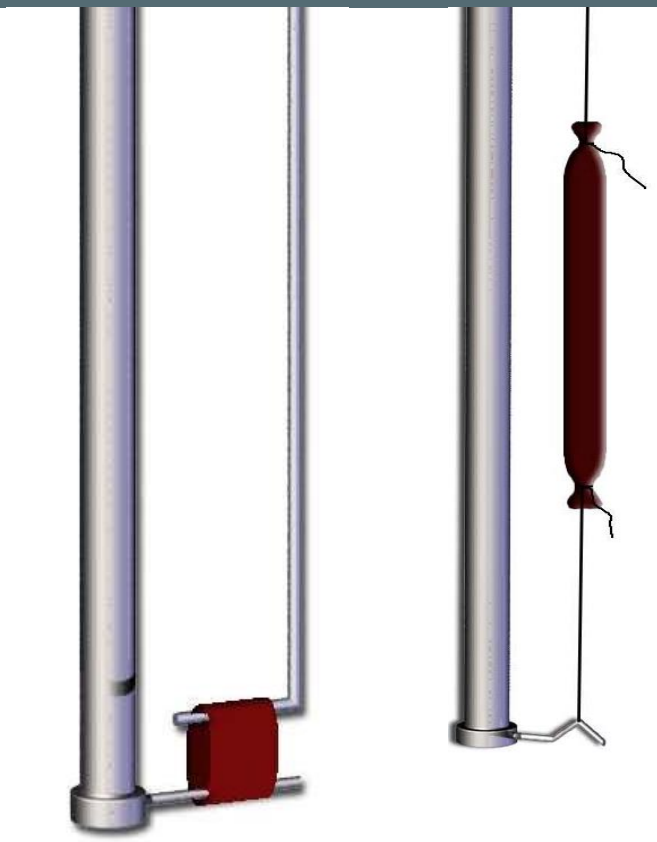
#ORGAN BATH STUDIES

- **Primarily used to perform in-vitro, high throughput studies in response to stimulus**
- **Tissues:**
  - **Large vessels (aorta, arteries, trachea)**
  - **Skeletal muscle strips (ileum, colon, gastric antral, sphincter, bladder strips, penile muscle)**
  - **Cardiac muscle tissue (papillary)**
- **Stimuli:**
  - **Drug agonists**
  - **Electrical stimulation**



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#ORGAN BATH SYSTEMS

BENEFITS

- HIGH THROUGHPUT
- EASILY REPEATABLE
- DRUG TESTING
- SIDE BY SIDE COMPARISONS
- ISOLATED PHARMACOLOGICAL RESPONSES

DRAWBACKS

- LIMITED EXPERIMENTS
- ORGAN BATHS CAN TAKE UP SIGNIFICANT LAB SPACE
- DOUBLE-JACKETED HEATING
- DIFFICULT TO AUTOMATE

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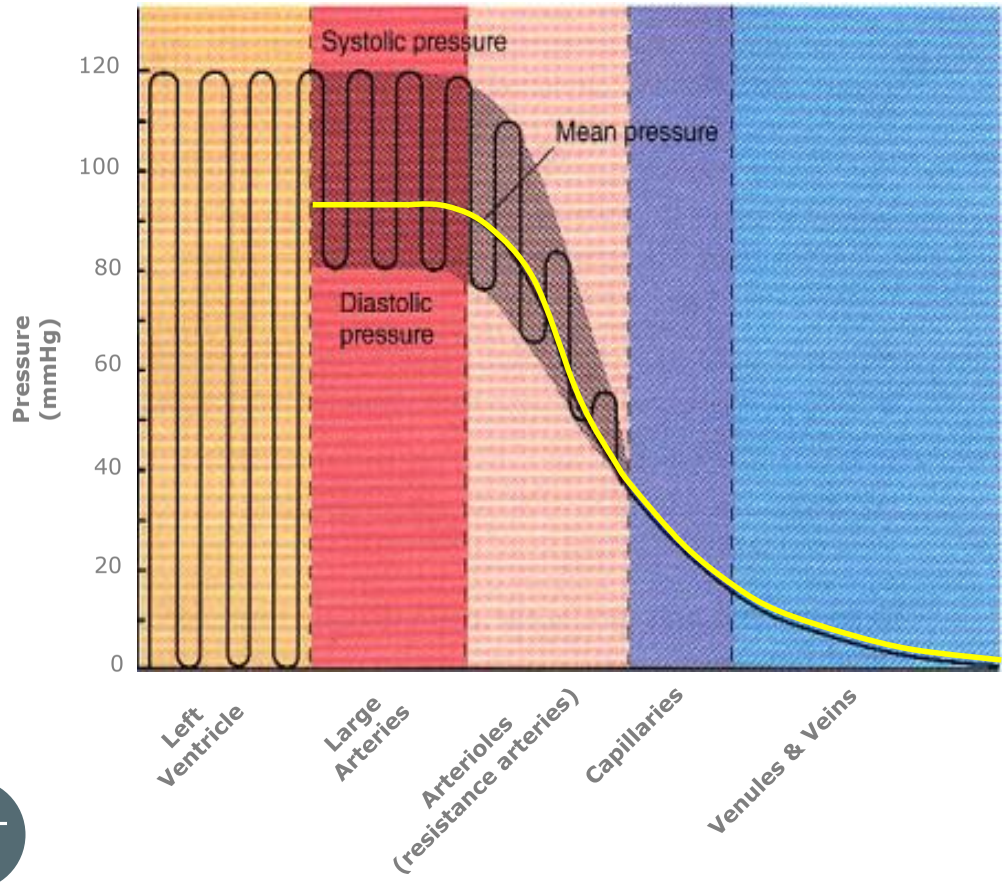
#ARTERIAL PRESSURE



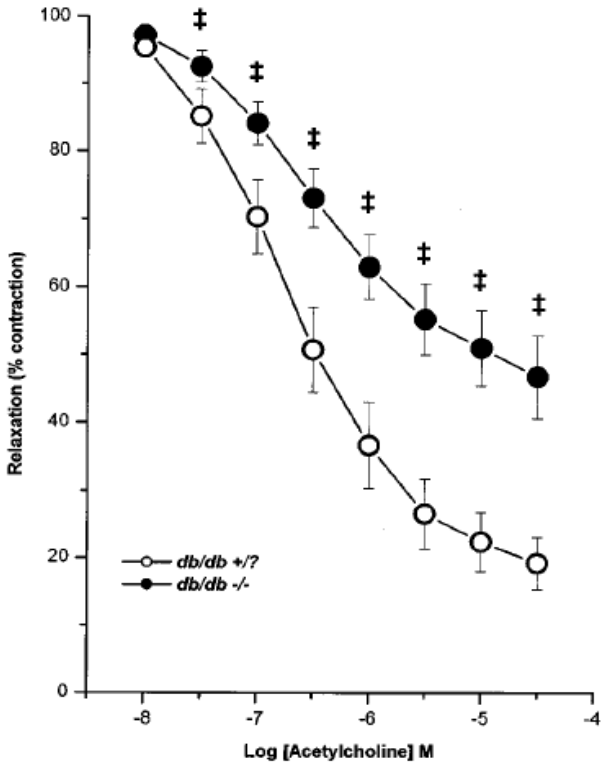
- **Left ventricle = driving force**
- **Highest in the aorta**

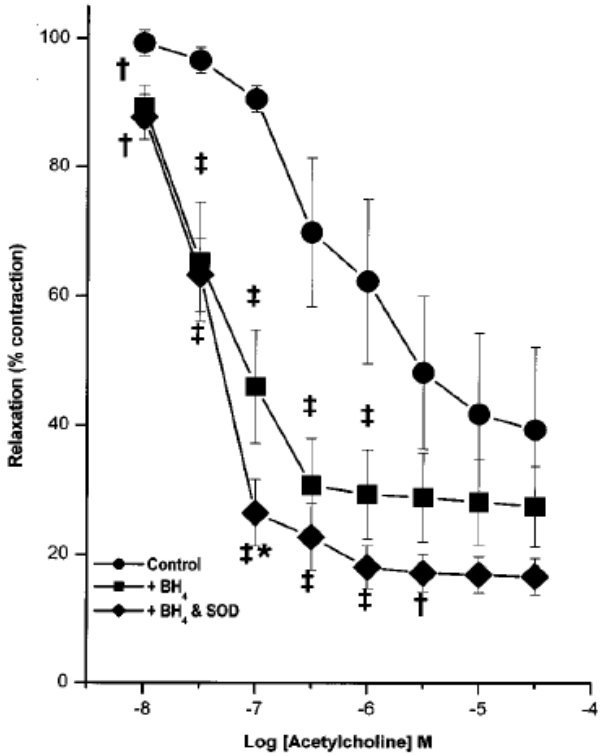
#WIRE MYOGRAPH (620M)

- **VESSELS MOUNTED ON WIRE**
  - Stainless steel (40µm)
  - Tungsten (10µm, 15µm or 25µm)
- **SMALL BATH VOLUMES**
  - 2.5ml-6ml

Click to learn more about the 620M

#ORGAN BATH STUDIES





**Adapted from Pannirselvam M, Verma S, et al. Br J Pharmacol. (2002) 136:255-263.*

#ORGAN BATH SYSTEMS

NECESSARY COMPONENTS:

1. FORCE TRANSDUCER
2. POSITIONER
3. EASY MOUNTING ACCESS
4. EASY ACCESS TO ADD AGONISTS
5. ABILITY TO WASHOUT BATH

#ORGAN BATH SYSTEMS

ORGAN BATH SHORTCOMINGS:

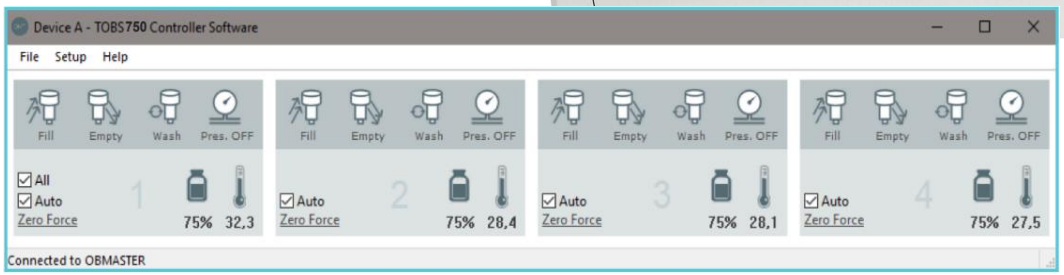
- BREAK EASILY
- DIFFICULT TO REPLACE PARTS
- LONG CLEANING PROCEDURES
- FILLING/EMPTYING BY HAND
- CUSTOM ELECTRICAL STIMULATION
- MAINTENANCE
- ADDITIONAL STEPS IN DATA ACQUISITION
- NECESSITY TO PHYSICALLY CHANGE TRANSDUCERS FOR DIFFERENT TISSUES

#DMT 750TOBS TISSUE ORGAN BATH

DMT ORGAN BATH SYSTEM

- RAPID AIR HEATING
- SINGLE WALLED GLASS CHAMBERS
- COMPUTER-AUTOMATED FILLING/EMPTYING- CONSISTENT VOLUME LEVELS
  - MULTIPLE SYSTEMS CAN BE LINKED
- 1/4TH SPACE OF TRADITIONAL ORGAN BATH
- QUICK CHAMBER AND TISSUE MOUNT REPLACEMENT
- PROGRAMABLE FORCE TRANSDUCERS- ISOMETRIC AND ISOTONIC

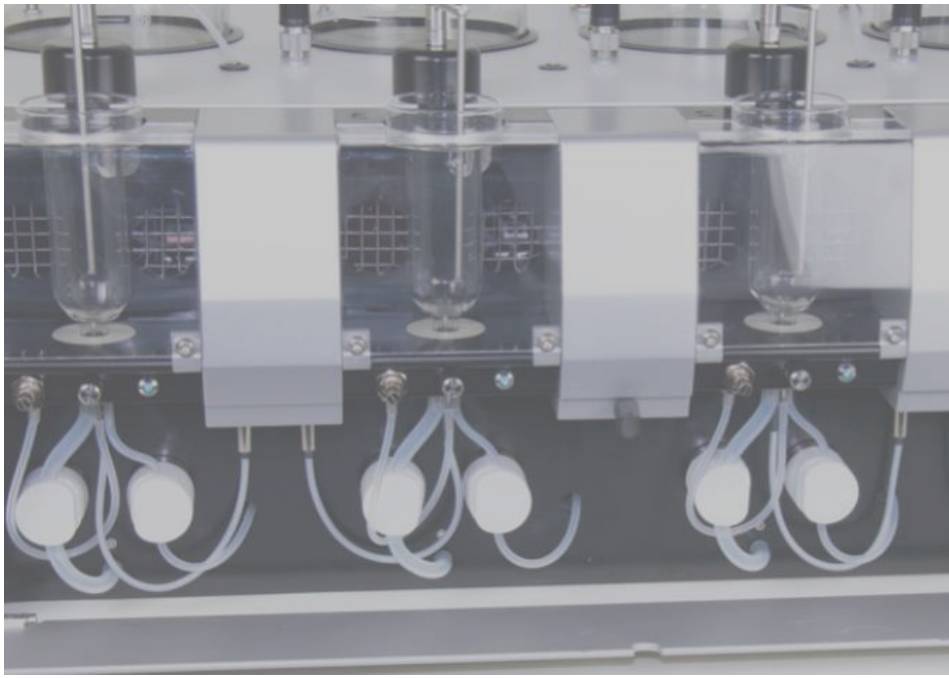
Click to learn more about the 750TOBS



#DMT 750TOBS TISSUE ORGAN BATH

DMT ORGAN BATH SYSTEM

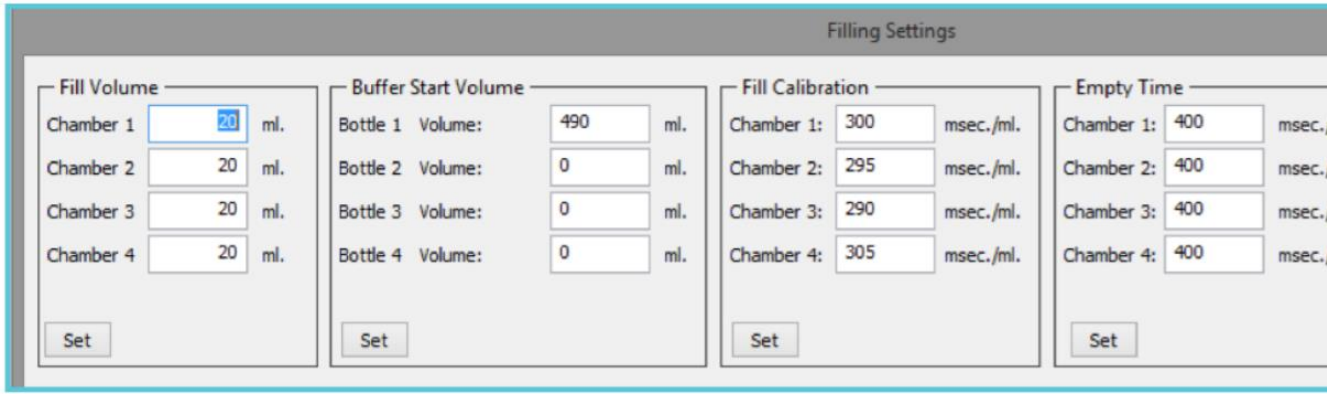
- MULTIPLE ELECTRODE OPTIONS
- AUTOMATED CLEANING
- MORE CONSISTENT/STREAMLINED EXPERIMENTS
- DATA GENERATION
- DURABLE FOR DAILY USE
- SECURE BUFFER HANDLING
- EASILY REPLACEABLE PARTS
- FEW UPKEEP/OPERATIONAL COSTS

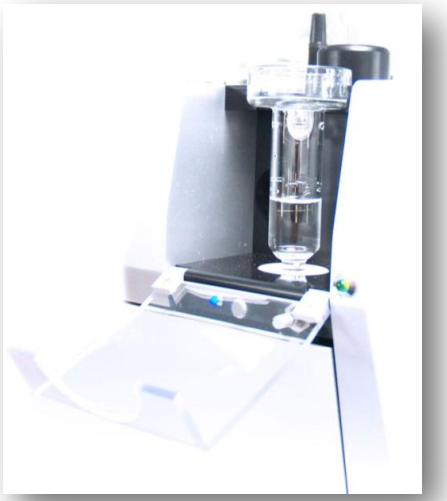


#750TOBS CHAMBER FEATURES

TOBS CHAMBERS

- EASY ACCESS FOR ADDING DRUGS
- 10, 20, 50mL OPTIONS
- CUSTOMIZED FILLING VOLUMES
- EASY SWAP
- AUTOCLAVABLE



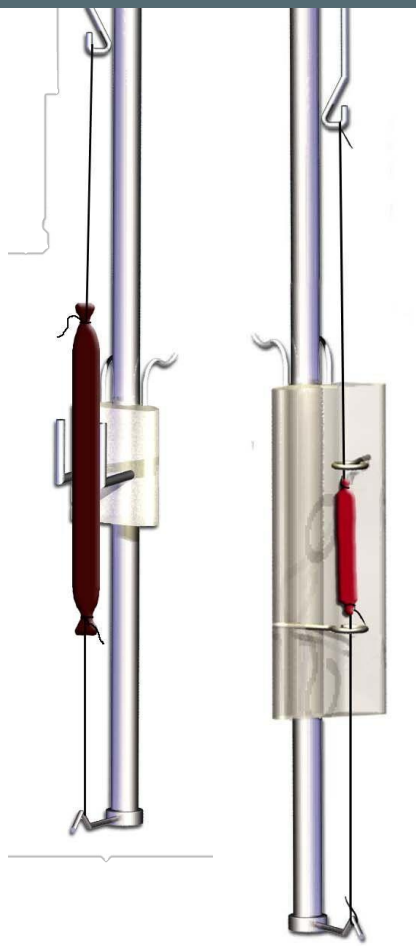


#ELECTRICAL STIMULATION

STIMULATION OPTIONS FOR TISSUE ORGAN BATHS:

- **DMT CS4/CS8**
  - COMPUTER CONTROLLED PROTOCOLS
- **OTHER STIMULATORS**

Click to learn more about DMT stimulators



#THE BASIC SETUP

Pressure Source-
Gas Tank or Bench-Top Air

Data Aquisition System
ADInstruments Labchart

DMT
750TOBS

#SUMMARY OF ORGAN BATH SYSTEMS

- DMT has redesigned the traditional organ bath system with developments in product design, generation of consistent results, and durability.
- System is adaptable for different tissues, varying forces, and stimulation options.
- Control software is minimal, but allows for customization and automated washes and cleaning.

Maternal Obesity Programming of Offspring Vascular Function

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Department of Pediatrics
University of Arkansas for Medical Sciences

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UNIVERSITY OF ARKANSAS FOR MEDICAL SCIENCES

Arkansas Children's
HOSPITALS · RESEARCH · FOUNDATION

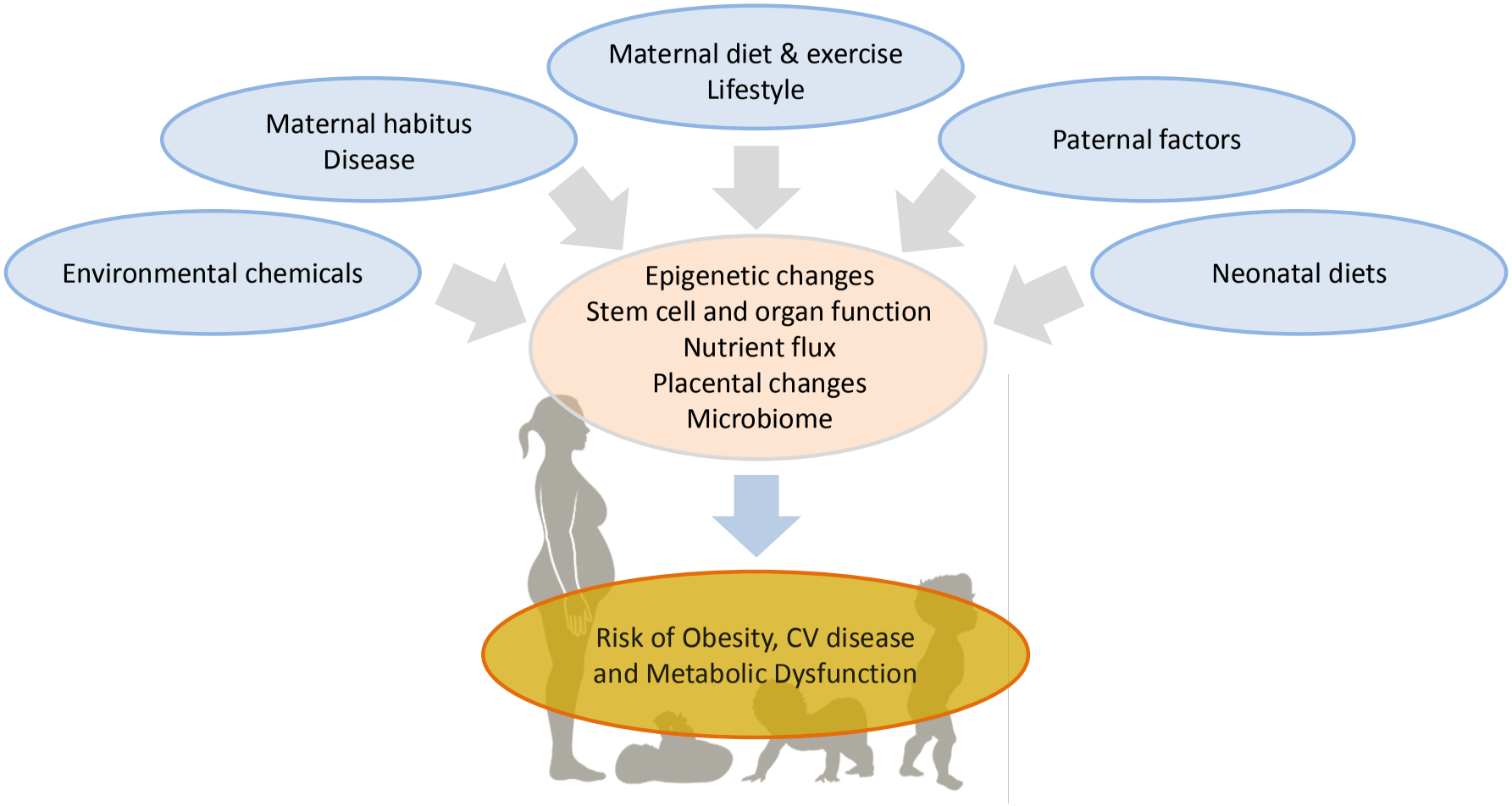
TODAY'S GOALS ARE TO DEMONSTRATE THAT:

1. Organ bath systems can be used to study the function of perivascular adipose tissue (PVAT)

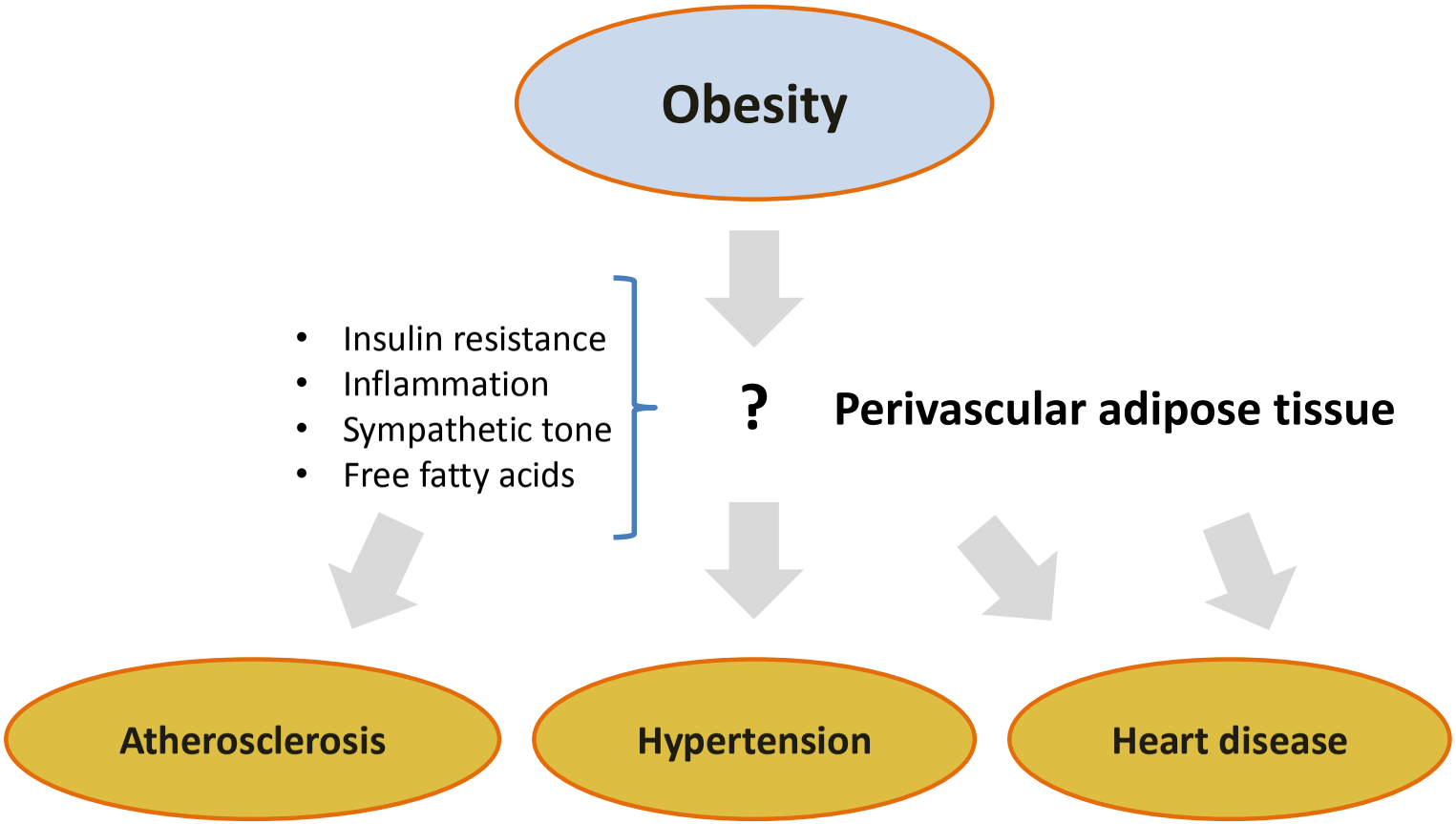
2. The adipose surrounding arteries (PVAT) regulates arterial function

3. Maternal obesity may program offspring vascular function

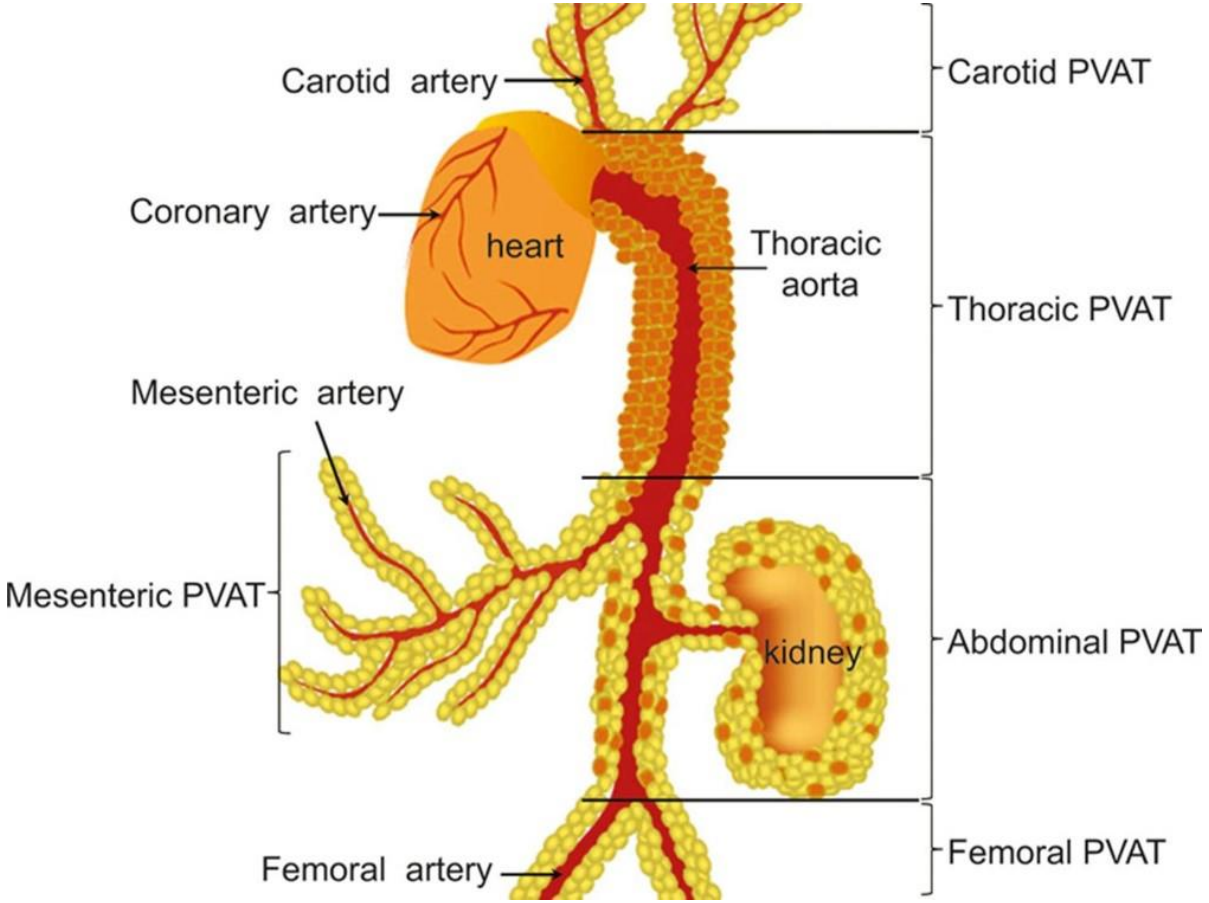
DEVELOPMENTAL PROGRAMMING



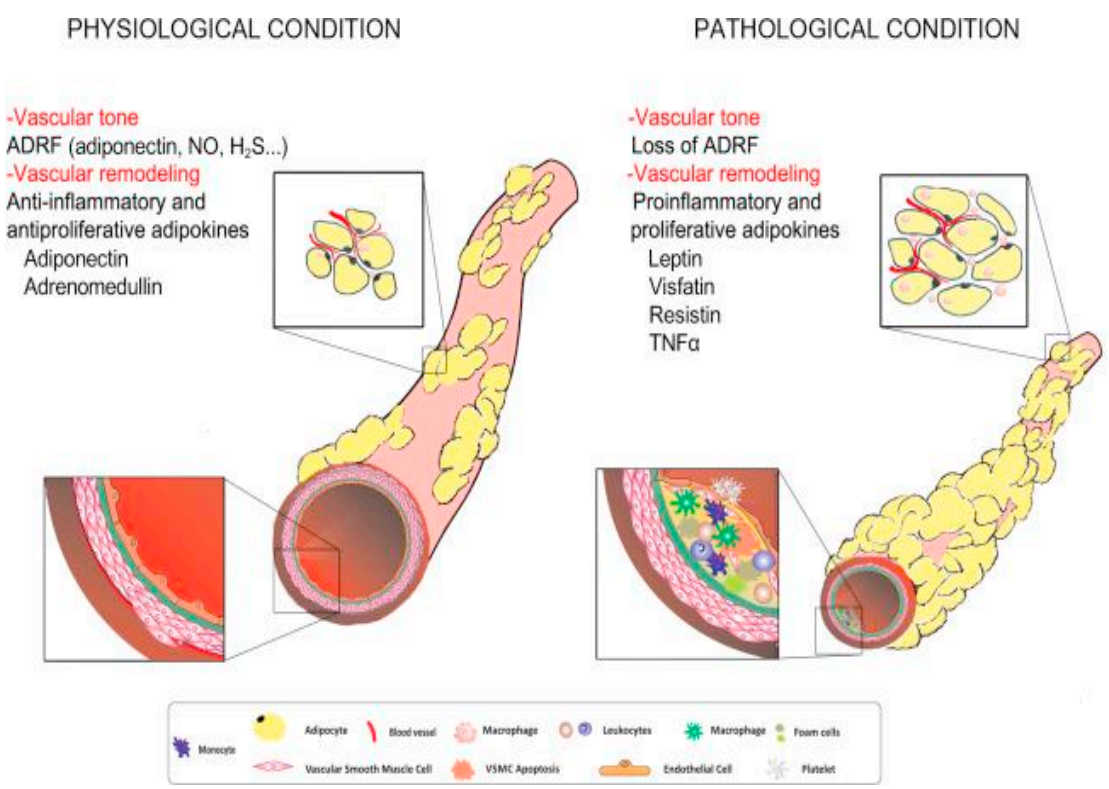
OBESITY AND CARDIOVASCULAR DISEASE

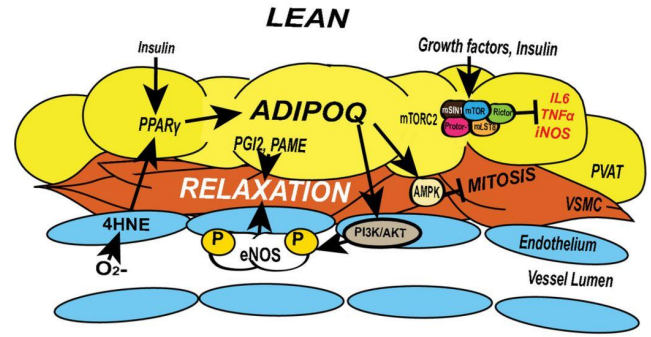


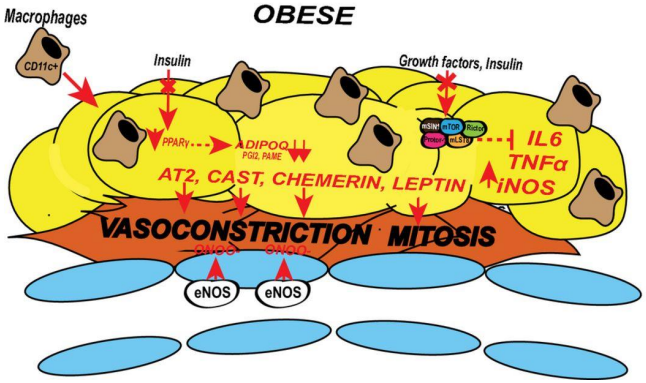
PERIVASCULAR ADIPOSE TISSUE (PVAT)



PVAT AND OBESITY







PERFORMING AN ISOMETRIC TENSION RECORDING EXPERIMENT:

1. Aortic tissue dissection and preparation
2. Placement of aortic helical strips in the organ bath
3. Pulling passive tension
4. Tissue Equilibration
5. Assess tissue viability
6. Experimental design
7. Data quantitation

PERFORMING AN ISOMETRIC TENSION RECORDING EXPERIMENT:

1. Aortic tissue dissection and preparation
   - Dissection dish with black silastic foundation
   - Cannulation wire
   - Physiological salt solution (PSS): (See next slide for composition)
   - Small vannas (iris) scissors and forceps
   - Silk suture to attach aorta to stationary metal rod and force transducer
2. Placement of aortic helical strips in the organ bath
   - Warmed (37° C)
   - Aerated: 95% O_2/5% CO_2 medical grade gas

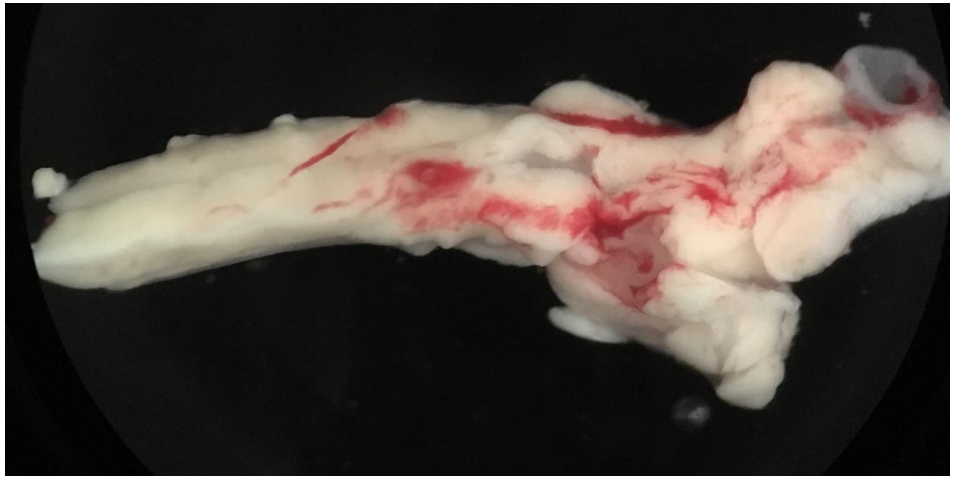
PHYSIOLOGICAL SALT SOLUTION (PSS) COMPOSITION:

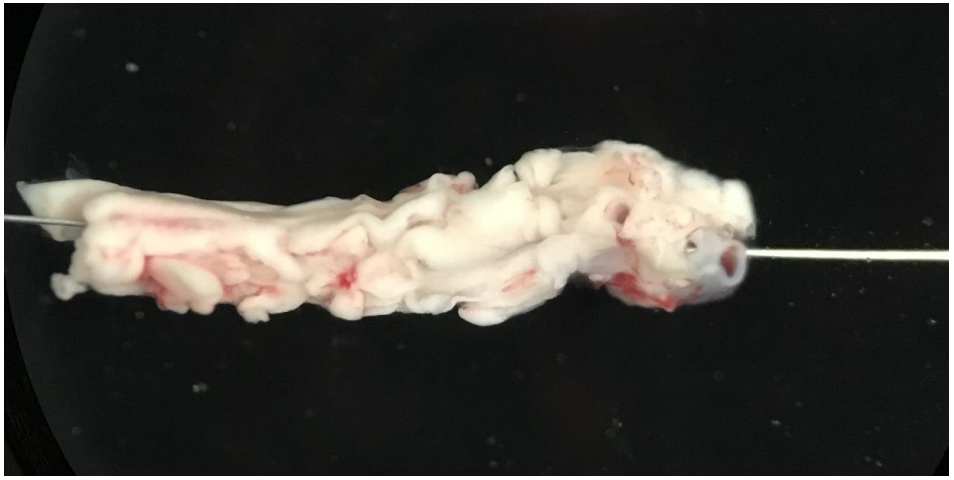
Chemical	Concentration (mM)
NaCl	130
KCl	4.7
$MgSO_4x7H_2O$	1.17
$NaHCO_3$	14.9
$CaNa_2EDTA$	0.03
KH_2PO_4	1.18
Glucose (Dextrose)	5.5
*$CaCl_2x2H_2O$	1.6

* Make a 1M solution, and add after mixing all other components and water

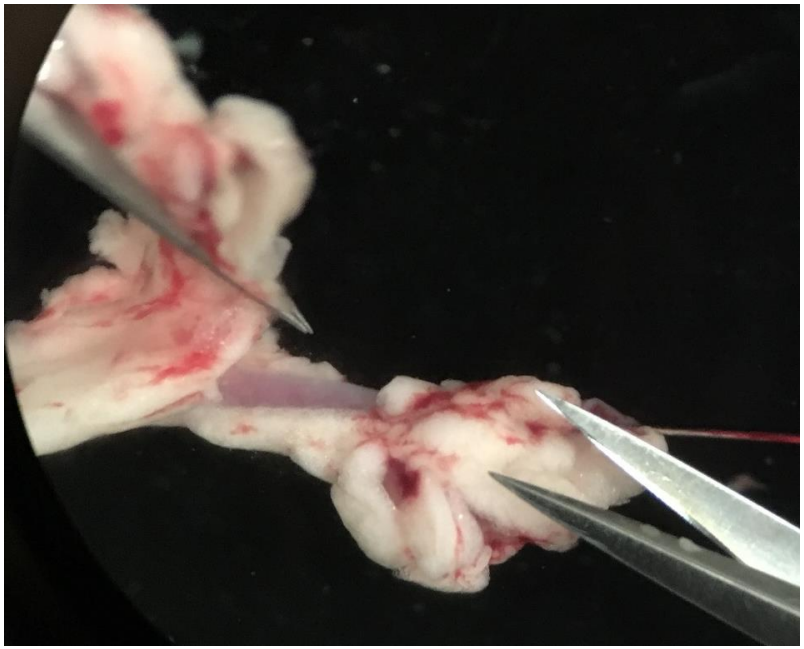
** pH 7.4 when warmed to 37°C and bubbled with 95% O_2/5% CO_2

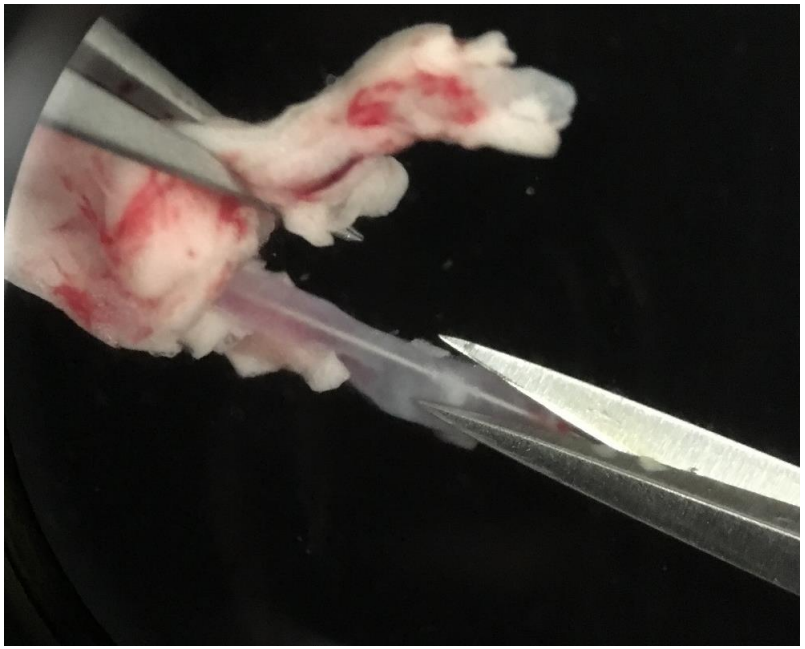
CANNULATING THE MOUSE AORTA





DISSECTING OFF PVAT

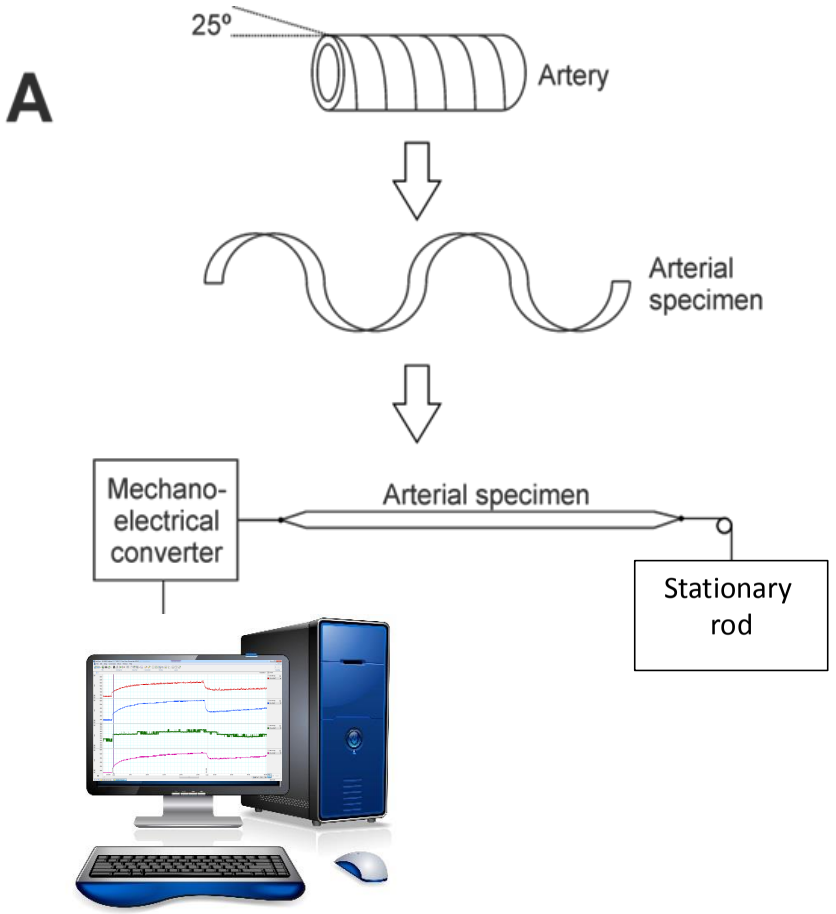




ISOMETRIC TENSION RECORDING :

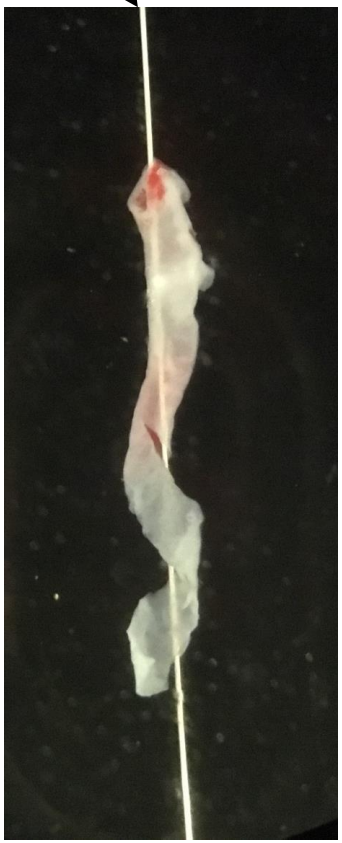
1. Aortic tissue dissection and preparation

2. Placement of aortic helical strips in the organ bath



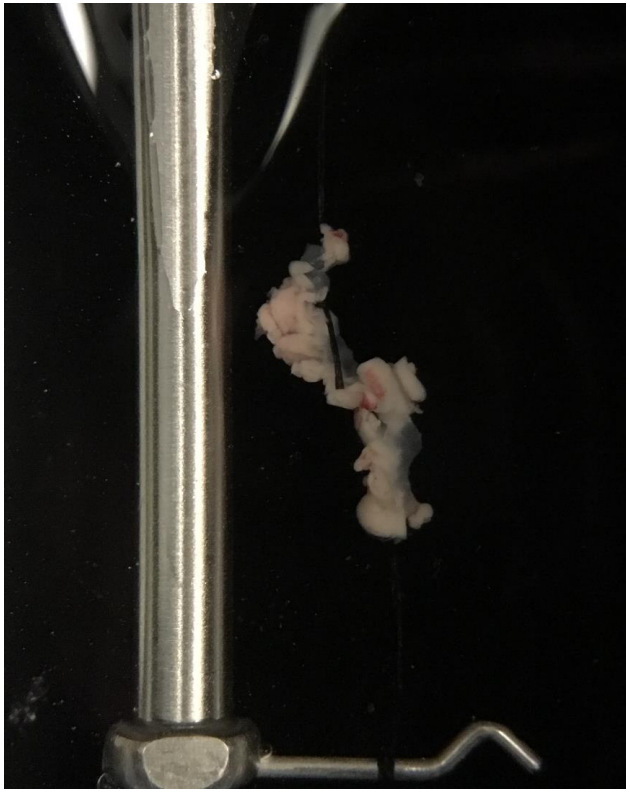
CUTTING HELICAL STRIPS

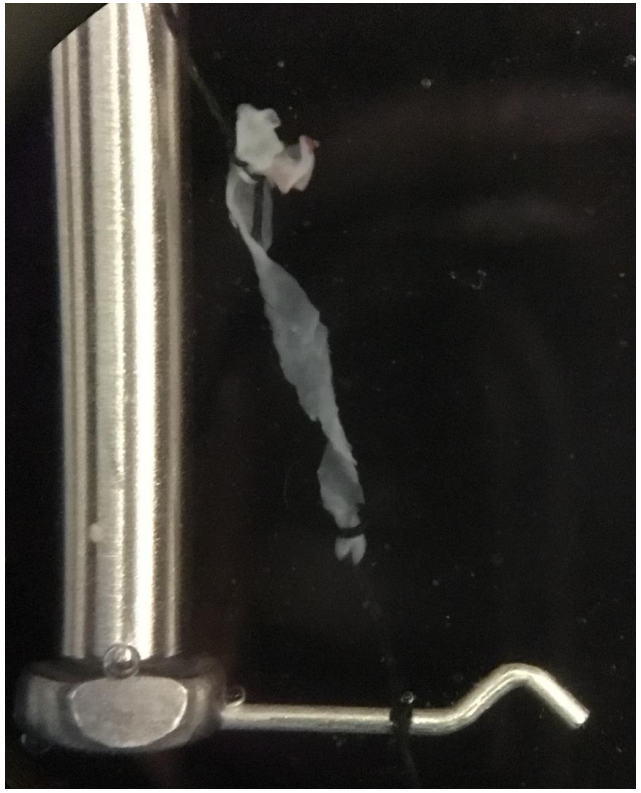
PVAT dissected off



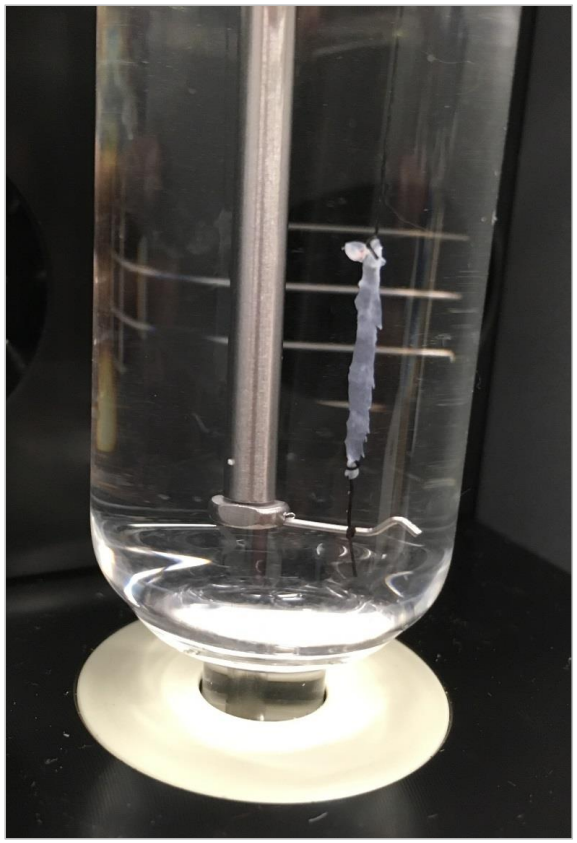
PVAT still attached

ATTACHING AORTIC STRIPS TO THE STATIONARY ROD



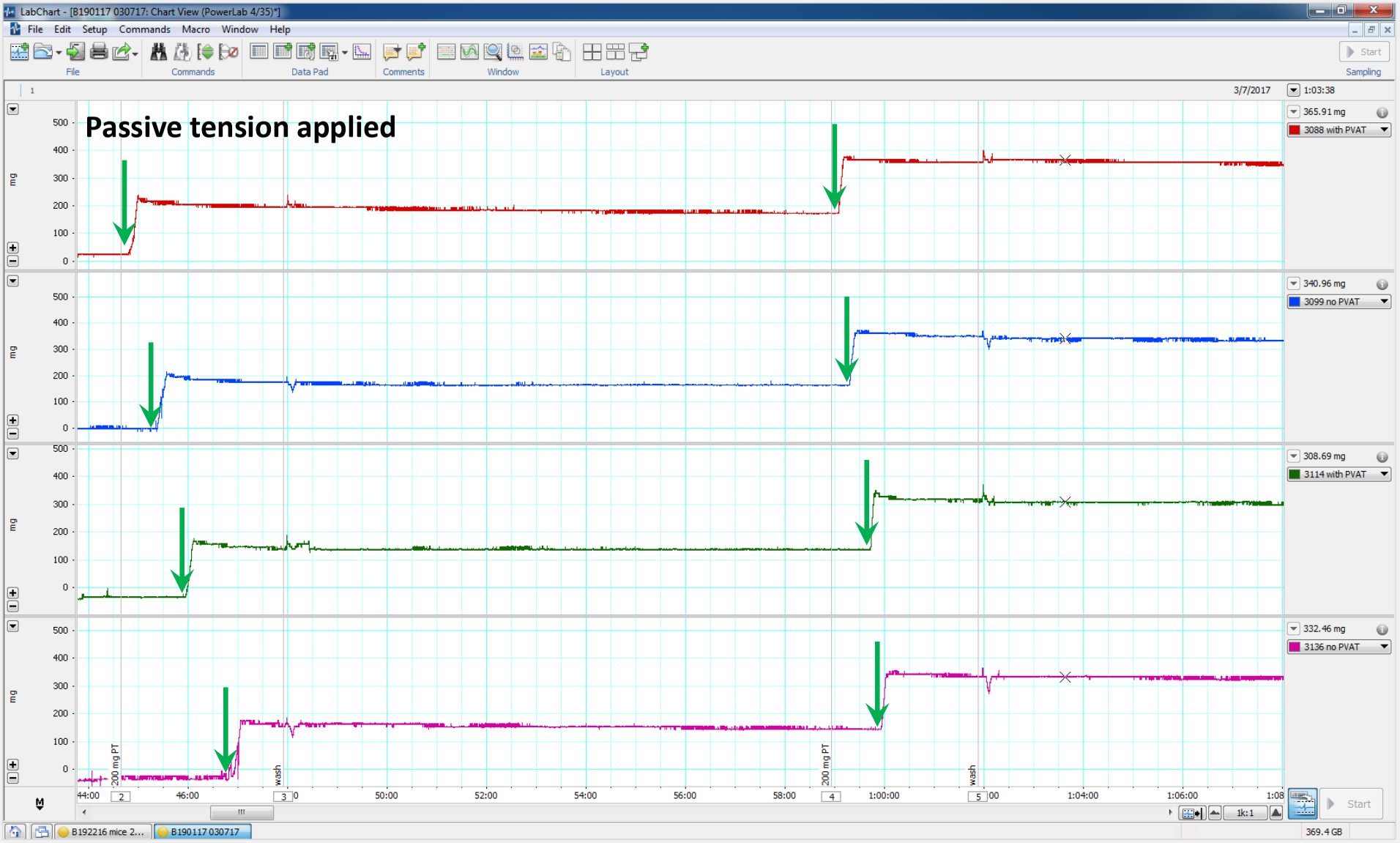


POSITIONING AORTIC STRIPS IN THE ORGAN CHAMBERS



PERFORMING AN ISOMETRIC TENSION RECORDING EXPERIMENT:

3. Pulling passive tension
   - Each tissue has a length (L_o) at which smooth muscle cells respond optimally
   - Preliminary experiments to determine the optimal stretching tension which achieves this length must be performed for each individual tissue type to be examined
   - Pull in 2 steps, note it is normal for the tissue to relax after pulling tension
4. Tissue equilibration
   - Equilibrate the tissue for 60 min after applying passive tension to the tissue.
   - During the equilibrium phase, wash the tissue every 15-20 min. Drain the tissue bath and replace the PSS with warmed PSS.


LabChart - [B190117 030717: Chart View (PowerLab 4/35)*]
File Edit Setup Commands Macro Window Help
Passive tension applied
3/7/2017
1:03:38
365.91 mg
3088 with PVAT
340.96 mg
3099 no PVAT
308.69 mg
3114 with PVAT
332.46 mg
3136 no PVAT
mg
200 mg PT
wash
200 mg PT
wash
44:00
46:00
50:00
52:00
54:00
56:00
58:00
1:00:00
1:04:00
1:06:00
1:08
Start
Sampling
B192216 mice 2...
B190117 030717
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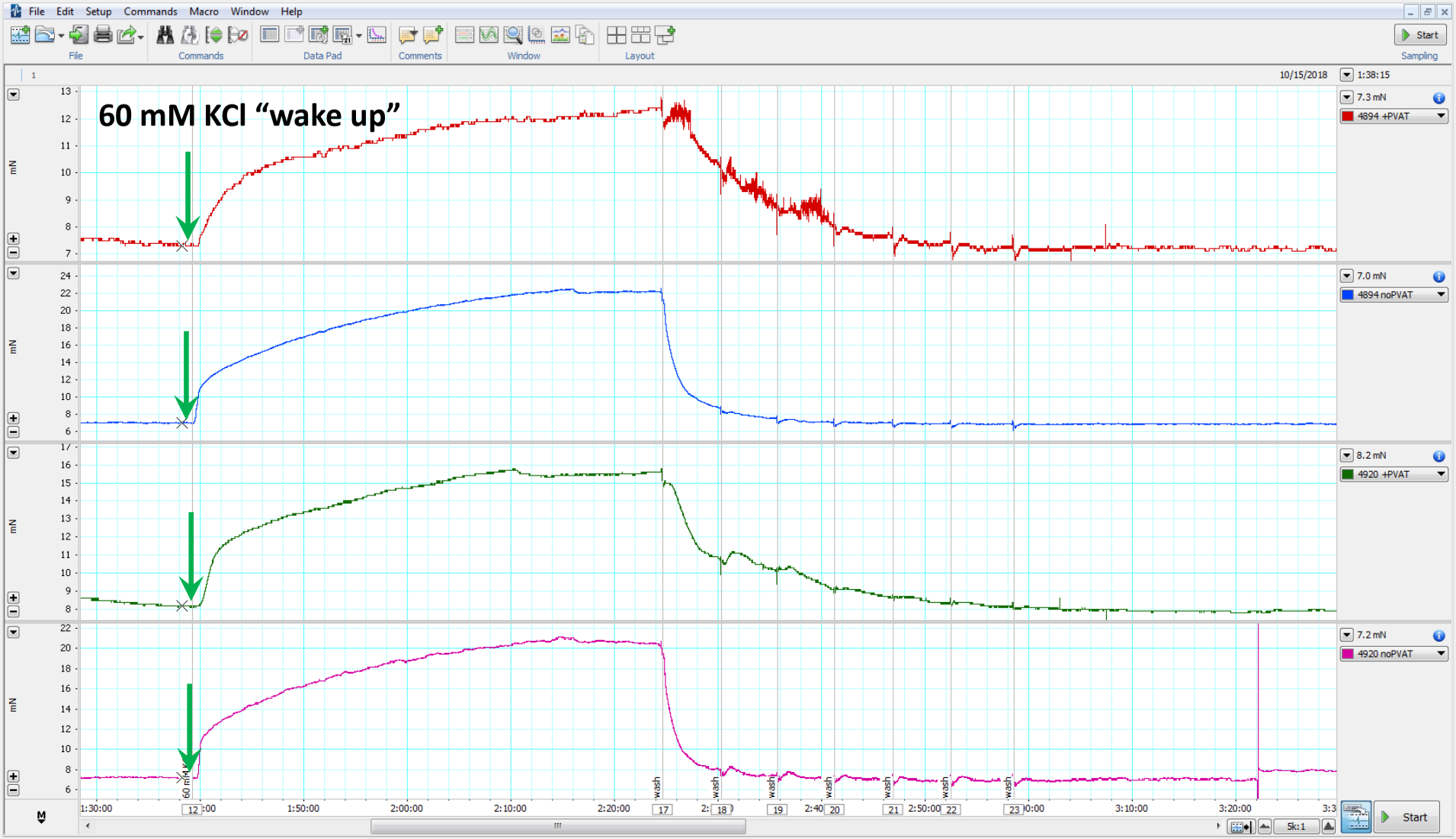
PERFORMING AN ISOMETRIC TENSION RECORDING EXPERIMENT :

5. Assess tissue viability
   - Pick an agonist (compound that will cause active contraction) to which the tissue responds


File Edit Setup Commands Macro Window Help
File
Commands
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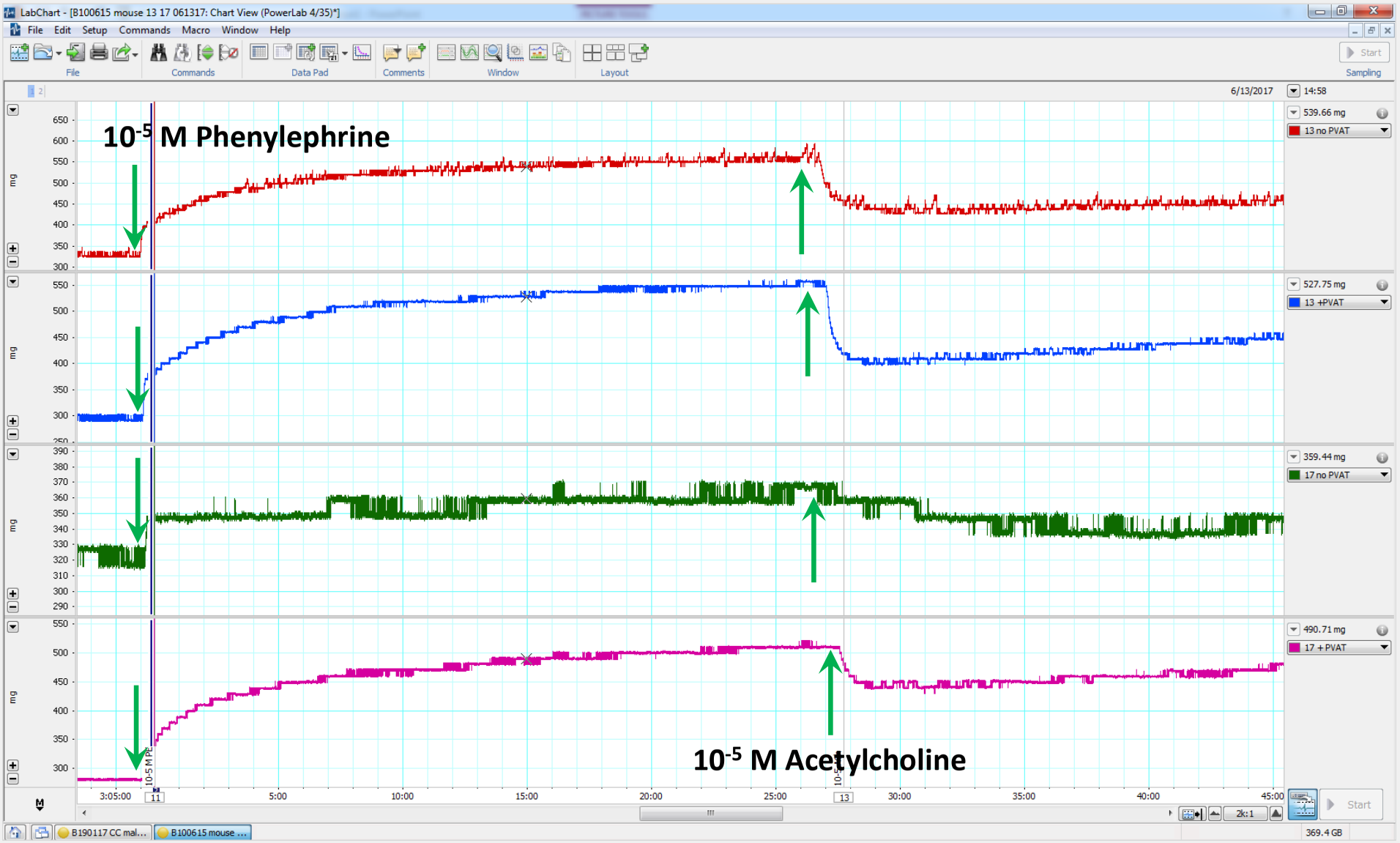
Start
Sampling
10/15/2018
1:38:15
60 mM KCl “wake up”
mN
7.3 mN
4894 +PVAT
7.0 mN
4894 noPVAT
8.2 mN
4920 +PVAT
7.2 mN
4920 noPVAT
60 mM K
wash
1:30:00
1:50:00
2:00:00
2:10:00
2:20:00
3:10:00
3:20:00
5k:1
Start

PERFORMING AN ISOMETRIC TENSION RECORDING EXPERIMENT :

5. Assess tissue viability

- Pick an agonist (compound that will cause active contraction) to which the tissue responds
- Perform the wake-up challenge by adding 10^{-5} M phenylephrine to the tissue bath. To achieve this concentration in 20 ml tissue bath, add 20 µl of a 10^{-2} M phenylephrine solution.
- NOTE: 20 µl into 20 ml of PSS is a 1/1,000 dilution, so the final concentration is 1,000x less than stock, or 10^{-5} M. Knowledge of volume and consistent maintenance of the volume within the tissue bath chamber is critical to determining the final drug concentration.
- Add 10^{-5} M phenylephrine to the tissue bath and allow the contraction to peak and then plateau, when the slope of the data becomes zero. Make sure to record each experimental event to aid post-experimental analysis.
- After plateau, wash the agonist out thoroughly by emptying and refilling the tissue bath chamber with new PSS. Make sure to record these events as well.
- NOTE: A rule of thumb is that the concentration of agonist in the bath is reduced 10 fold with each wash.
- Let the tissue rest at baseline tone for ~10 min before proceeding


File Edit Setup Commands Macro Window Help
File
Commands
Data Pad
Comments
Window
Layout
Start
Sampling
10/15/2018
1:38:15
60 mM KCl “wake up”
7.3 mN
4894 +PVAT
7.0 mN
4894 noPVAT
8.2 mN
4920 +PVAT
7.2 mN
4920 noPVAT
mN
Wash out
60 mM KCl
wash
1:30:00
1:50:00
2:00:00
2:10:00
2:20:00
2:40
2:50:00
3:10:00
3:20:00
5k:1
Start


LabChart - [B100615 mouse 13 17 061317: Chart View (PowerLab 4/35)*]
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File
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Comments
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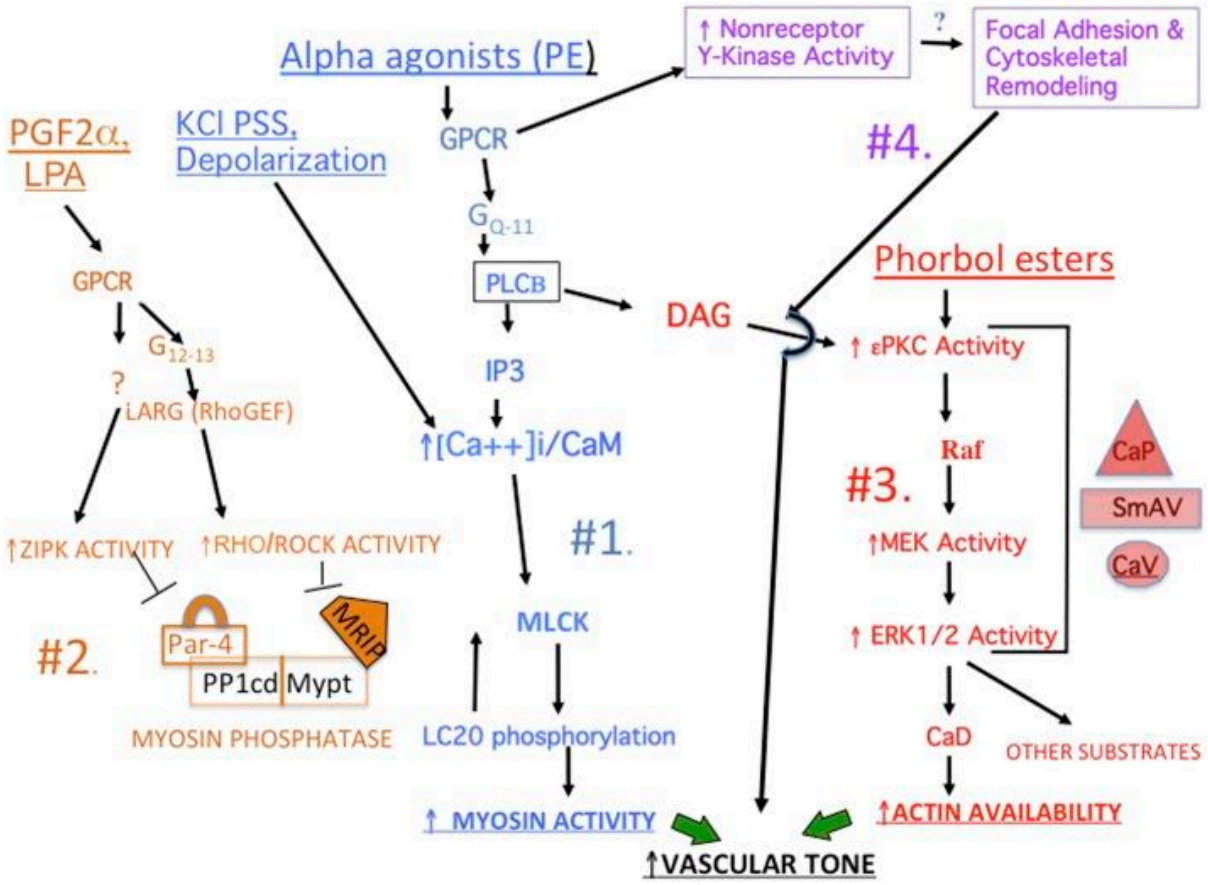
Start
Sampling
6/13/2017
14:58
10-5 M Phenylephrine
539.66 mg
13 no PVAT
527.75 mg
13 +PVAT
359.44 mg
17 no PVAT
490.71 mg
17 + PVAT
10-5 M Acetylcholine
mg
3:05:00
5:00
10:00
15:00
20:00
25:00
30:00
35:00
40:00
45:00
2k:1
Start
B190117 CC mal...
B100615 mouse ...
369.4 GB

STEPS TO PERFORMING AN ISOMETRIC TENSION RECORDING EXPERIMENT USING MOUSE THORACIC AORTA:

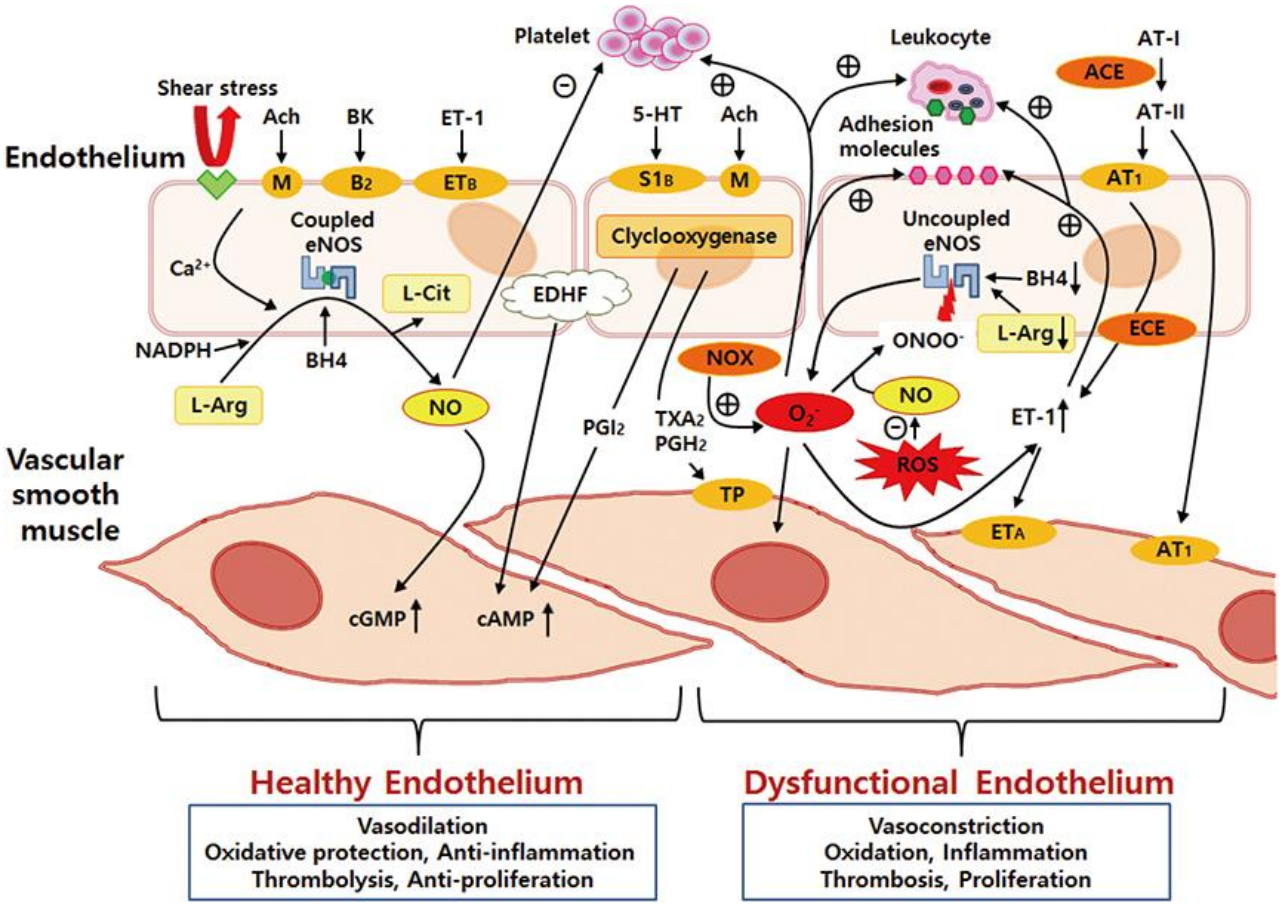
6. Experimental design

7. Data quantitation

PATHWAYS REGULATING VASCULAR TONE



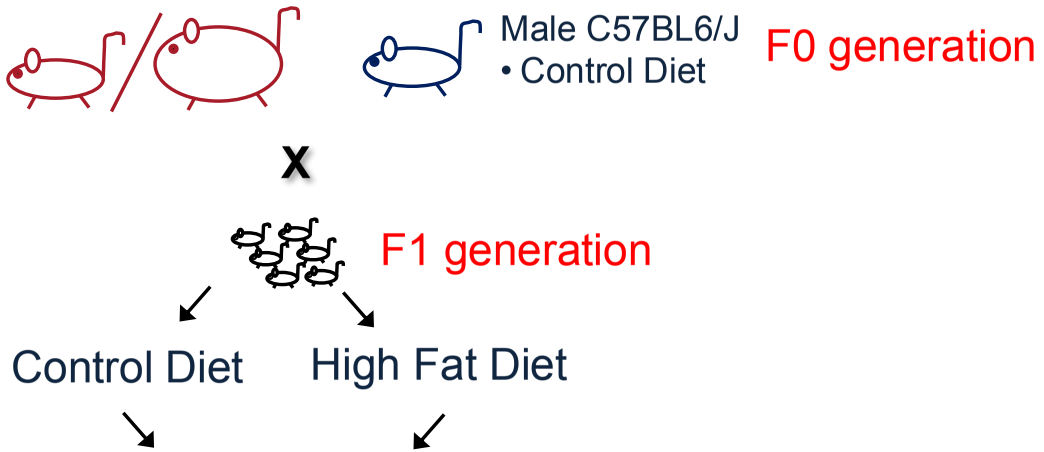
ENDOTHELIUM/SMOOTH MUSCLE SIGNALING



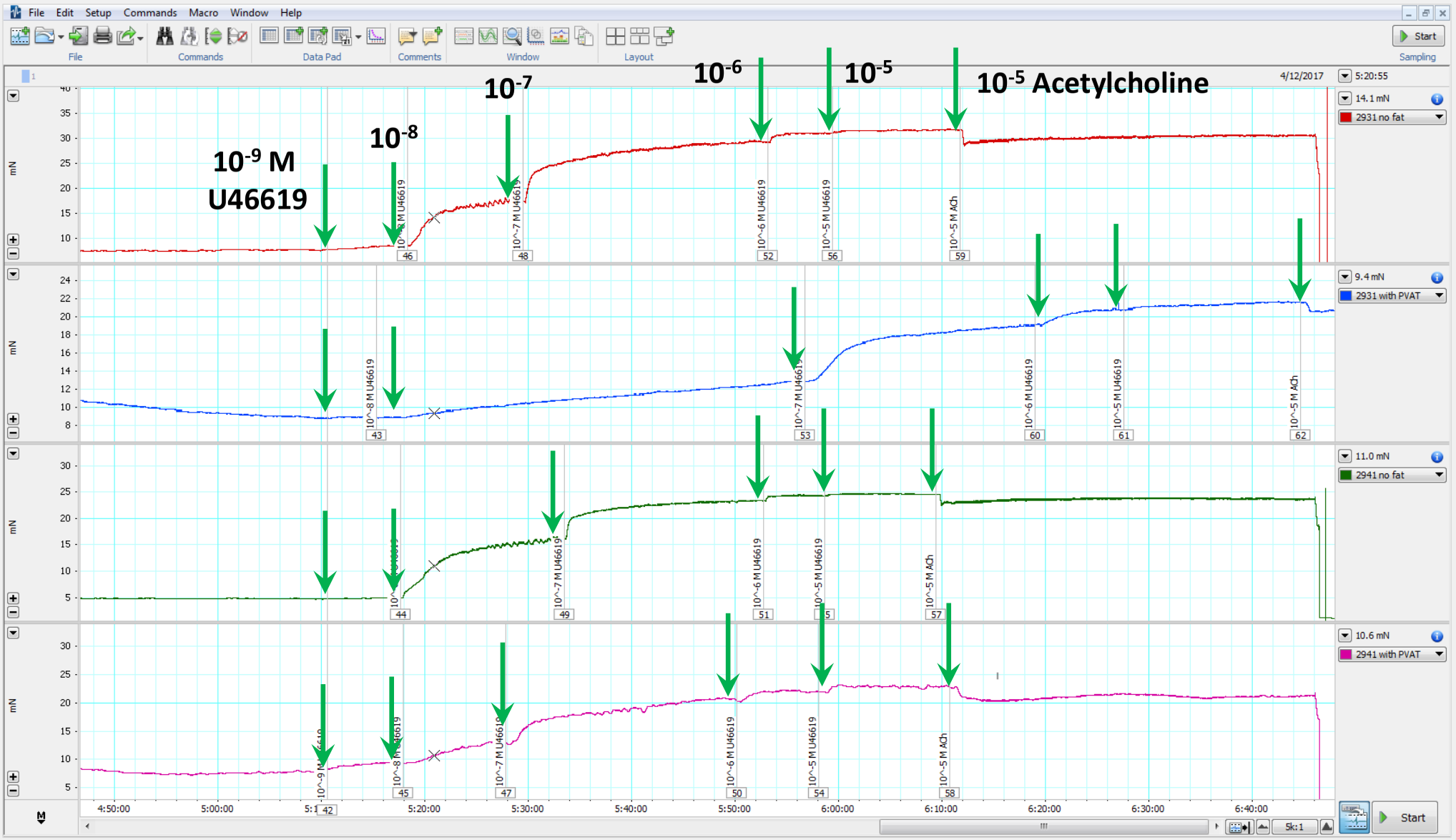
EXPERIMENTAL DESIGN FOR STUDYING PVAT FUNCTION

Female C57BL6/J

- Control Diet:
  - 17% calories from fat
- High Fat Diet (HFD):
  - 45% calories from fat
- On diets for 12 weeks



- Four groups of offspring (for each sex): CC, CH, HC, HH
- Contractility of thoracic aorta ± PVAT
- Questions:
  1. Is PVAT anti-contractile in our model?
  2. Does maternal HFD program smooth muscle, endothelial cell, or PVAT function?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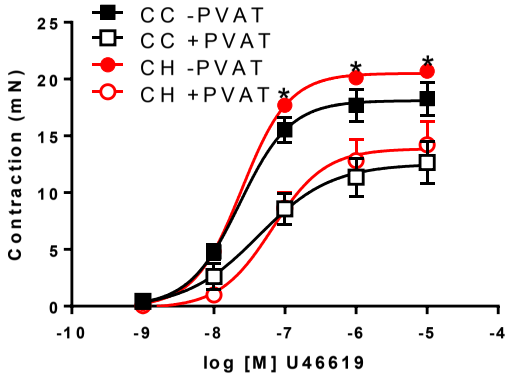
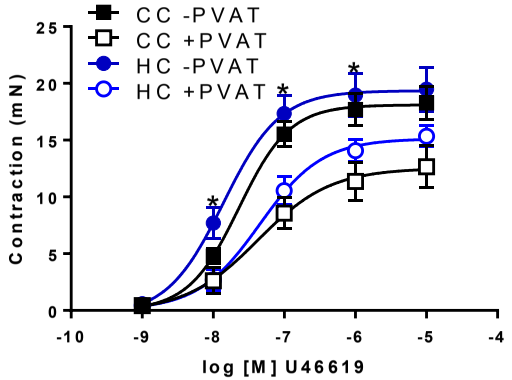
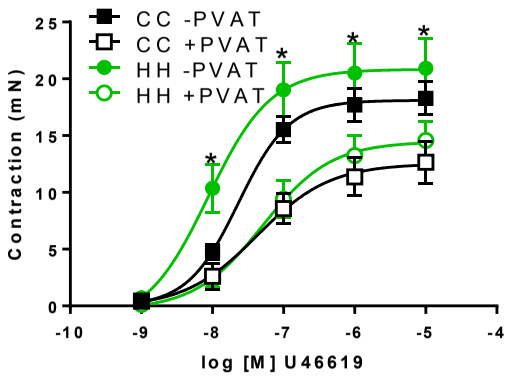
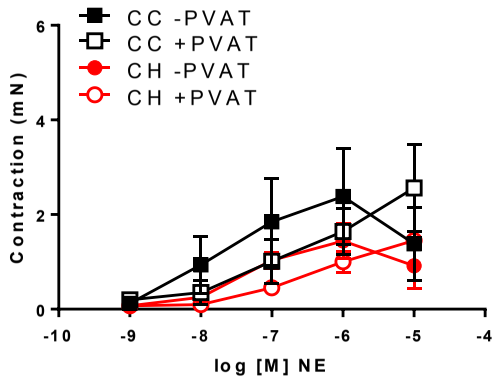
Window
Layout
Start
Sampling
4/12/2017
5:20:55
10^{-9} M U46619
10^{-8}
10^{-7}
10^{-6}
10^{-5}
10^{-5} Acetylcholine
14.1 mN
2931 no fat
9.4 mN
2931 with PVAT
11.0 mN
2941 no fat
10.6 mN
2941 with PVAT
mN
10^-8 M U46619
10^-7 M U46619
10^-6 M U46619
10^-5 M U46619
10^-5 M ACh
4:50:00
5:00:00
5:20:00
5:30:00
5:40:00
5:50:00
6:00:00
6:10:00
6:20:00
6:30:00
6:40:00
5k:1
Start

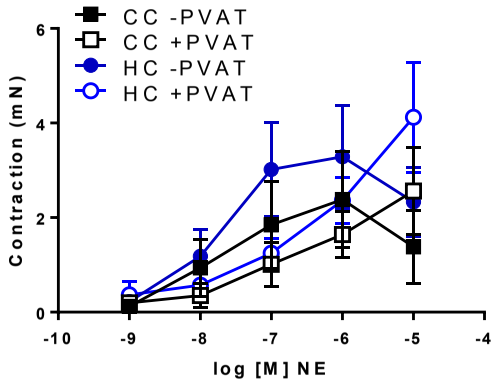
OBESITY AND CARDIOVASCULAR DISEASE

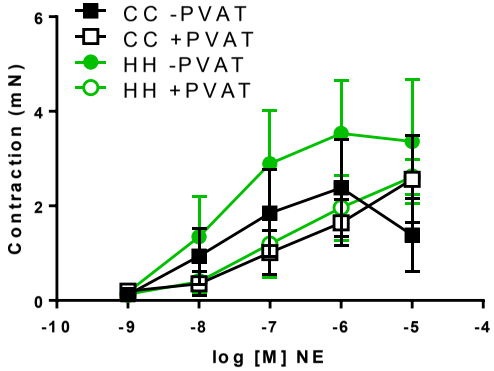












CONCLUSIONS

1. The 750TOBS can be used to study PVAT function;
2. The thoracic aortic PVAT normally reduced the magnitude of aortic contraction;
3. Maternal obesity may program offspring vascular function.

Suggested Resources

- Jespersen, B., Tykocki, N. R., Watts, S. W., Cobbett, P. J. Measurement of Smooth Muscle Function in the Isolated Tissue Bath-applications to Pharmacology Research. *J. Vis. Exp.* (95), e52324, doi: 10.3791/52324 (2015).
- Russell, A., Watts, S. Vascular reactivity of isolated thoracic aorta for the C57BL/6J mouse. *J Pharmacol Experimental Therapeutics*. (294), 598-604, (2000).
- Brozovich, F.V., Nicholson, C.J., Degen, C.V., Yuan, Z.G., Aggarwal, M., Morgan, K.G. Mechanisms of vascular smooth muscle contraction and the basis for pharmacologic treatment of smooth muscle disorders. *Pharmacol Rev*. (68), 476-532, doi: 10.1124/pr.115.010652 (2016).

Thank You

For additional information on the products and applications presented during this webinar please visit www.dmt.dk

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