THOMPSON RIVERS UNIVERSITY

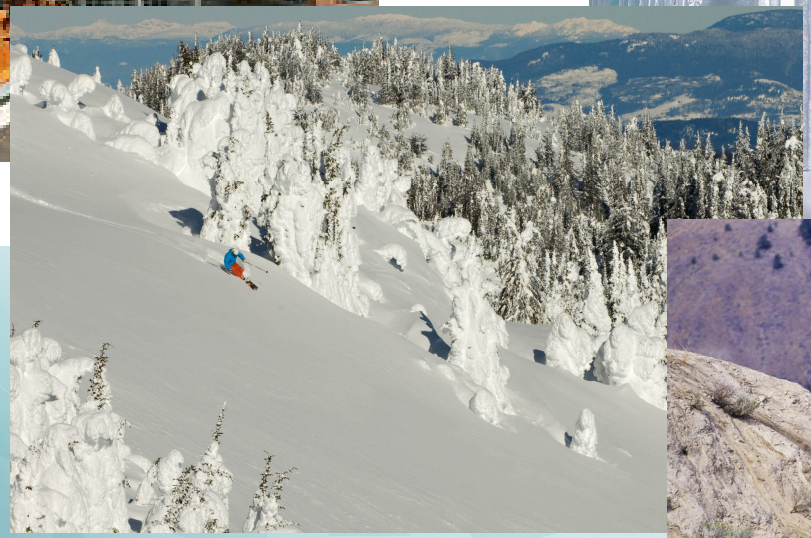
Exercise derived extracellular vesicles and vascular adaptations

Research

Mark Rakobowchuk, PhD
Assistant Professor
Biological Sciences

tru.ca/research

Thompson Rivers University, Kamloops, Canada



Sport, Adventure and Science in British Columbia

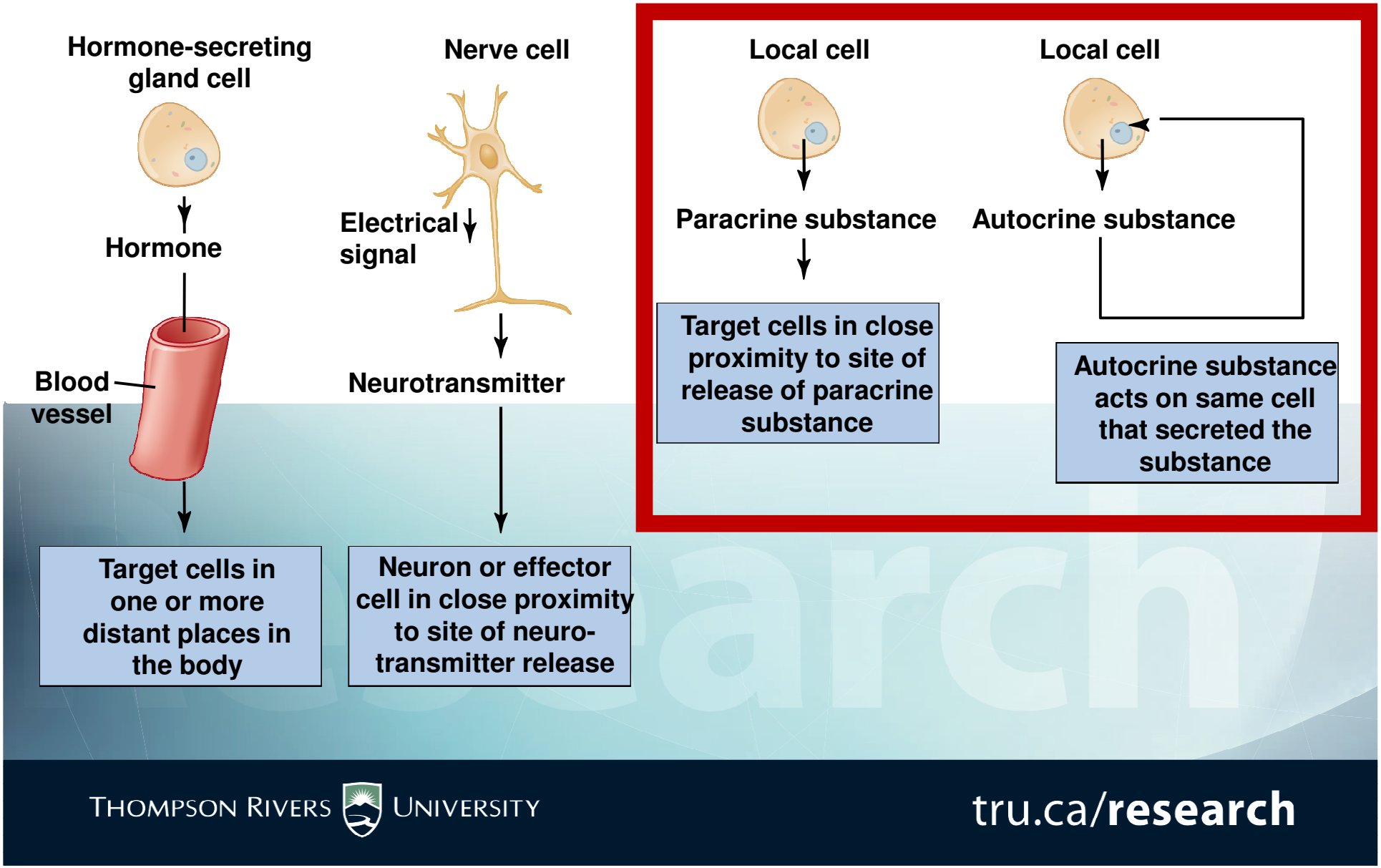
THOMPSON RIVERS UNIVERSITY

tru.ca/**research**

Outline of this talk

- What are extracellular vesicles?
- How are they involved in cell to cell communication?
- Which are released with acute exercise and what factors influence their release?
- What do they do within the body?
- Future directions and unanswered questions

Typically communication between cells in humans



Most cells shed vesicles of different shapes and sizes

Three major types

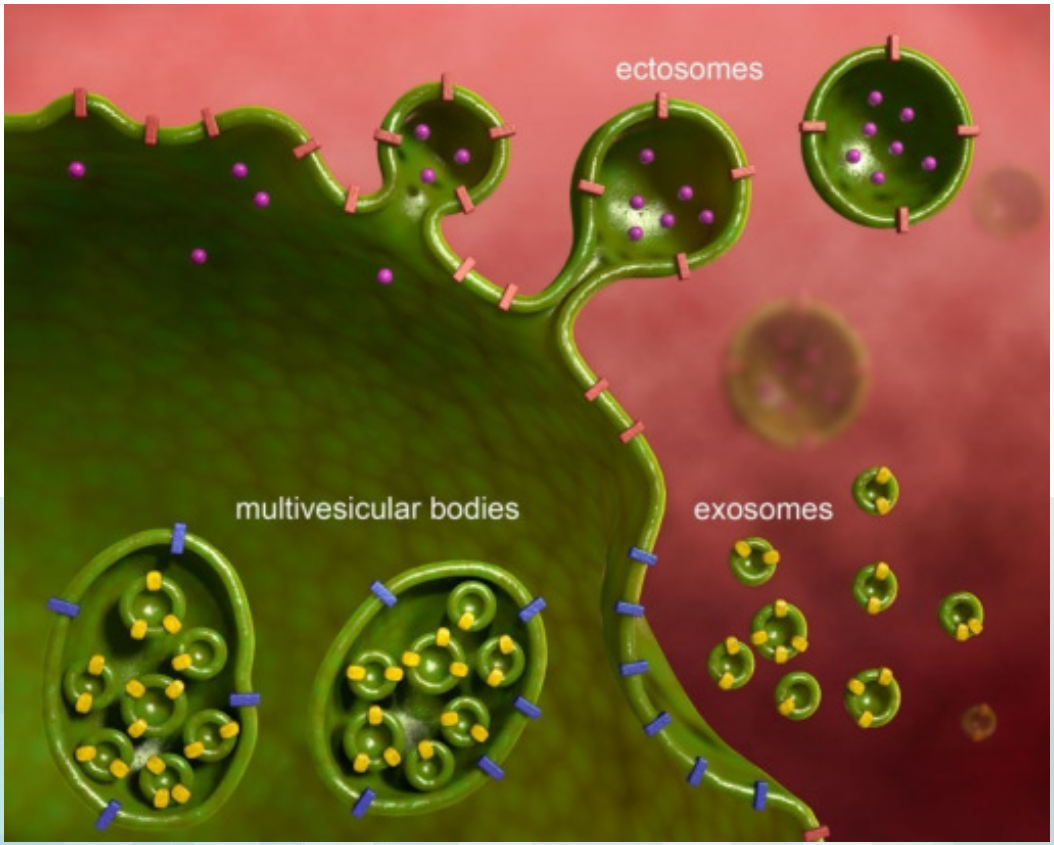
Ectosomes (microvesicles or microparticles)

Exosomes (released from MVBs)

Apoptotic bodies (not illustrated)

Released from:
All human tissues/cells

Bacteria, yeast and other organisms (interactions with humans?)



Colombo et al. (2012) Front Physiol

What are they and why might they be important?

Composed of a lipid membrane

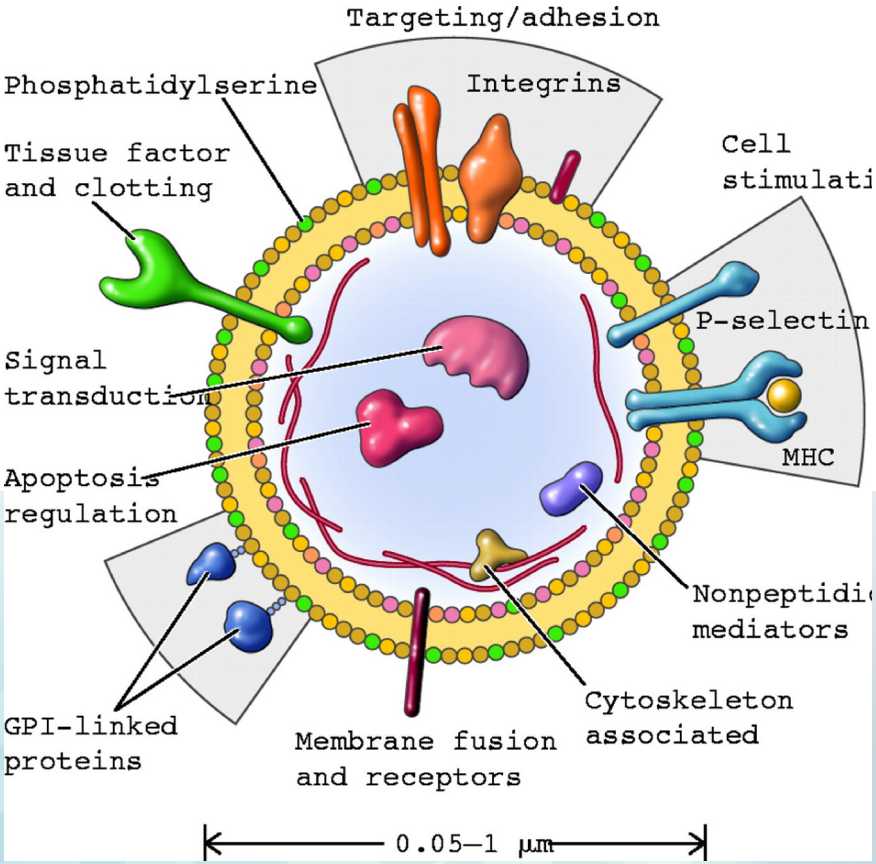
Integral proteins (adhesion, signaling)

GPI-linked proteins

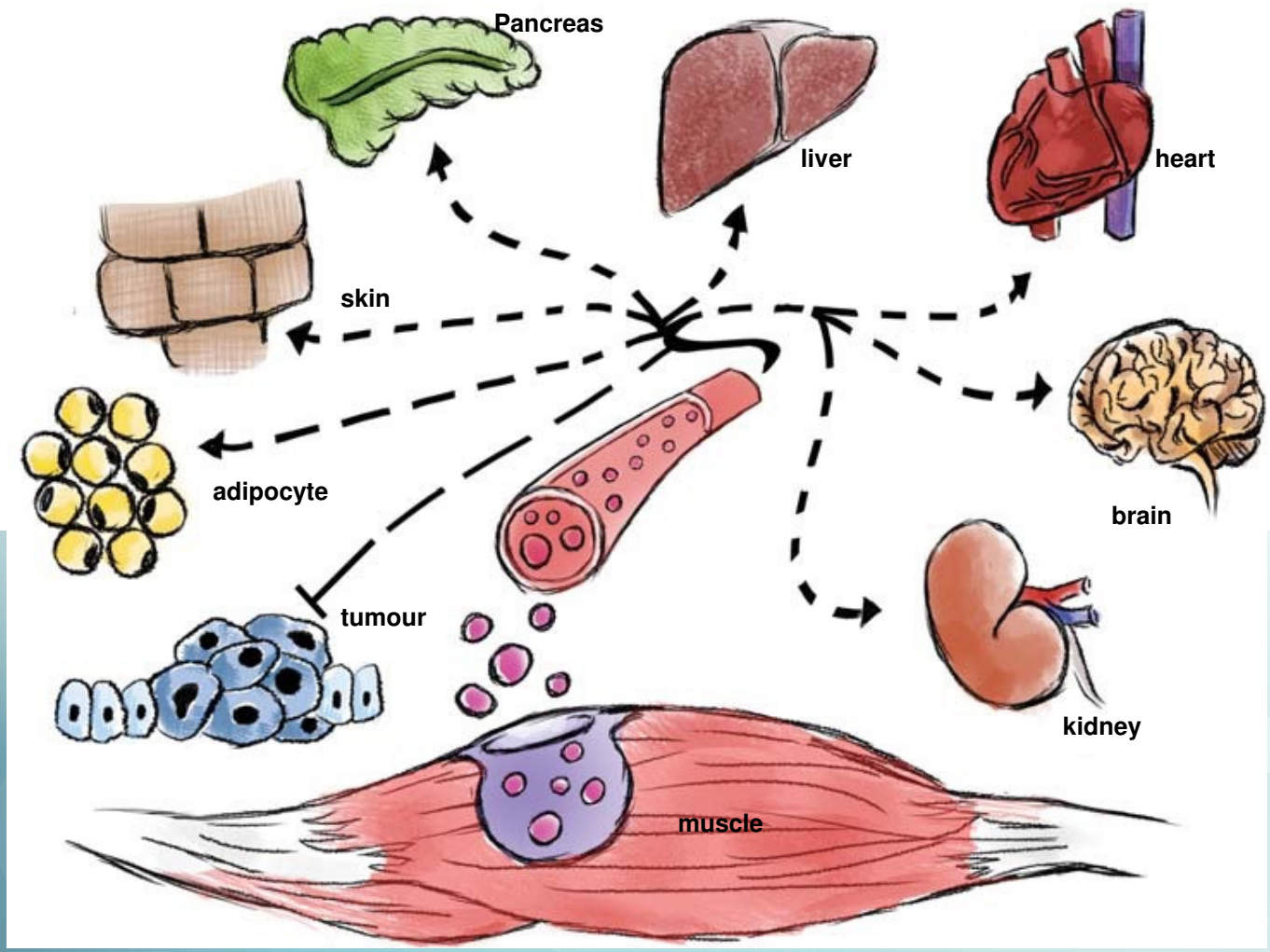
mRNA, miRNA, soluble proteins

Phospholipids

<u>All can interact with neighboring cells or distant cells when circulating</u>

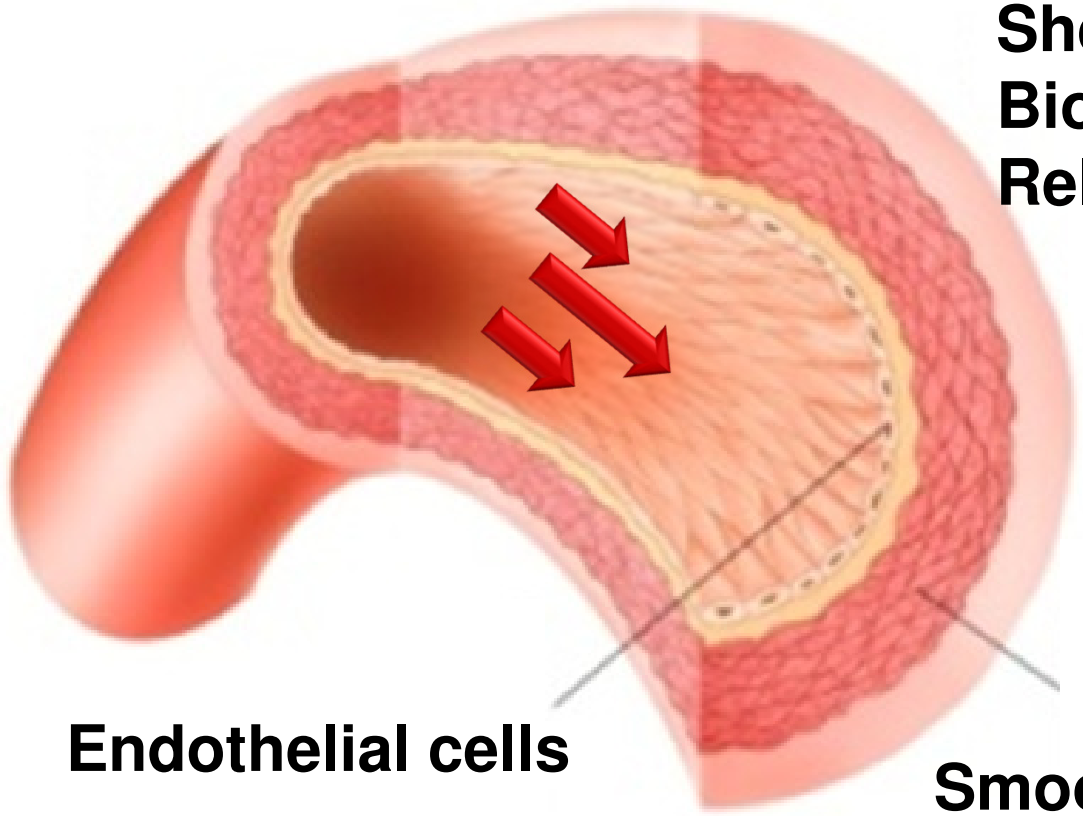


Theoretical impacts of exercise and the “exerkine” derived from skeletal muscle



Safdar and Tarnopolsky (2017) Cold Spring Harbour Perspective in Med

What are the stimuli responsible for this platelet response?



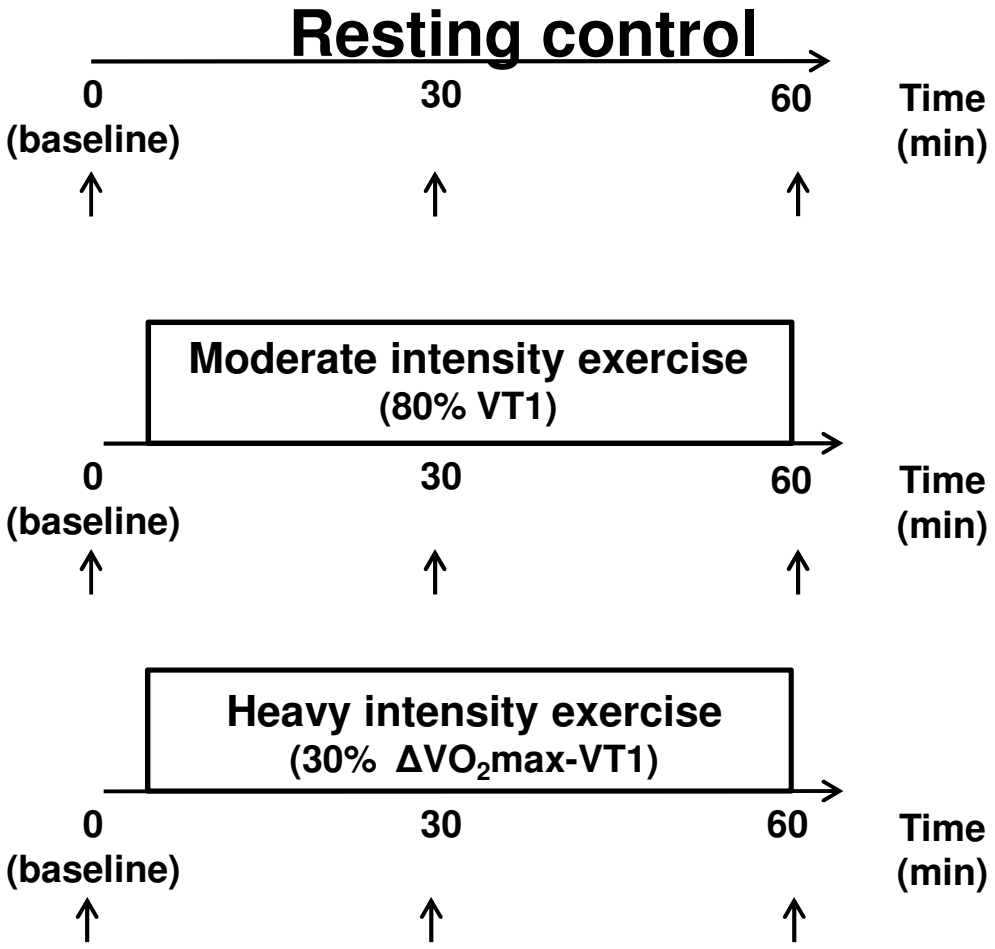
We assessed which stimuli are involved and whether MVs are potentially relevant in angiogenesis

<u>Study 1:</u> Is the exercise intensity an important modulator and is it related to shear stress?

<u>Study 2:</u> Is the stress placed upon the muscle important in the modulating the increase of MVs with exercise?

<u>Study 3:</u> What is the the biological relevance? Are they important in exercise adaptations like angiogenesis?

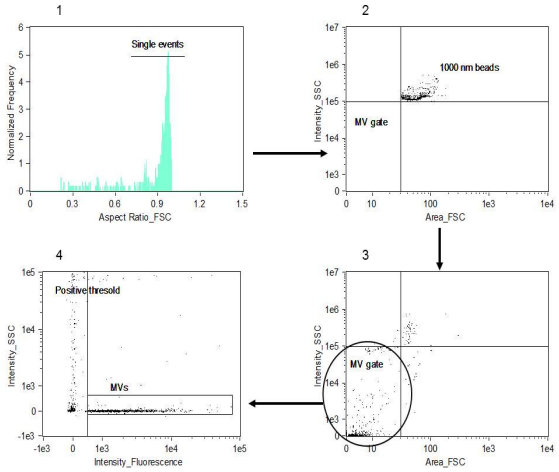
Is the intensity of exercise an important and what about EMVs?



Methodology to study microvesicle dynamics

Plasma Microvesicle Quantification:

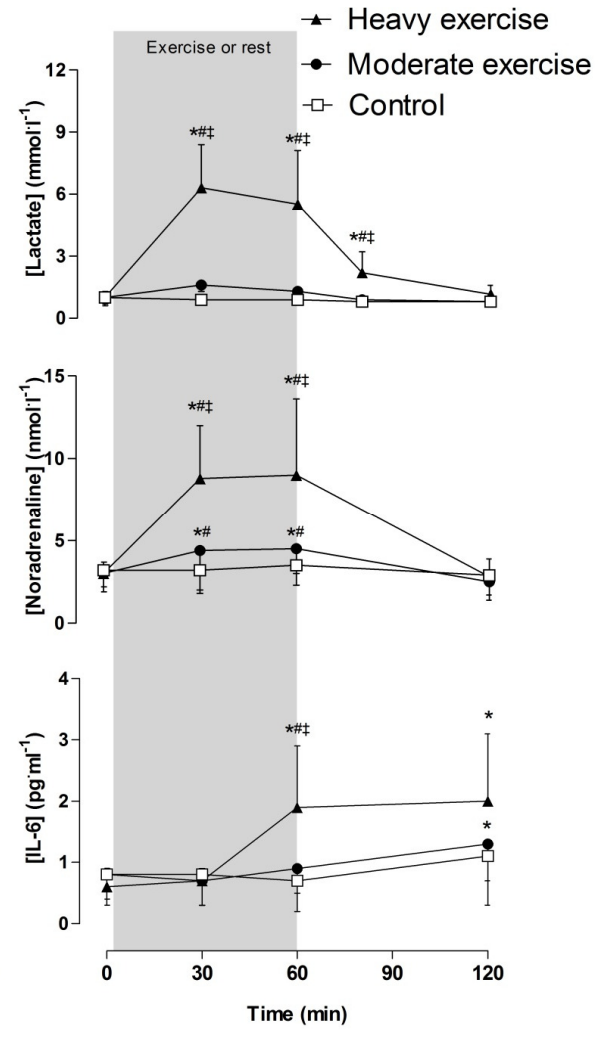
- Image Flow Cytometry
  (ImageStream, Amnis,USA)
- CD41+ (PMVs)
- CD62E+ (EMVs)
- Size calibration beads

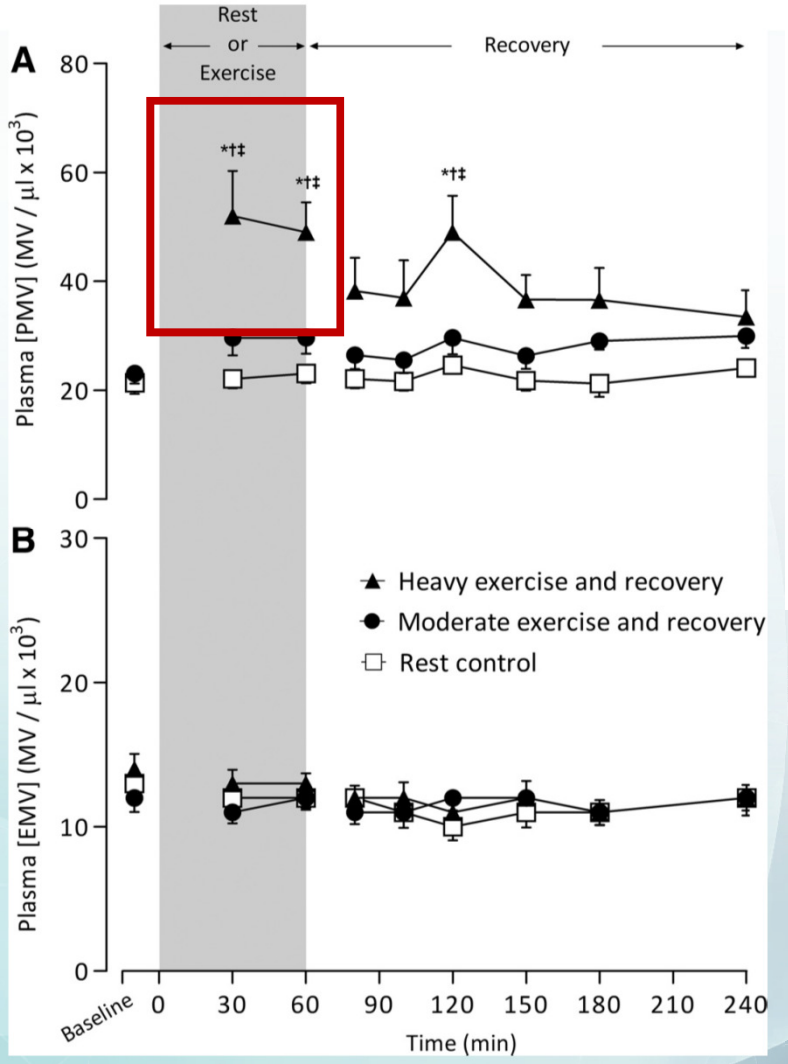


Haemodynamic measurements:

- Ultrasonography
- Shear rate at the brachial artery

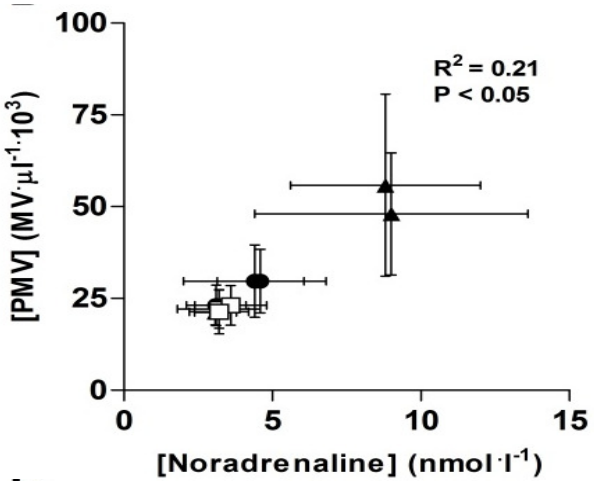
A biphasic response in PMVs with intense exercise

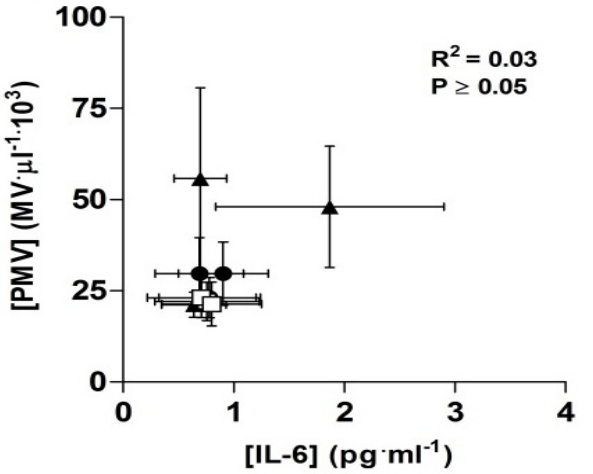




Wilhelm et al (2016) Am J Physiol: Heart Circ Physiol

Modest relationship with norepinephrine but no relationship with IL-6





Individual subject regressions

NorEp has modest relationship

Increased IL-6 does not relate to changes in PMV

No increase in EMVs so no relationships exist with exercise

Do aspects of the muscle contraction influence the appearance of both PMVs and EMVs?

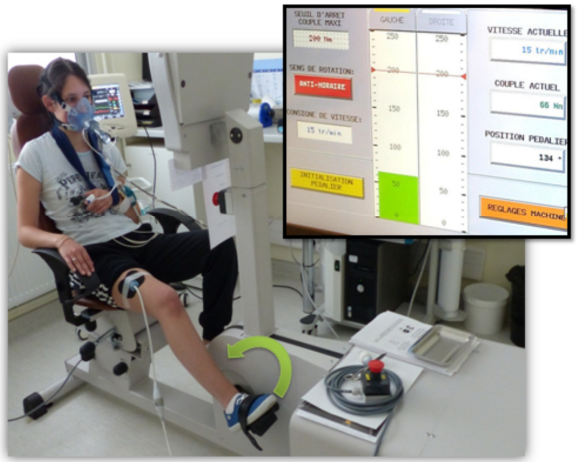
Lindstedt et al. (2001) Physiol

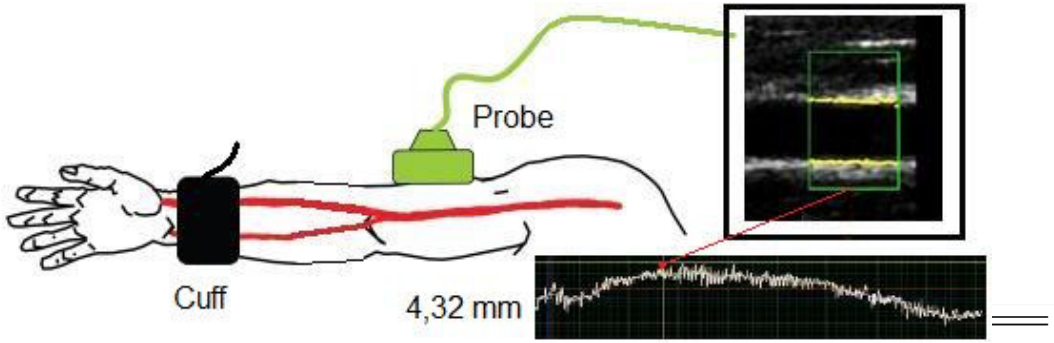
Is the downhill walking a viable intervention?

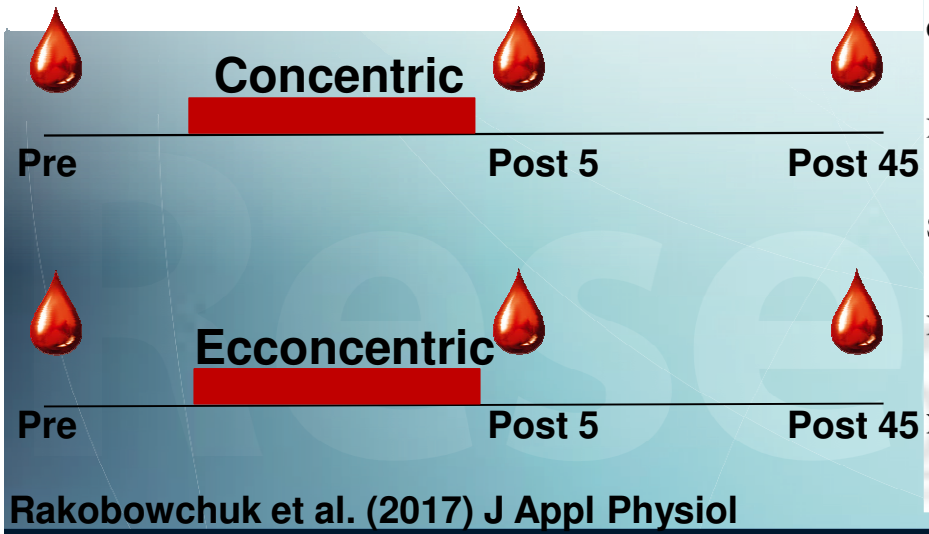
What benefits are there for the vasculature?

What are the risks to practitioner?

Do aspects of the muscle contraction influence the appearance of both PMVs and EMVs?



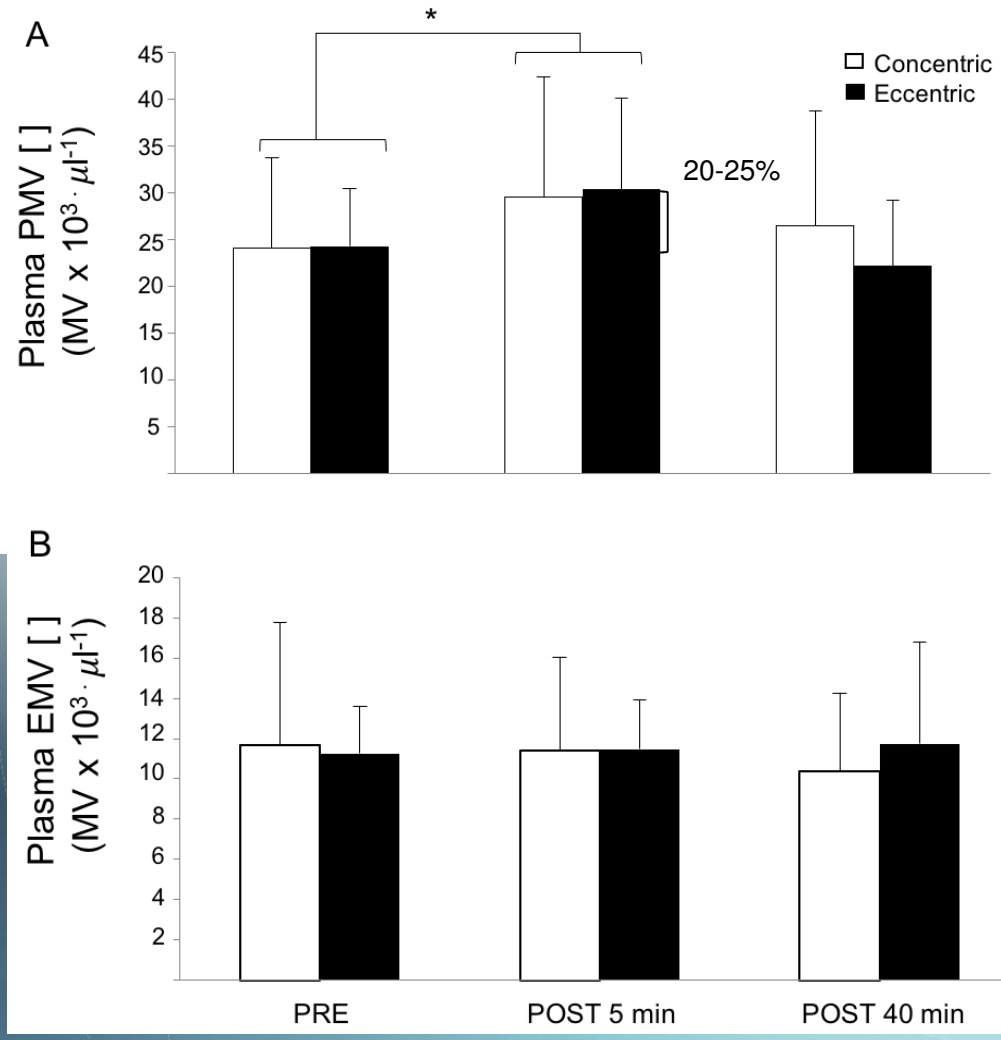




Rakobowchuk et al. (2017) J Appl Physiol

	Pre	Post 5 min	Post 40 min
Heart rate, beats/min			
Concentric	58.5 ± 7.6	104.2 ± 9.2*	59.1 ± 8.0
Eccentric	61.7 ± 8.8	117.4 ± 19.0*	62.4 ± 10.4
Cardiac output, l/min			
Concentric	7.6 ± 1.2	14.1 ± 1.8*	7.5 ± 1.2
Eccentric	7.9 ± 1.4	14.4 ± 2.7*	7.5 ± 1.3
Mean arterial pressure, mmHg			
Concentric	80 ± 13	93 ± 13*	76 ± 11
Eccentric	73 ± 10	102 ± 9*	72 ± 12
Systolic blood pressure, mmHg			
Concentric	118 ± 19	141 ± 21*	114 ± 14
Eccentric	109 ± 14	145 ± 13*	107 ± 17
Diastolic blood pressure, mmHg			
Concentric	59 ± 9	68 ± 10*	57 ± 8
Eccentric	54 ± 7	77 ± 8*	54 ± 9
Norepinephrine, pg/ml			
Concentric	184.1 ± 61.4	536.5 ± 144.7*	215.2 ± 87.4
Eccentric	217.0 ± 139.5	642.5 ± 301.8*	243.6 ± 97.5

Both methods of exercising increase modestly the circulating concentrations of PMV



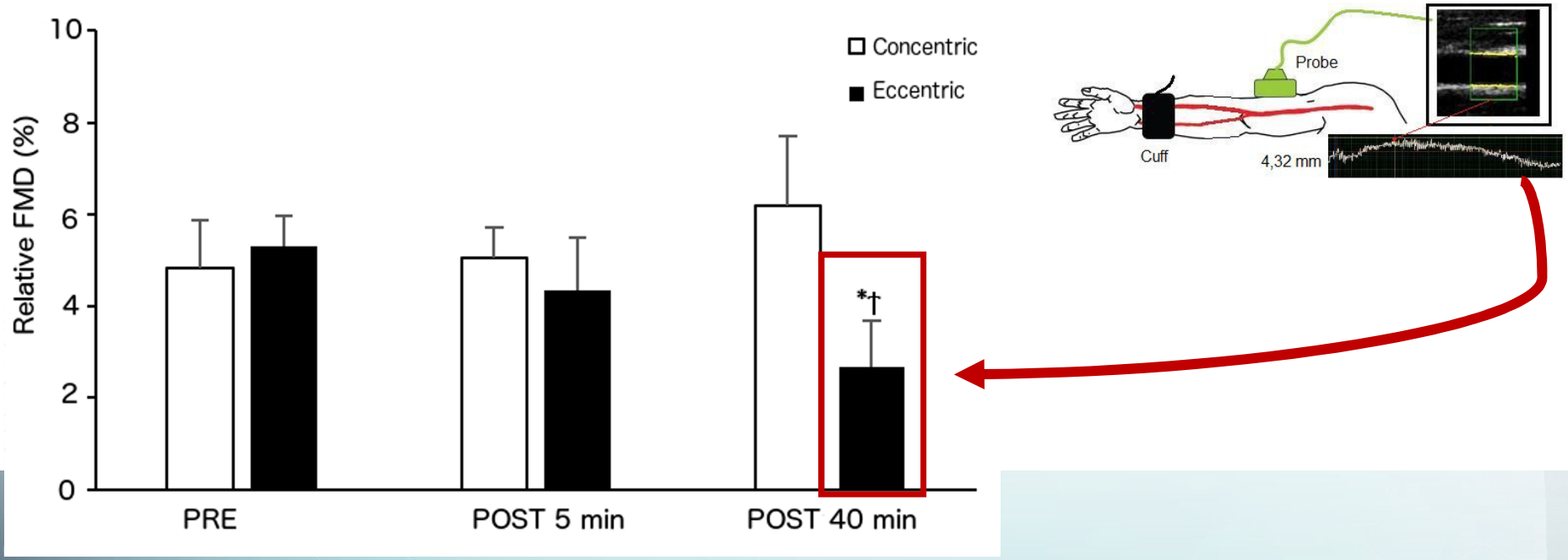
Platelet MVs increases in both modalities

Brief, returning to baseline quickly

Endothelial MVs were unchanged

Suggests no endothelial activation or damage

The function of arteries during recovery may be reduced with eccentric exercise



The maximal dilation caused by shear stress is reduced

Likely a systemic effect since the arms were not involved

Linked to late recovery oxidative stress

Summary of mobilization and appearance with exercise

1. Platelets (possibly megakaryocytes) release MV with exercise

2. Modest changes with moderate intensity, but substantial, and consistent with high intensity

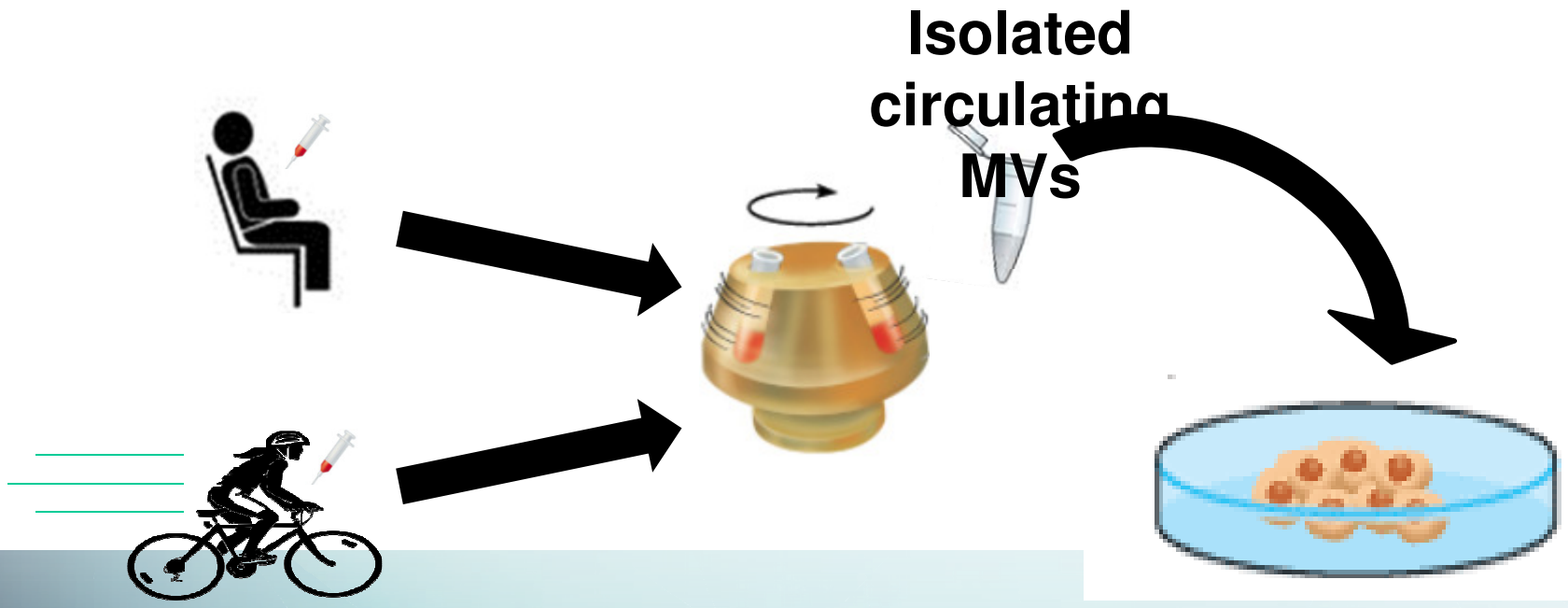
3. EMVs do not seem to be created

4. Shear stress not likely a major moderator of this response (results not shown Wilhelm et al. Physiol Rep)

Many unanswered questions remain

So PMVs increase after exercise, but what might they do?

Methods for in vitro assessments of the effects of MVs on endothelial cells



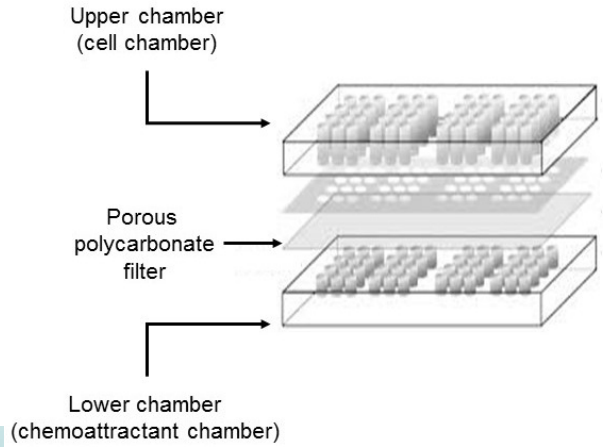
Events in capillary growth can be mimicked

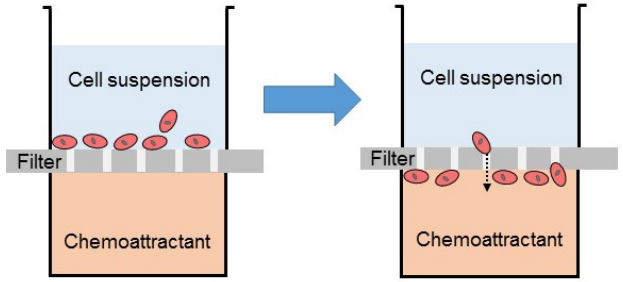
- **proliferation of cells (MTT assay)**
- **Migration towards angiogenic stimuli (VEGF or EVs)**
- **Combined proliferation and migration(scratch wound assay)**
- **Formation of capillary-like structure (matrigel tubule assay**

What might they be MVs being doing in the body?

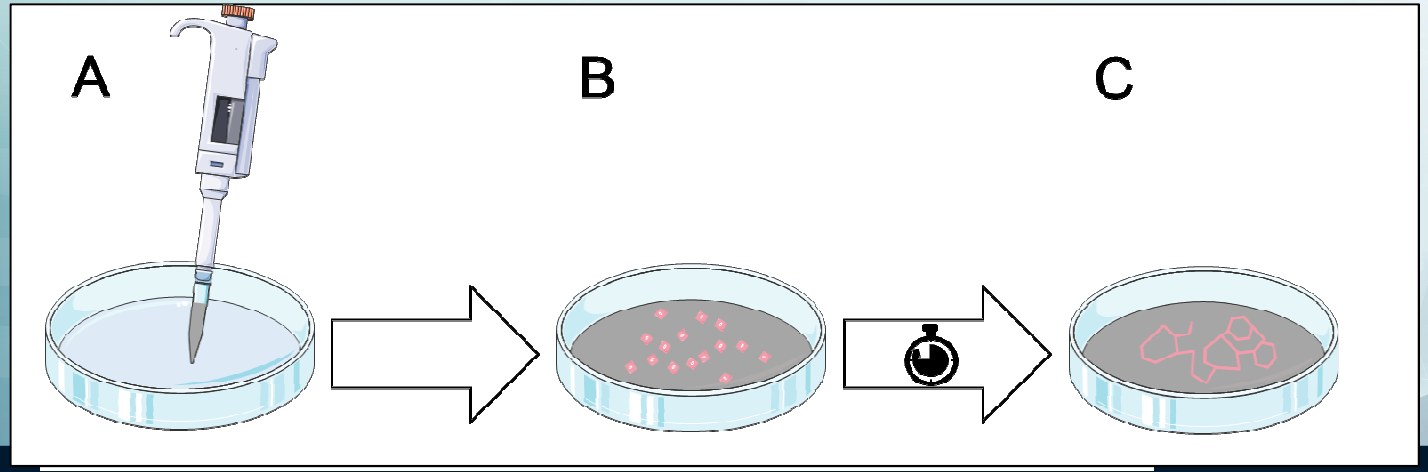
Conducted experiments using isolated MVs from rest and exercise

Migration:

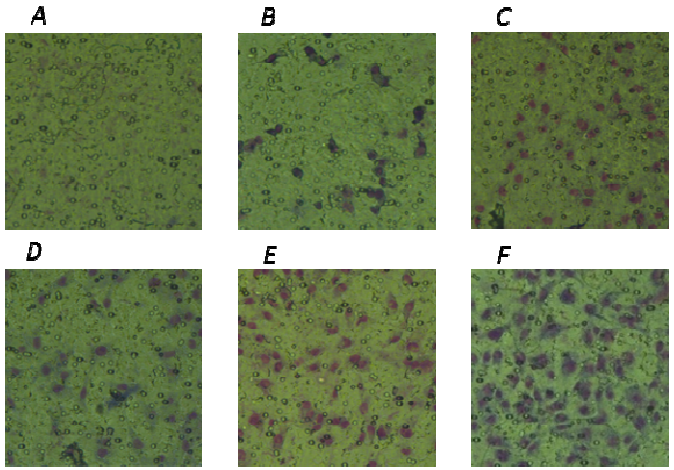


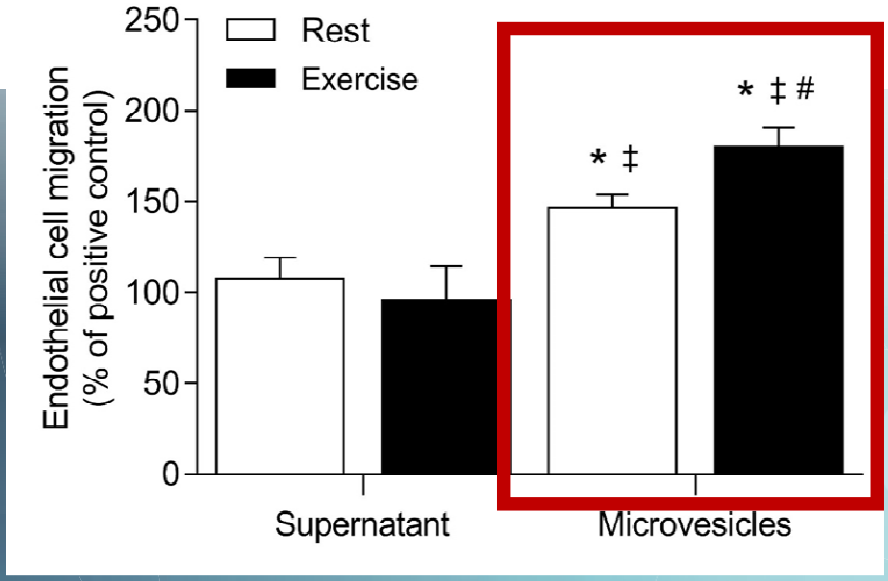


Wound healing formation:



What might they be MVs doing in the body?





Endothelial cells

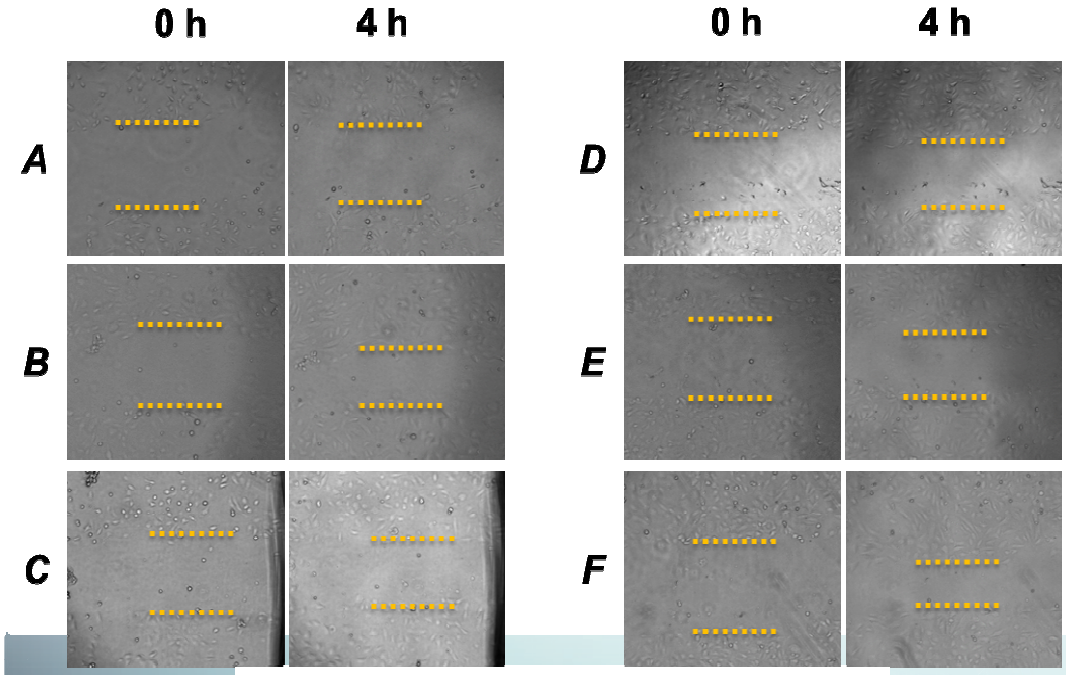
Migrate/move when stimulated with MVs

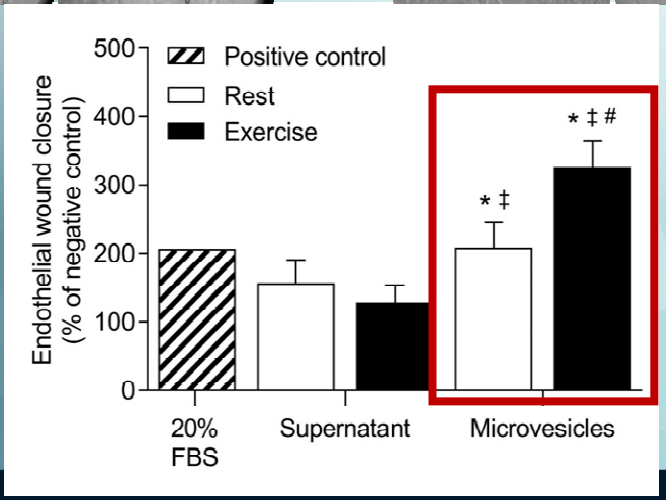
Effect is potentiated with MVs derived from blood obtained during exercise

Concentration or content?

Wilhelm et al. Am J Physiol 2016

What might they be MVs being doing in the body?





<u>Endothelial cells</u>

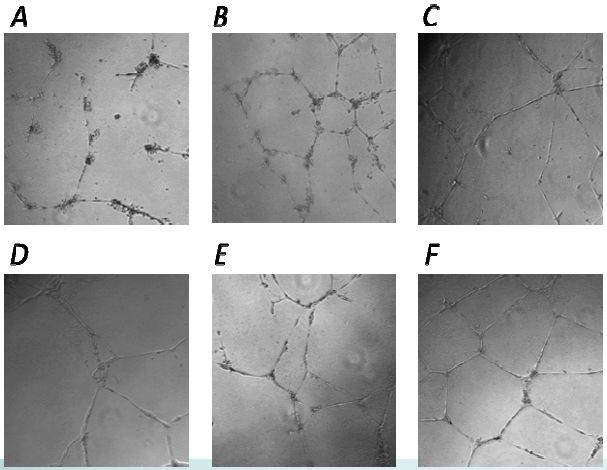
Heal a disturbed monolayer when stimulated by MVs

Wound healing is quicker with MVs derived from blood obtained during exercise

Concentration or content?

Wilhelm et al. Am J Physiol 2016

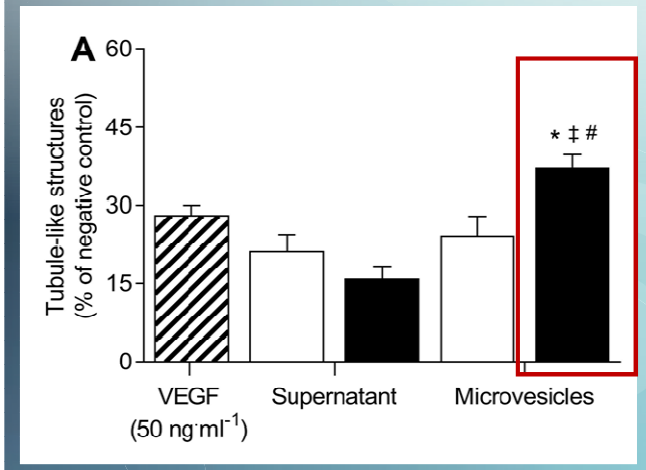
What might they be MVs being doing in the body?

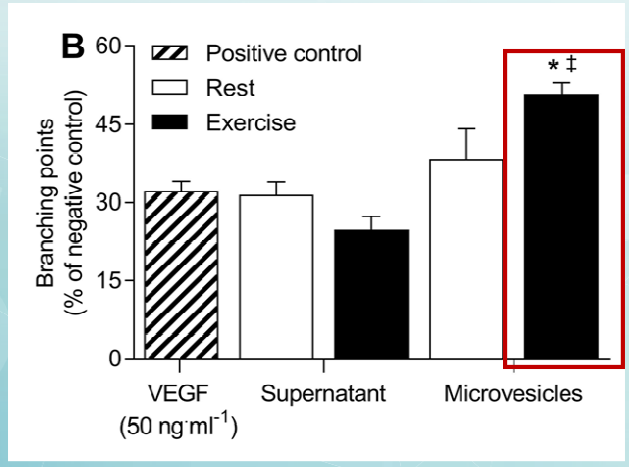


Endothelial cells

Create more tubule like structures with exercise derived MVs

Content or Concentration?





Wilhelm et al. Am J Physiol 2016

Take home points and future directions

Future Directions

What are the stimuli that lead to MV formation in health, with exercise and does MV communication deteriorate with aging?

Does the content of MVs change with acute exercise and does this impact other cells?

What mechanism leads to the change in endothelial cell function when exposed to MV derived from exercise?

Acknowledgements

Collaborators related to this work:
Dr Laurent Mourot, Université de Franche-Comté
Dr Nicolas Tordi, Université de Franche-Comté
Dr Laurie Isacco, Université de Franche-Comté
Ophélie Ritter, Université de Franche-Comté
Dr Eurico Wilhelm, Brunel University London
Dr Jon Van Hamme, TRU
Prof José González-Alonso, Brunel University London
Dr Bruno Cinel, TRU
Dr Bruno Degano, Université de Franche-Comté

Previous research environments
Brunel University London
University of Essex
University of Leeds
McMaster University

Thank You
for joining us today

Research

tru.ca/**research**

THOMPSON RIVERS UNIVERSITY

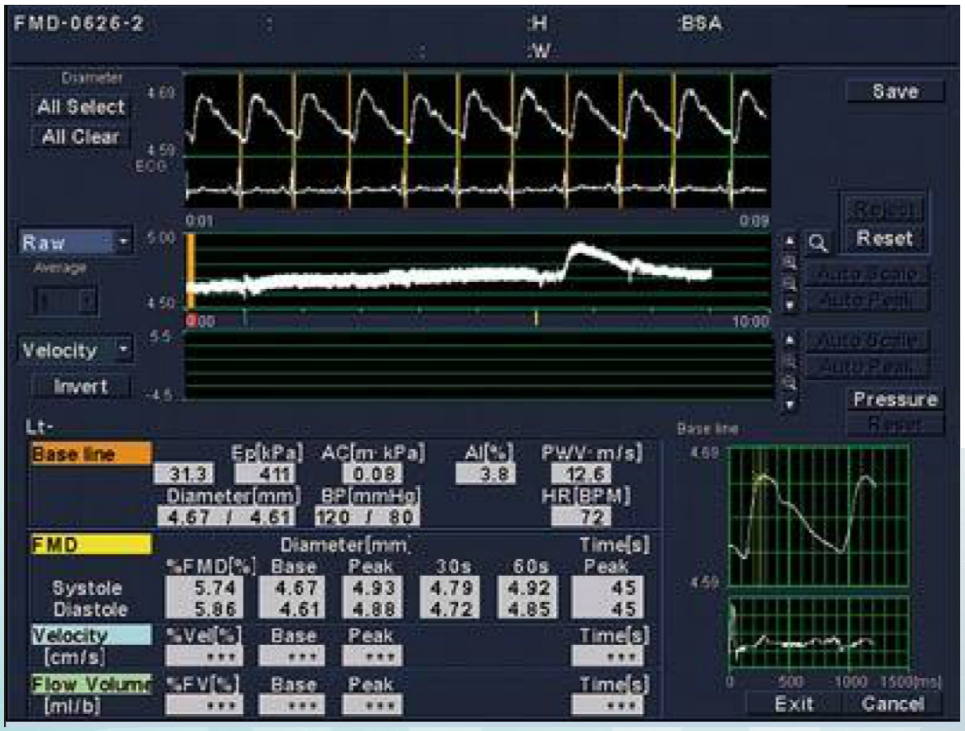
THOMPSON RIVERS UNIVERSITY

tru.ca/**research**

How do we evaluate blood vessel health and function in humans non-invasively?

Induce ischemia to the forearm or leg

Monitor reactive hyperaemia and dilation



Blockade of signaling pathways possible to determine treatments and responses

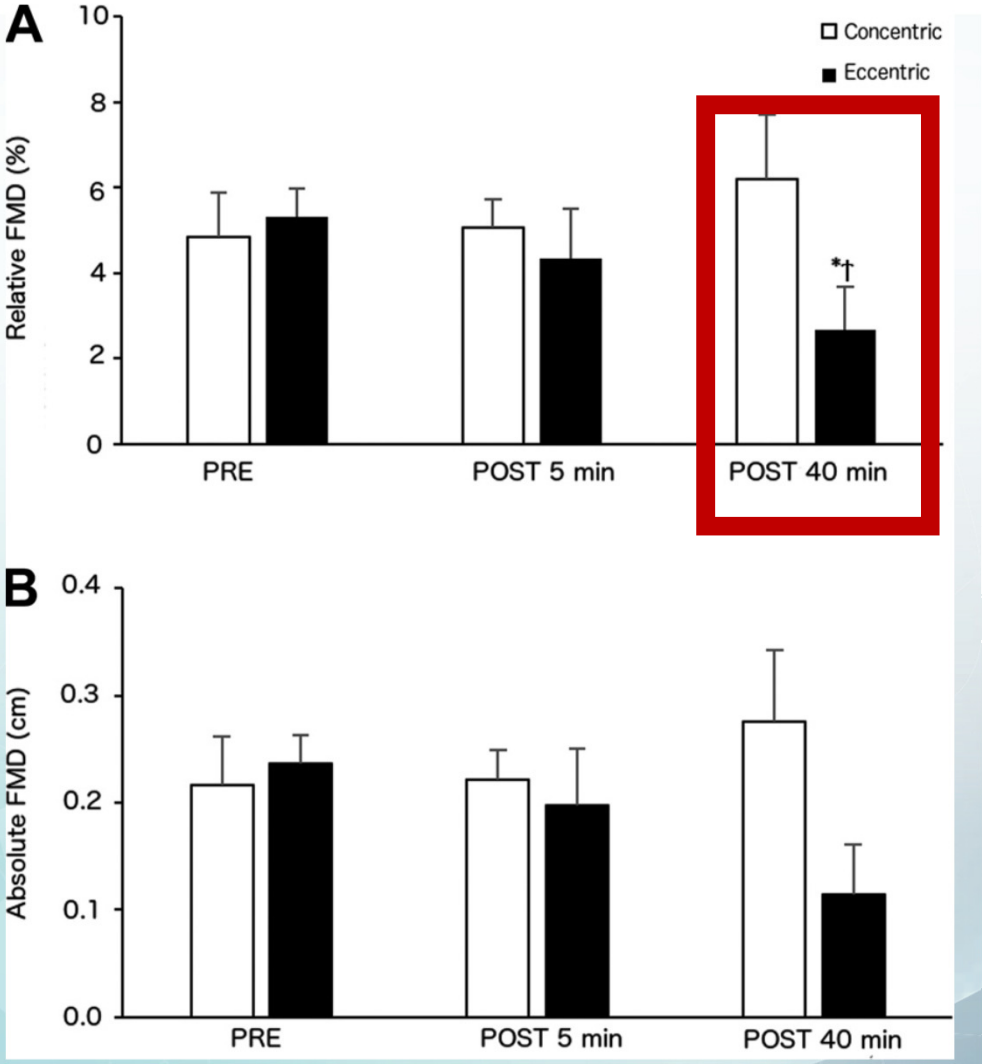
tru.ca/**research**

Eccentric exercise and endothelial function

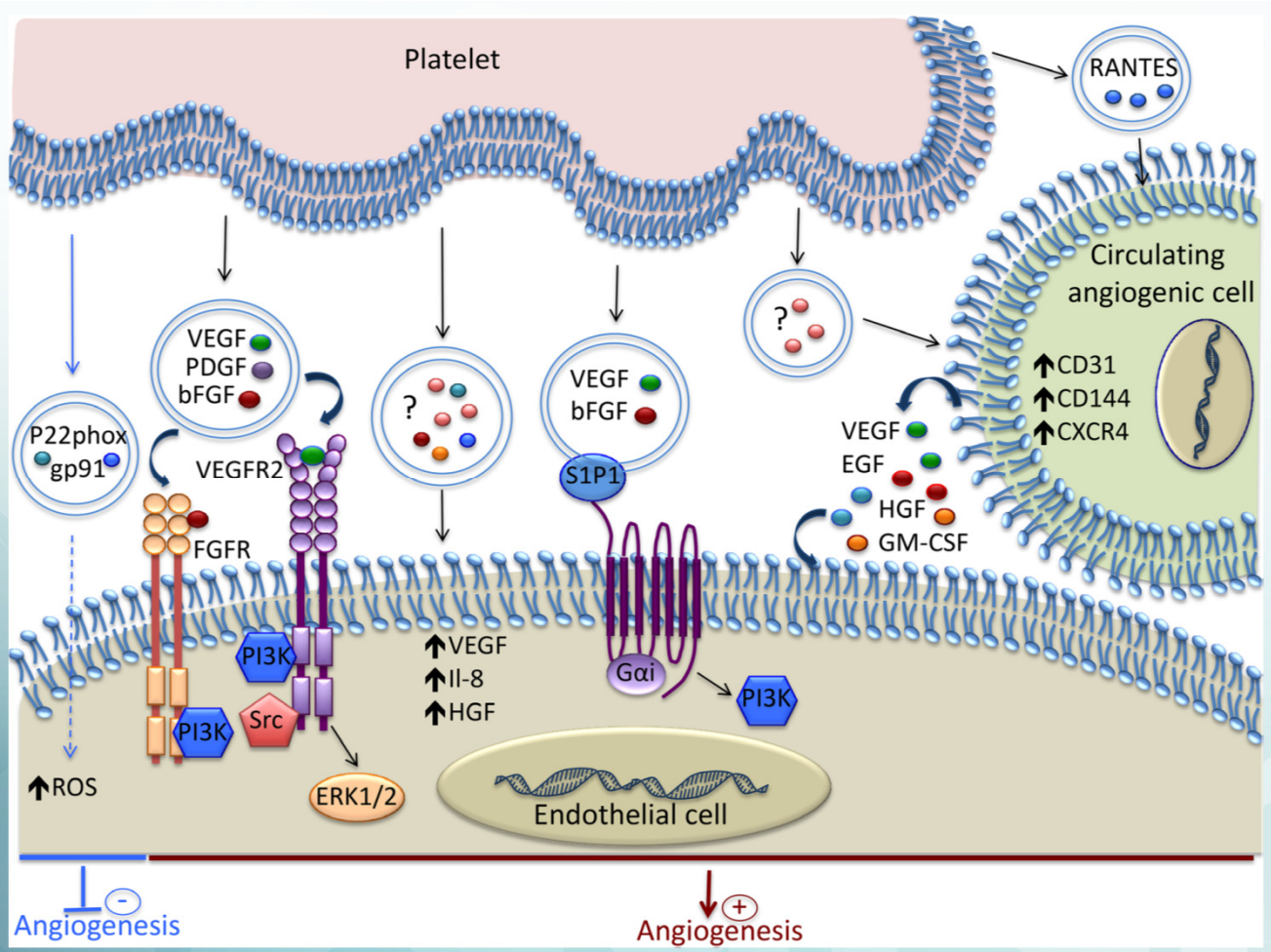
No change in FMD with concentric cycling

Reductions with eccentric cycling late in recovery

Unrelated to oxidative stress induced by the exercise, possibly immune related during recovery

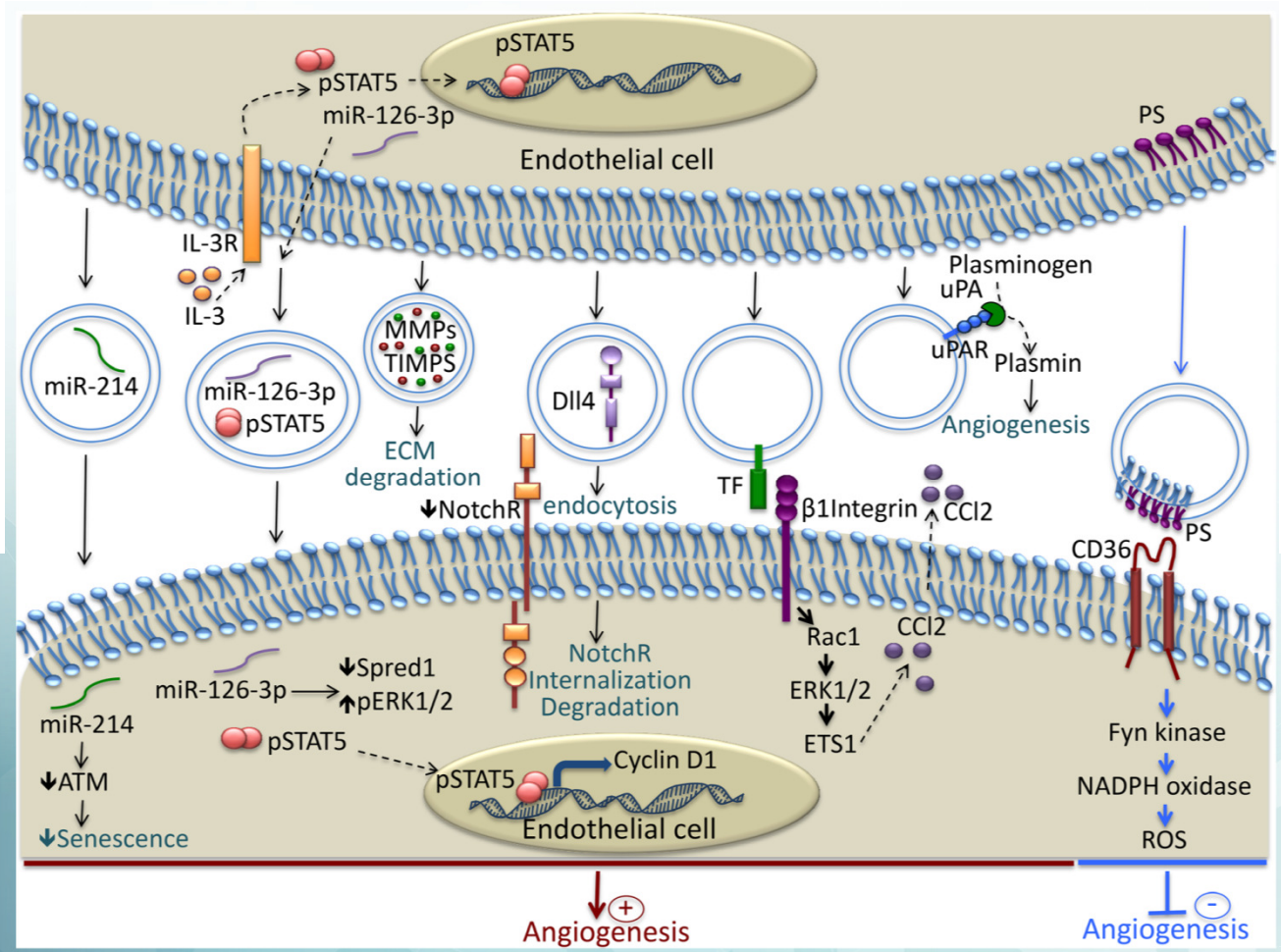


Platelet EV mechanism of angiogenic action



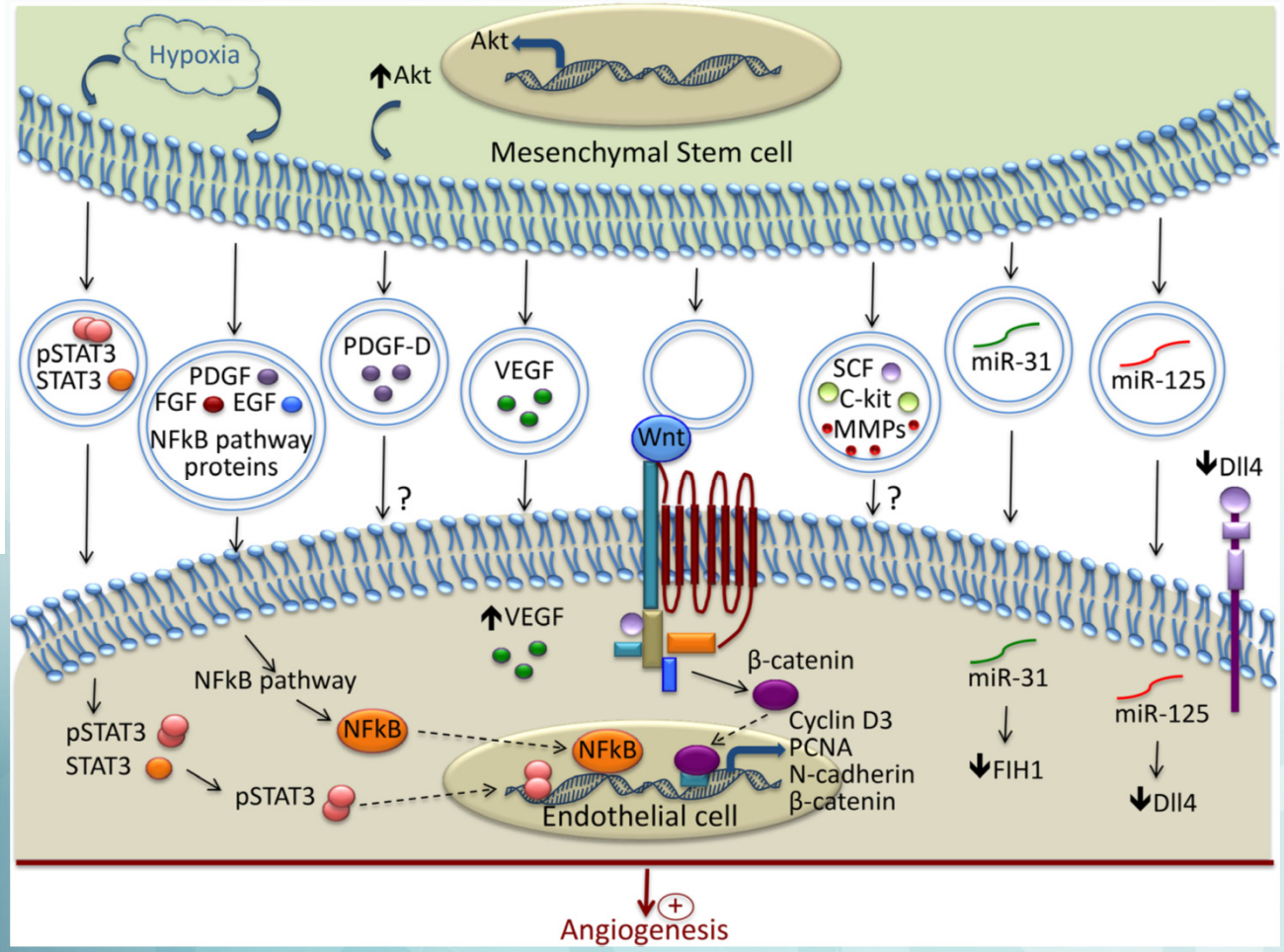
Tordorova et al. (2017) Circ Res

Endothelial EV mechanism of angiogenic action



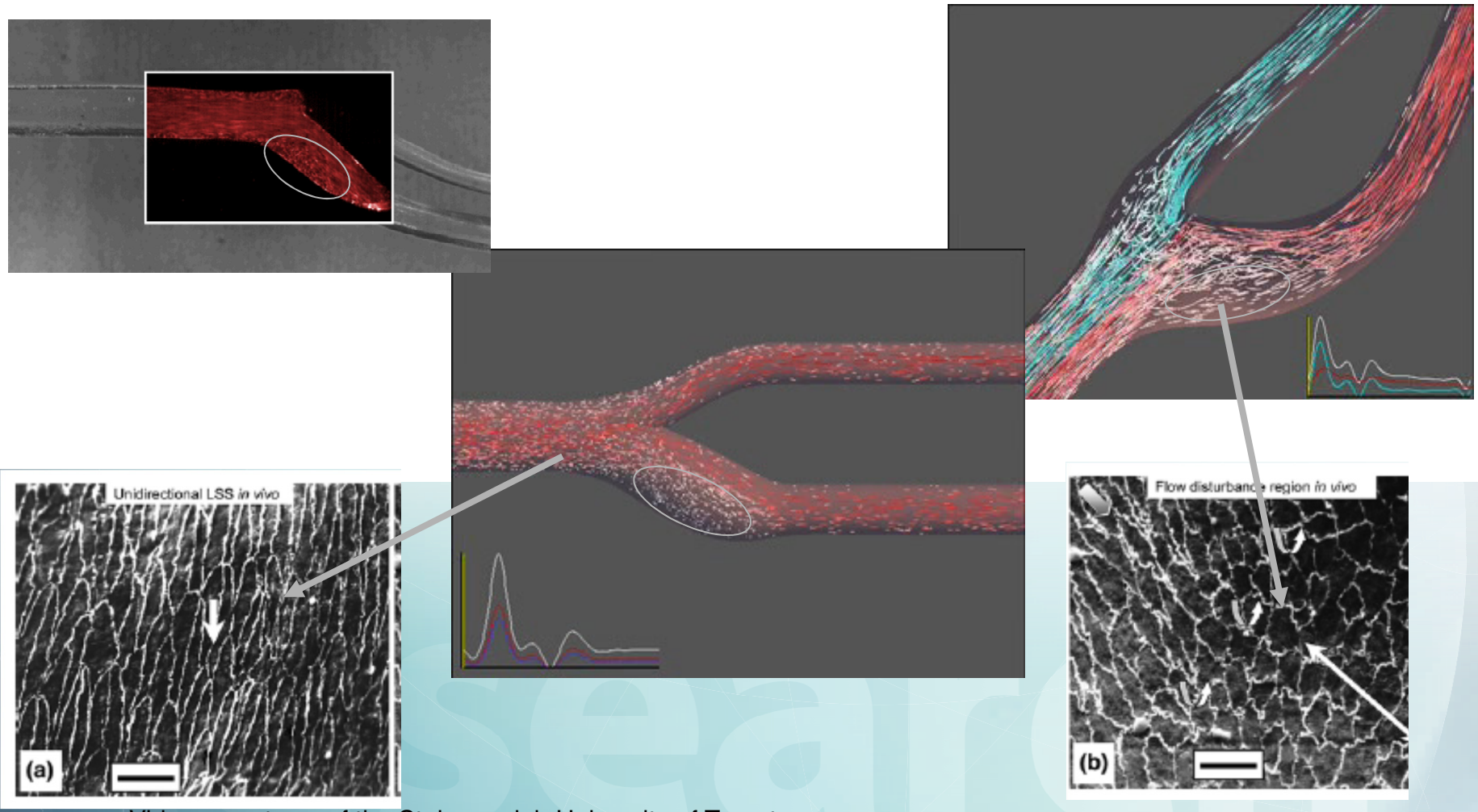
Tordorova et al. (2017) Circ Res

Mesenchymal stem cell EV mechanism of angiogenic action



Tordorova et al. (2017) Circ Res

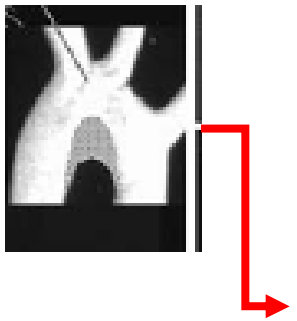
The vascular endothelium: an endocrine organ regulating vascular processes and disease progression



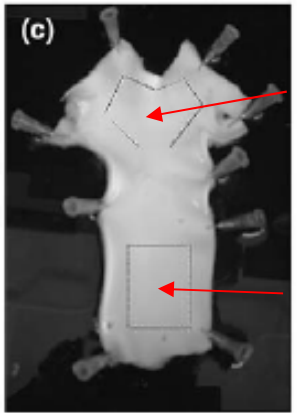
Videos courtesy of the Steinman lab University of Toronto
http://www.mie.utoronto.ca/labs/bsl/

Davies *Ann Biomed Eng 36(4):563-570*, 2008.

Disturbed flow patterns alter the phenotype of endothelial cells

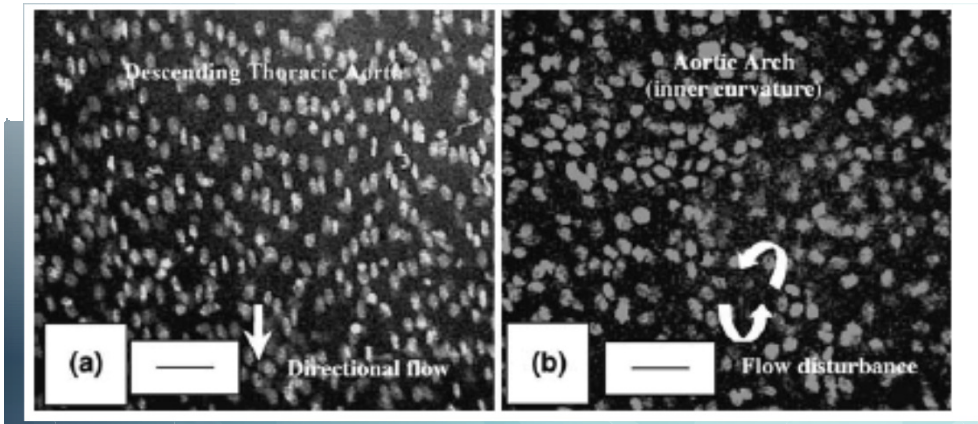


Aortic arch



Disturbed flow region

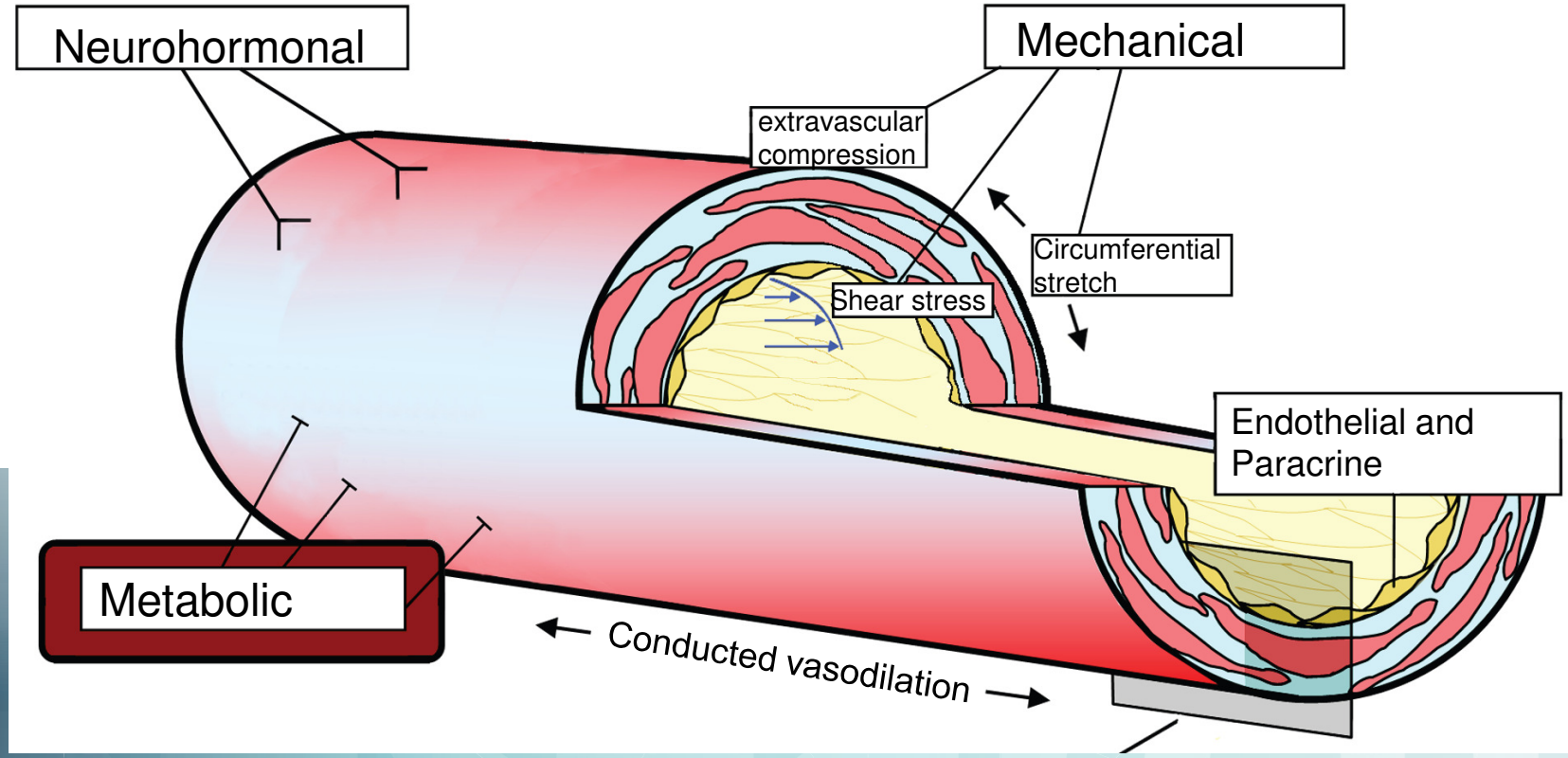
Directional flow region



Biological Classification	Number on array	Number differentially expressed
Inflammatory	150	18
Coagulation	63	5
Apoptosis	244	38
Adhesion	298	38
Superoxide	18	5

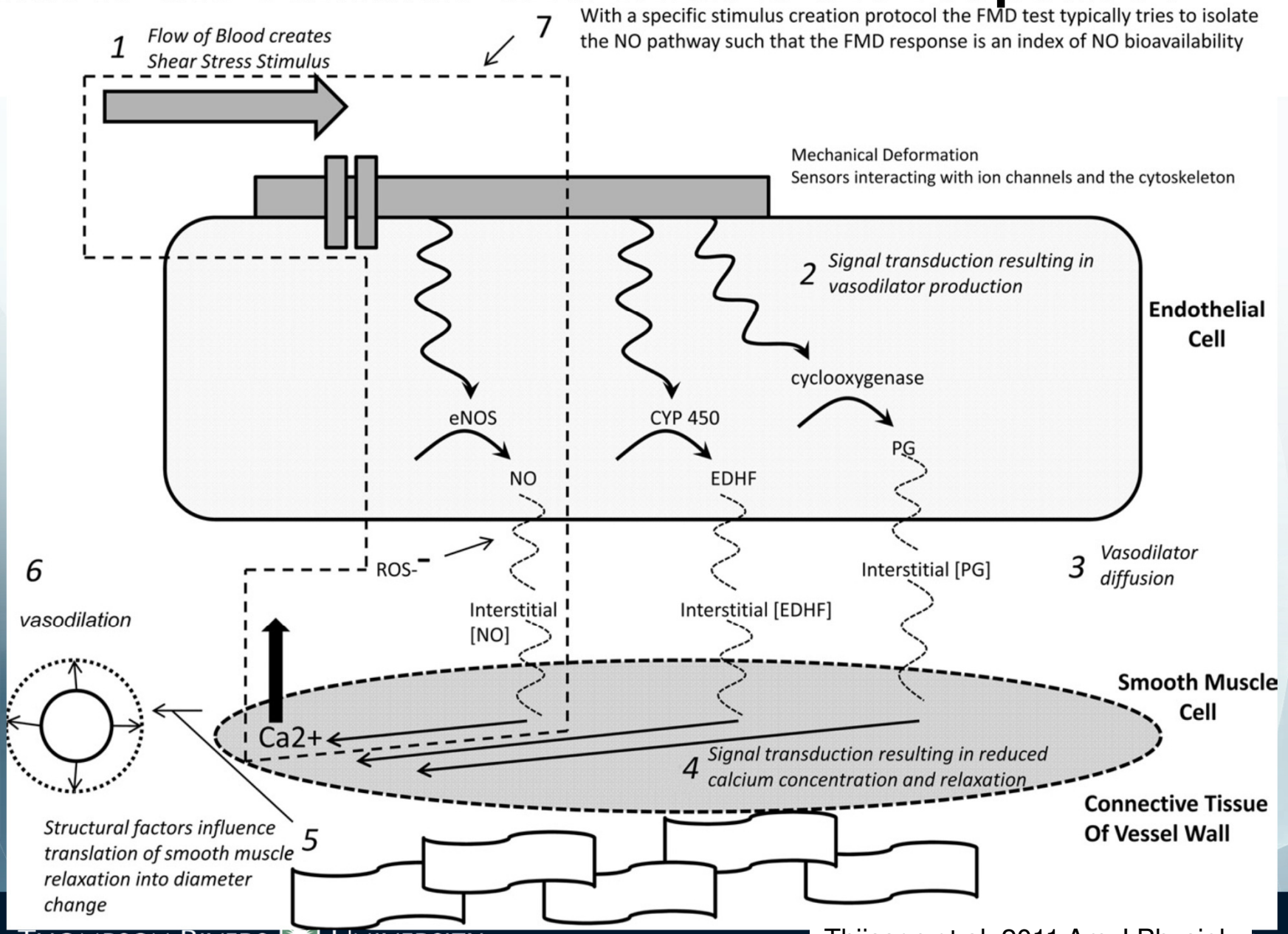
Davies *Ann Biomed Eng 36(4):563-570*, 2008.

The vascular endothelium: an endocrine organ regulating vascular processes and disease progression



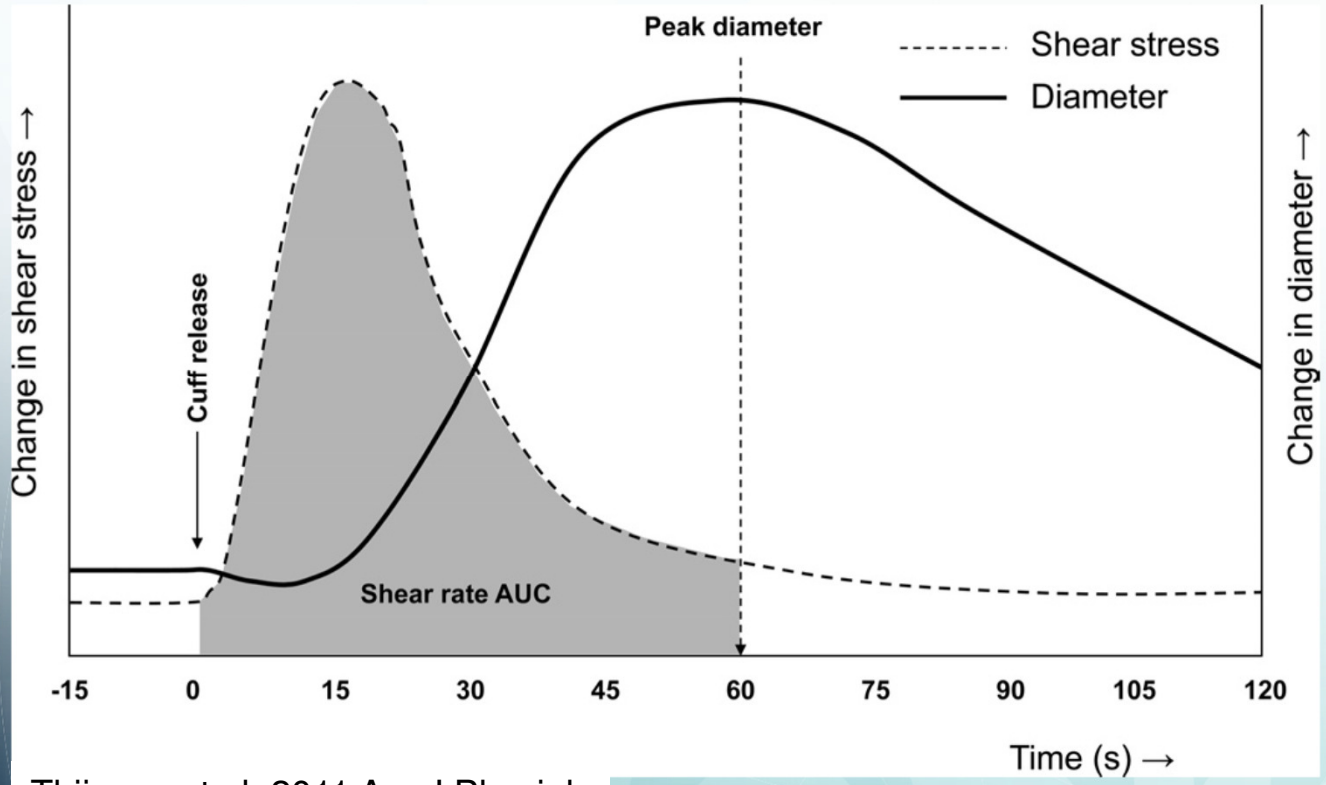
Laughlin et al. 2012 Comp Physiol

What is the stimulus and what is the response?



Thijssen et al. 2011 Am J Physiol

The typical time course of an FMD response at the brachial artery



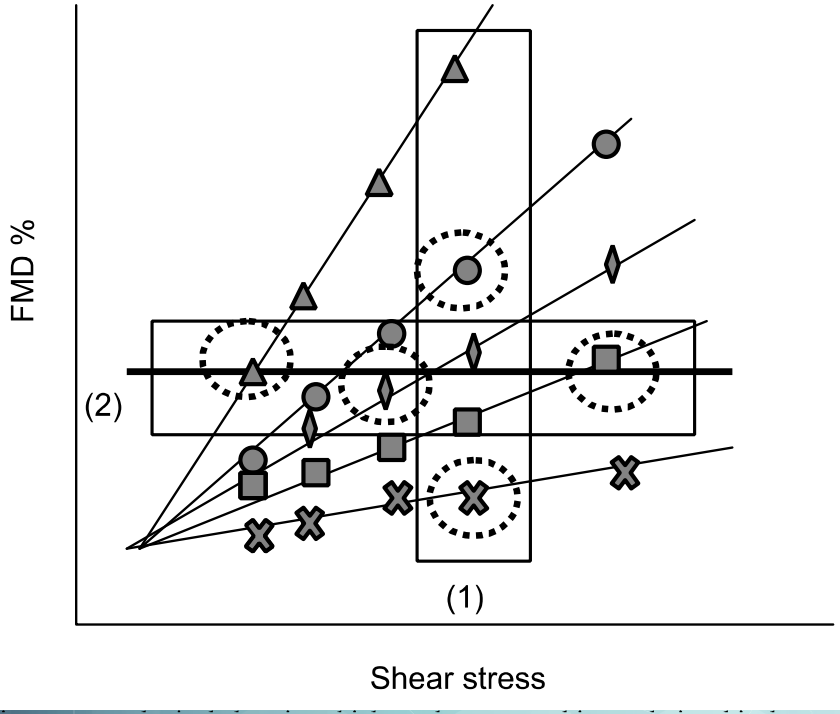
Thijssen et al. 2011 Am J Physiol

What parameters can be measured?

Shear stress induces the response and dictates its magnitude

Quantifying the shear stress is important and not simple to measure accurately

The shear stress may differ from one participant to another yet the FMD be similar



1. Participants have same shear but different dilation
2. Participants have same dilation but different shear
3. Baseline diameter influences both factors and their relationship