Studying Flow Mediated Responses in Isolated Vasculature

Éric Thorin, PhD

Professor of Surgery,
Université de
Montréal

Gerry Herrera, PhD

President,
Living Systems
Instrumentation

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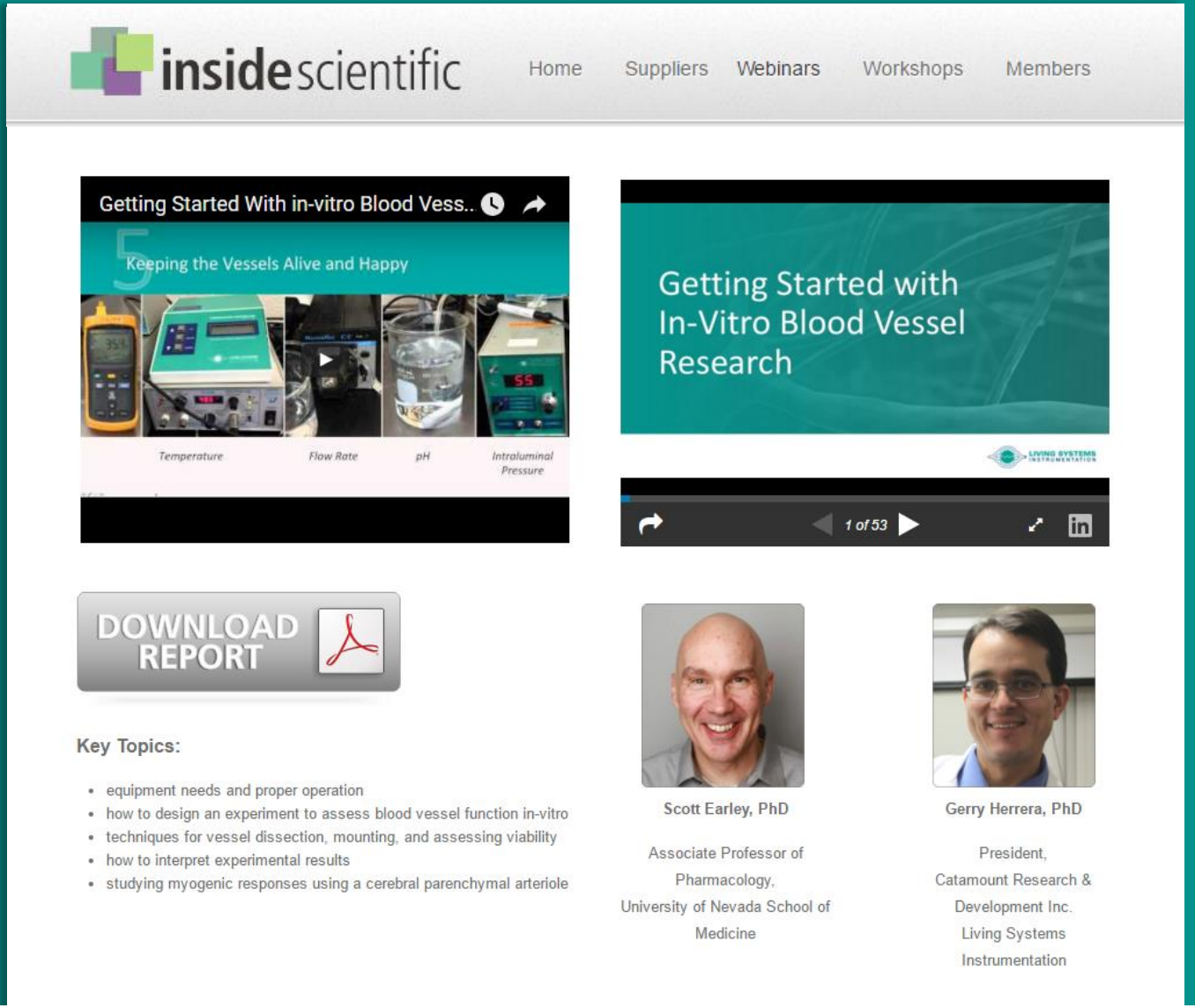
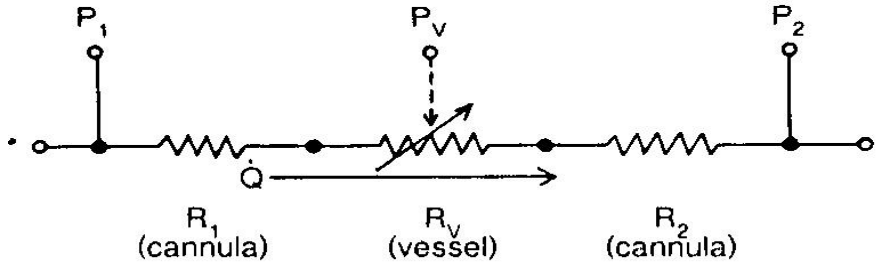
To learn how to mount an arteriole on pipets, please refer to the previous Webinar:

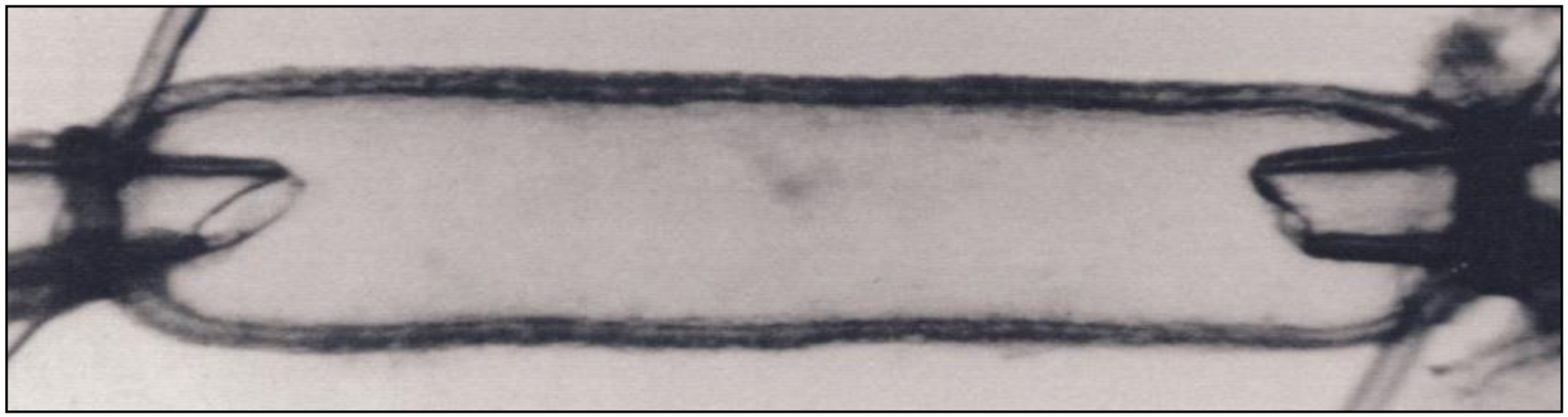
Getting Started with In-Vitro Blood Vessel Research



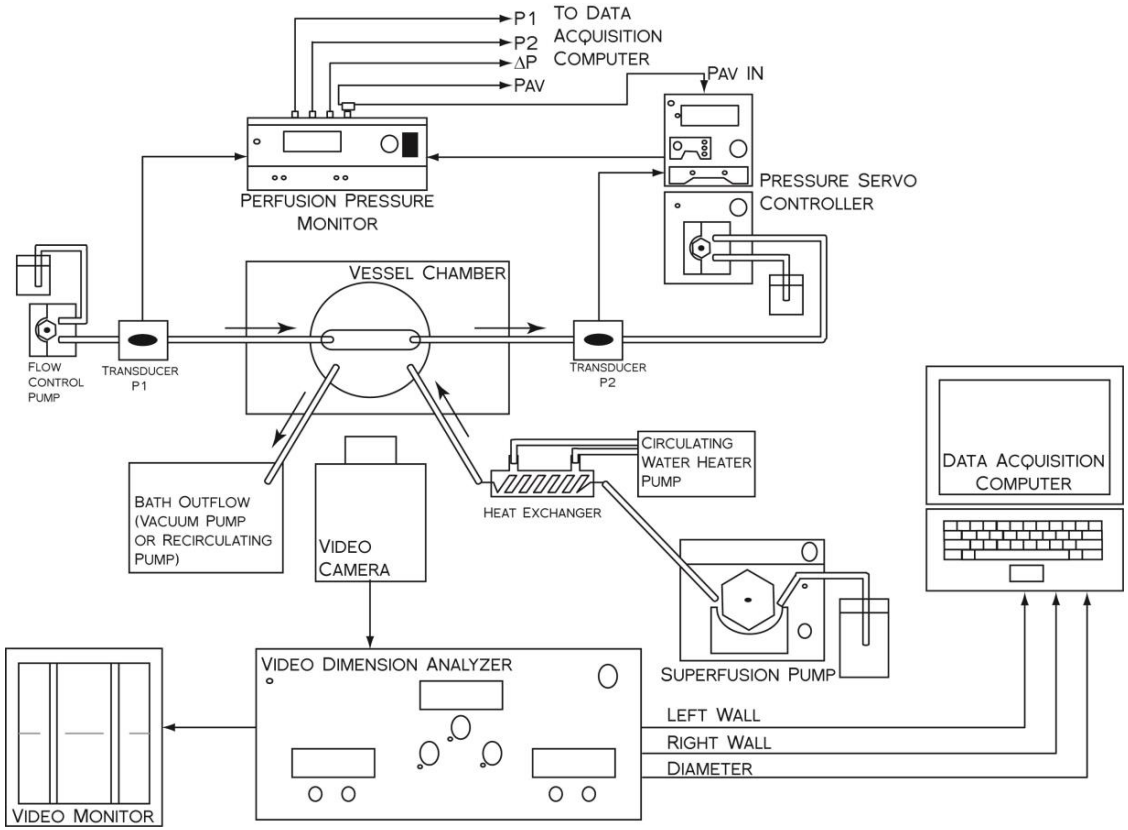
Perfused Resistance Vessels

Advanced Application:
Vascular reactivity can be studied under conditions of constant or variable intraluminal flow (shear stress)





Anatomy of a Pressurized Arteriograph System



Constant Pressure & Flow

- Key components include a pressure source, vessel chamber, means for temperature control, and data acquisition hardware/software
- Distal end of vessel remains open
- In addition to the basic items for regulating intravascular pressure, you will also need a pump to control intraluminal solution flow and a way to monitor pressure on both sides of the cannulated vessel

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AFFILIÉ À Université de Montréal

MONTREAL HEART INSTITUTE

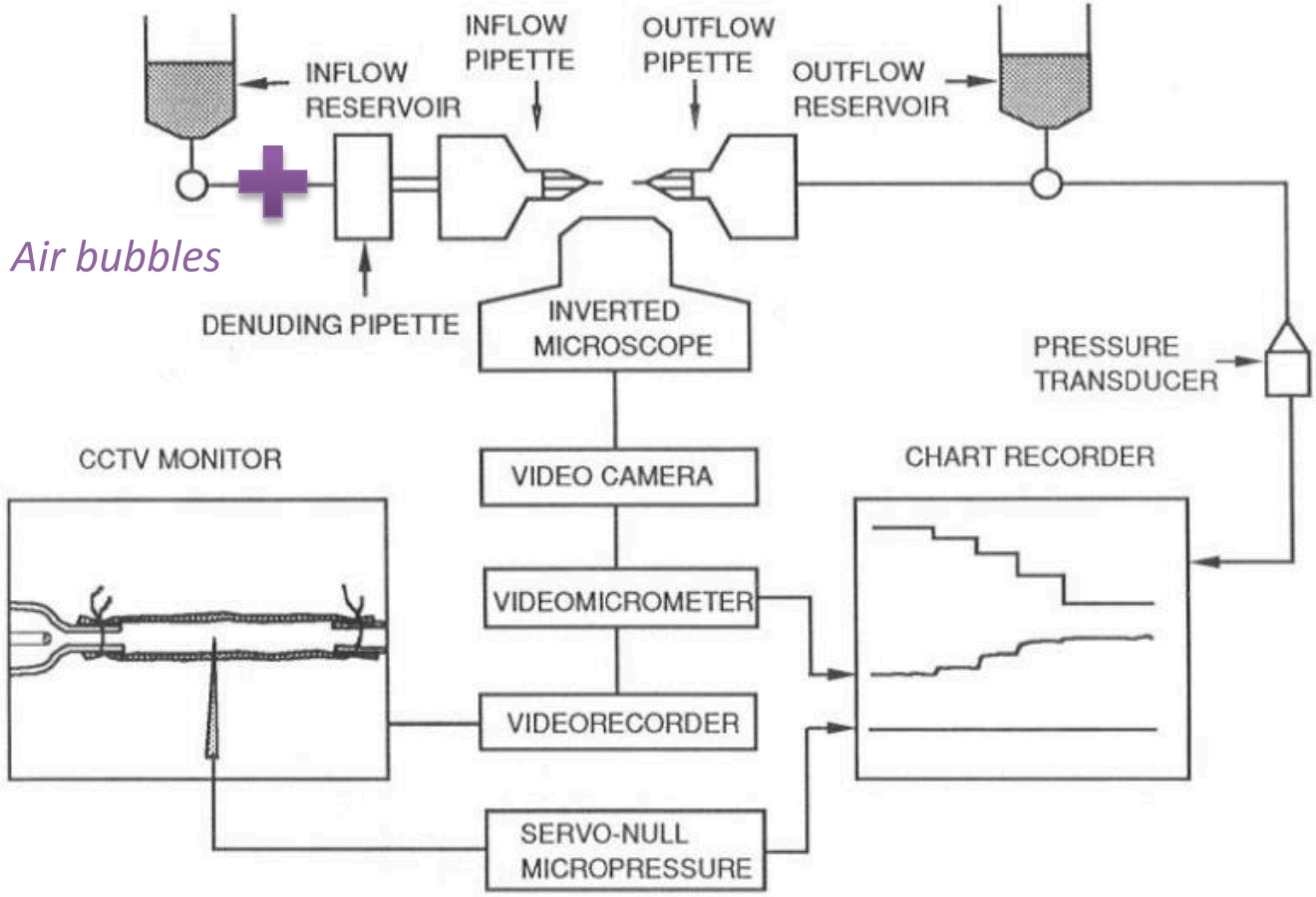
What are we going to cover today?

1. Required equipment
2. How to setup and calibrate instrumentation
3. Best practices, including how to minimize cannulae resistance
4. Sample experimental protocols and applications


There are two types of equipment…
Mechanic & Automatic

Mechanic

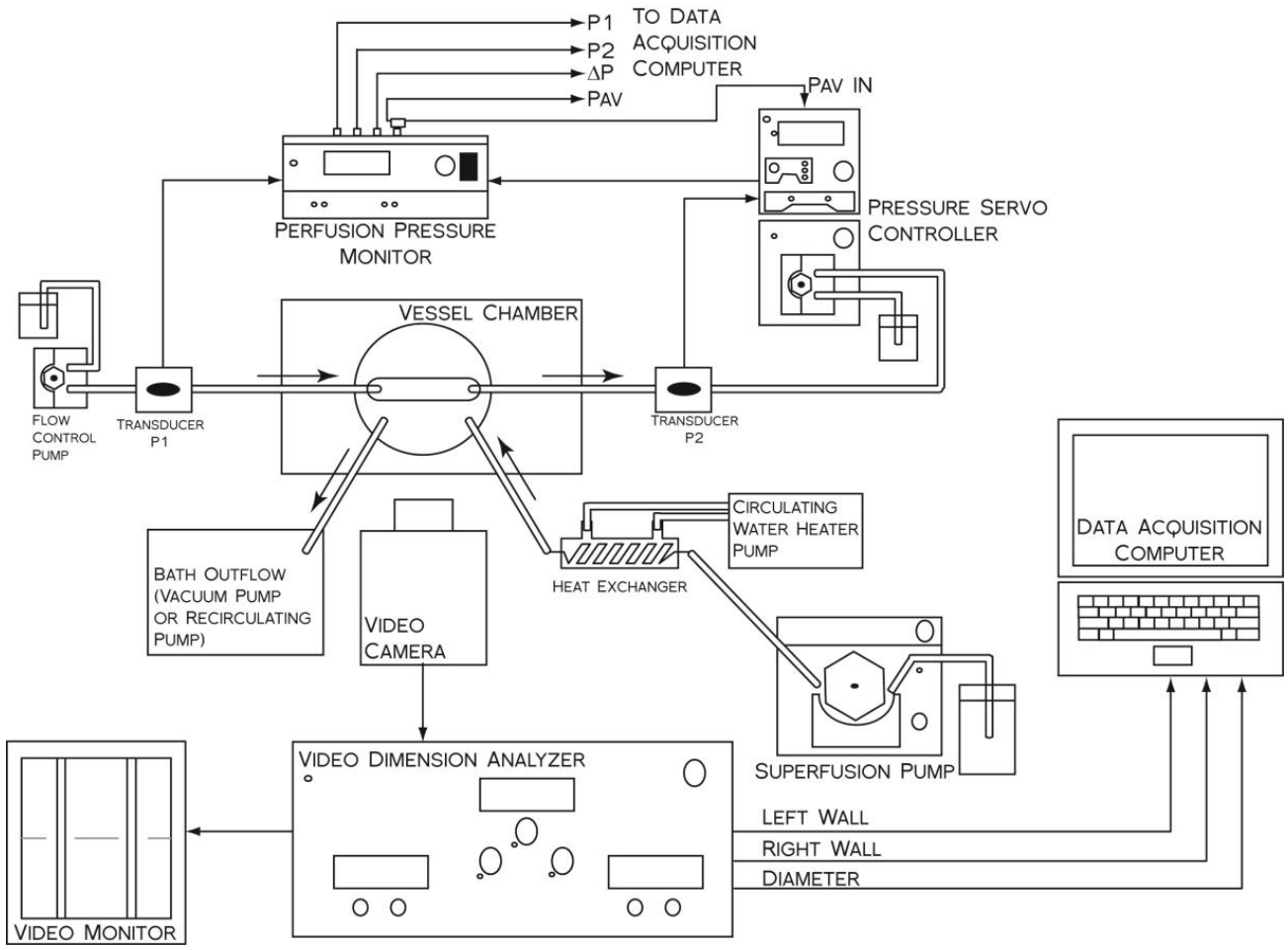
1. Pipettes with equal diameter
2. Two reservoirs
3. Pressure transducers



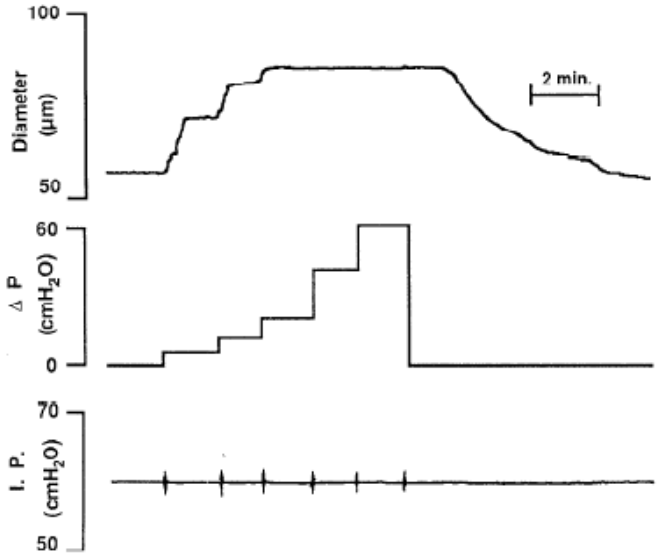
Kuo, Davis & Chilian. Am J Physiol 1990; 259(28): 1063-1070.

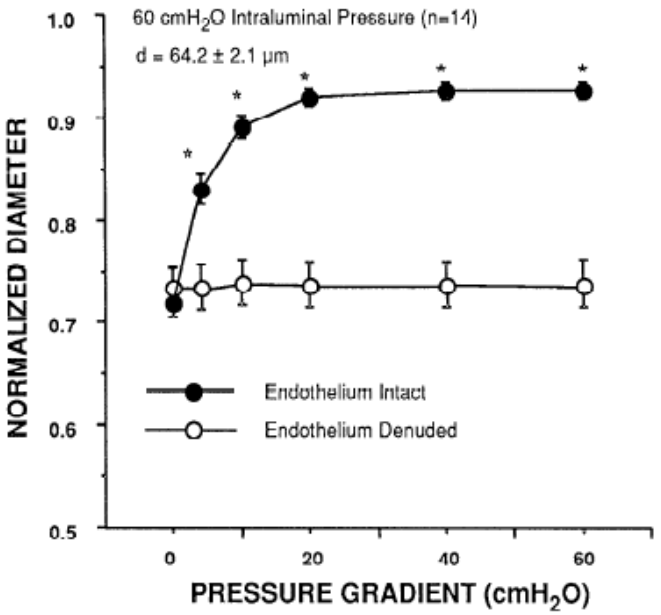
Automatic

1. Pipettes with equal diameter.
2. Pressure Servo
3. Flow pump



Endothelium-dependent, flow-induced dilation of isolated coronary arterioles





- Increasing pressure gradient leads to an increase in flow
- Increase in flow stimulates the endothelium
- The endothelium releases dilatory factors
- This leads to a dilation

Kuo, Davis & Chilian. Am J Physiol 1990;259(28):1063-1070.

An increase in flow increases shear stress

Ex vivo, when using the arteriograph, it is better to express the flow rates that you impose to the arterioles as shear stress values. It will help others to compare their own results to your results

How to calculate shear stress?

$$\tau = 4\eta Q/\pi r^3$$

Where...

τ = dynes/cm2

η = viscosity (0.009 P)

Q = applied flow rate through the lumen (ml/sec)

r = lumen radius (cm)

Excel template to calculate mean shear stress

Shear stress tends to be constant in the body…

Any change in shear stress will be transitory and will induce a dilation. Hence, shear stress will return to baseline.

Langille BL, O'Donnell F, 1986. *Science* 231, 405–407

La Barbera, 1990. *Science* 249, 992-1000

Malek AM, *et al*, 1999. *JAMA* 282, 2035–2042

Bolduc *et al*, 2013. *Am J Physiol* 305, H620–H633

***Ex vivo*,**
the change in flow (shear stress) is the stimulus to induce an arterial response

1. An increase in shear stress stimulates the endothelium

2. The endothelium releases factors primarily NO, but also, depending on the experimental conditions, EDHF, MMPs, transglutaminase, bradykinin, etc…

Shear stress is constant in the body...

Exception: *ex vivo* in diseased animals with a dysfunctional endothelium, a rise in flow will dilate much less and therefore will increase shear stress outside the “normal” range.

Drouin *et al*. Am J Physiol; 300: H1032–H1043, 2011

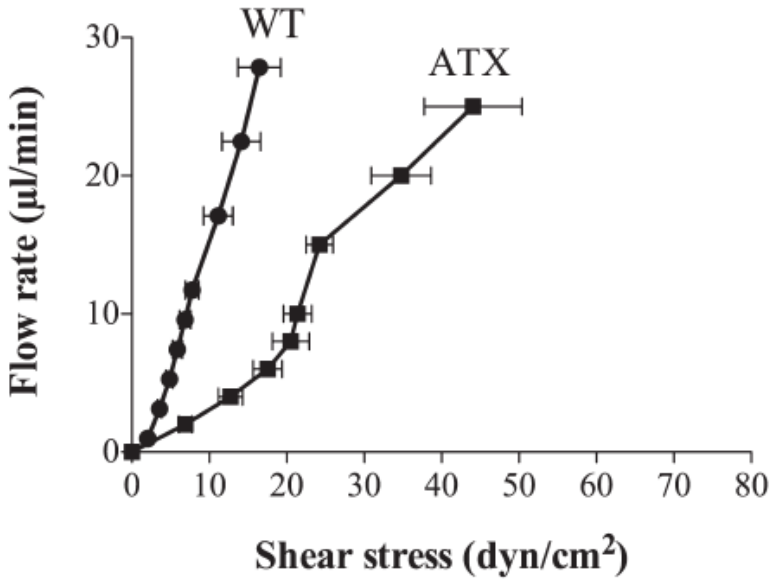


Fig. 7. Correlation between the flow rate (from 0 to 25 µl/min) applied to pressurized cerebral arteries isolated from 3-mo-old WT (n = 10) or ATX (n = 6) mice and the shear stress induced (from 0 to 50 dyn/cm^2). Data are means ± SE of n mice.

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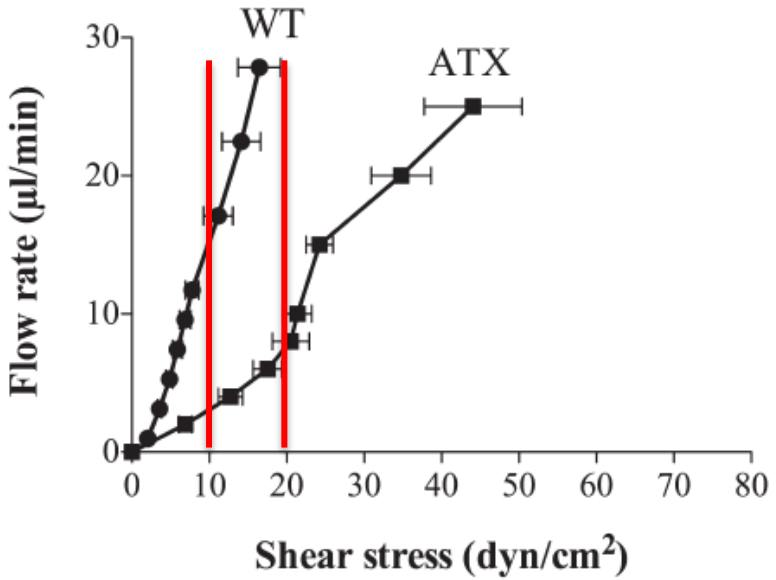

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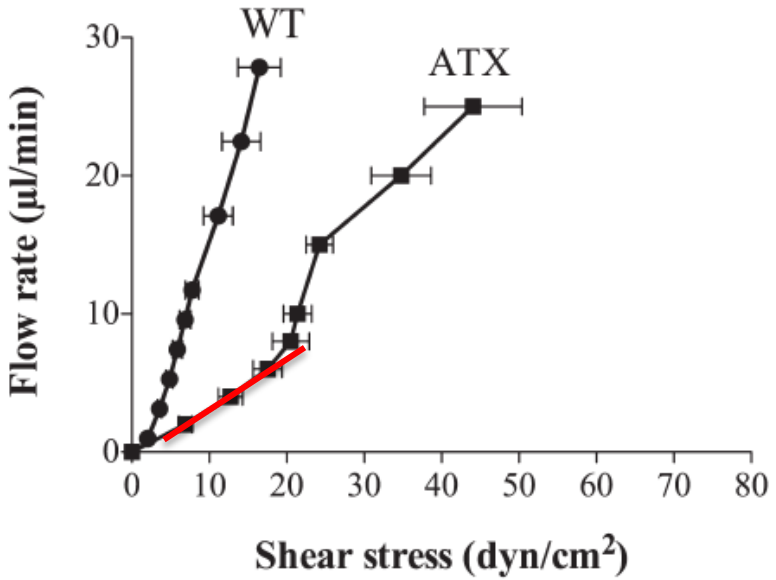

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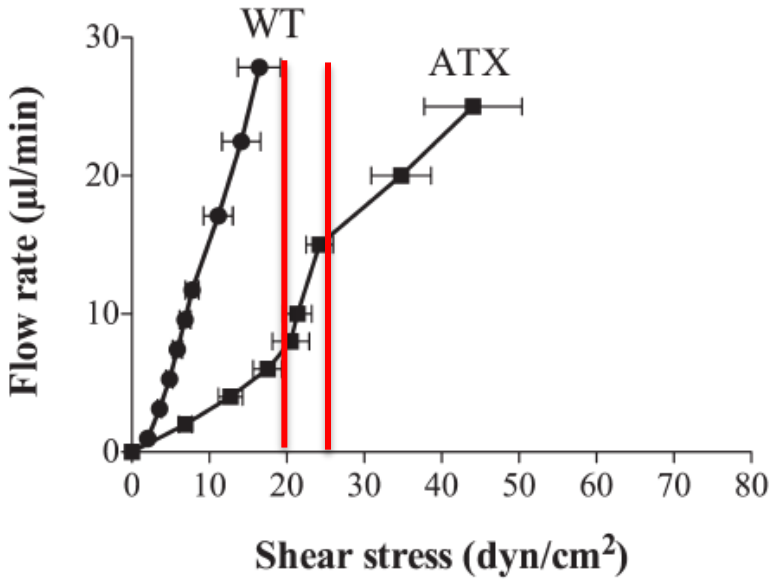

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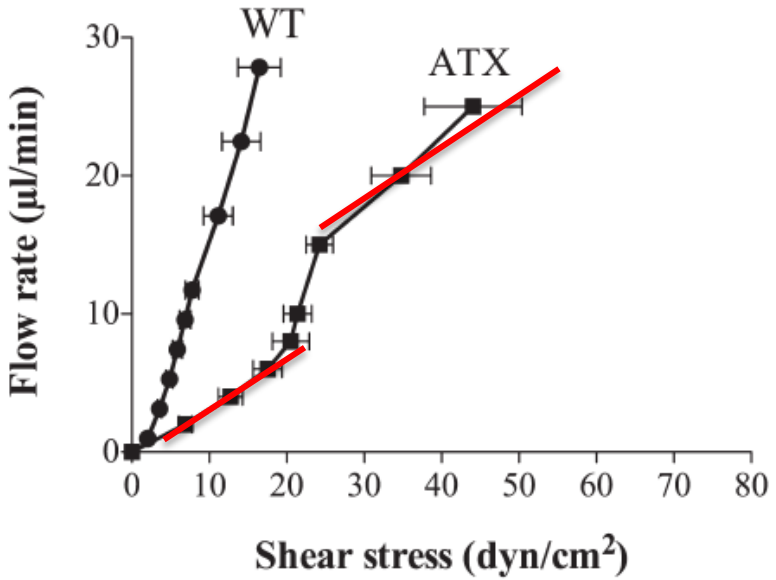

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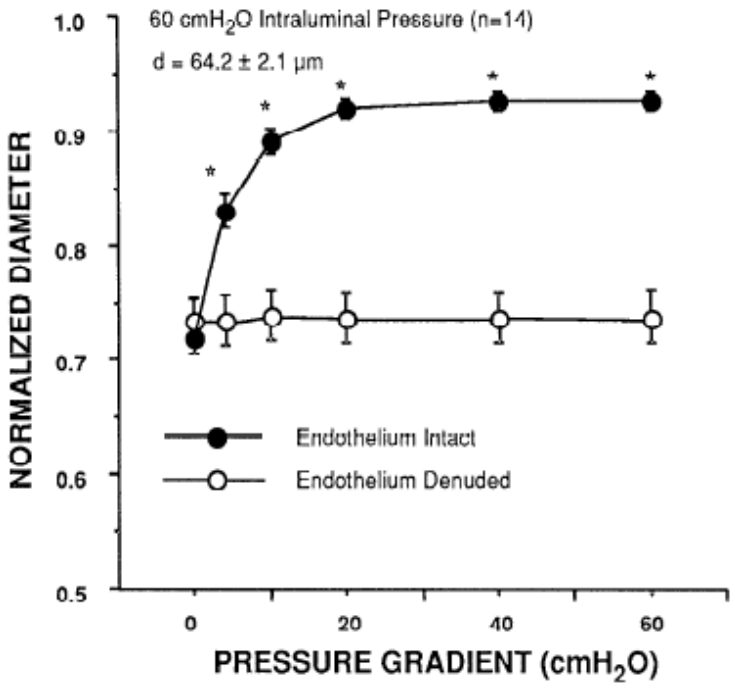
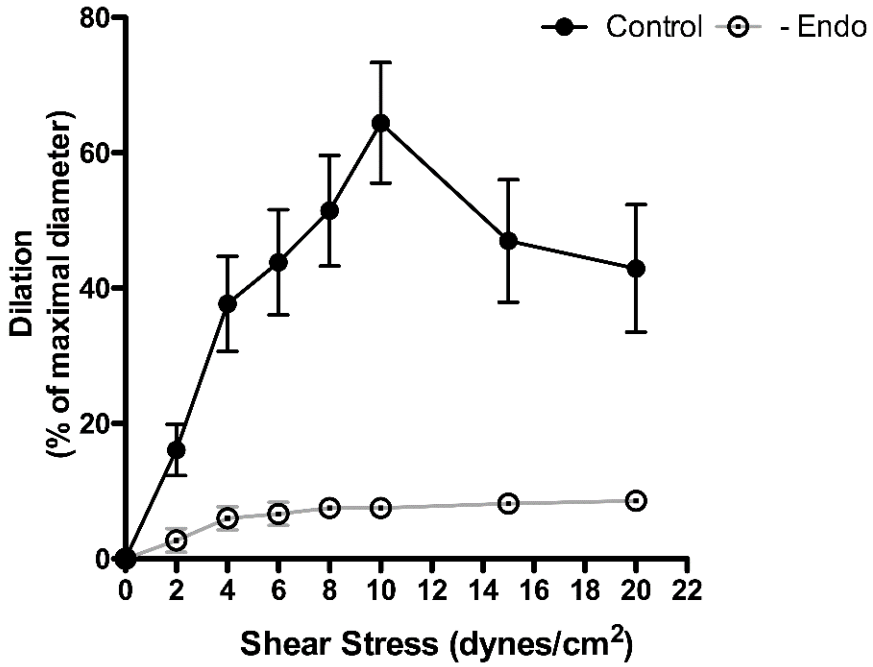
An increase in shear stress may dilate to a stable maximum



- In pig coronary arterioles, increased flow stimulates the endothelium that leads to a dilation
- The dilation reaches a stable maximum despite increasing flow
- Removing the endothelium eliminates the dilatory response

Kuo, Davis & Chilian. Am J Physiol 1990;**259**(28):1063-1070.

An increase in shear stress may dilate to a maximum and then lead to a reduction in diameter



- In mouse cerebral arteries, Increased flow stimulates the endothelium that leads to a dilation
- This dilation declines above shear stress values higher than 10 Dyn/cm^2
- Removing the endothelium eliminates the dilatory response

Raignault, Bolduc, Lesage, Thorin. JCBFM 2016; Jan 28, Epub.

Different responses to a rise in shear stress are possibly due to the following:

1. Difference in vascular bed
2. Shear stress induces the release of dilatory factors only (as in coronary arteries of the previous example)
3. Shear stress induces the release of dilatory factors associated at high shear stress to the release of constricting factors and/or the inhibition of the release of dilatory factors (cerebral arteries)

Summary

- Make sure you have the right equipment and that you master it
- Have two pipettes with similar diameter
- Practice, practice, practice
- Until there is a direct relationship between shear stress and diameter

Langille BL, O'Donnell F, 1986. *Science* 231, 405–407

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Malek AM, *et al*, 1999. *JAMA* 282, 2035–2042

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Thank You!

For additional information on Pressure Arteriograph Systems, Wire Myographs and solutions for studying vessel function in-vitro please visit: **www.livingsys.com**

Eric Thorin, PhD
eric.thorin@umontreal.ca

Gerry Herrera, PhD
info@livingsys.com

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