A. James Clark School of Engineering
Fischell Department of Bioengineering

Biomarkers and therapeutics for women's cardiovascular health

Alisa Morss Clyne, Keystone Professor

Disclaimers

Dr. Clyne's Funding:

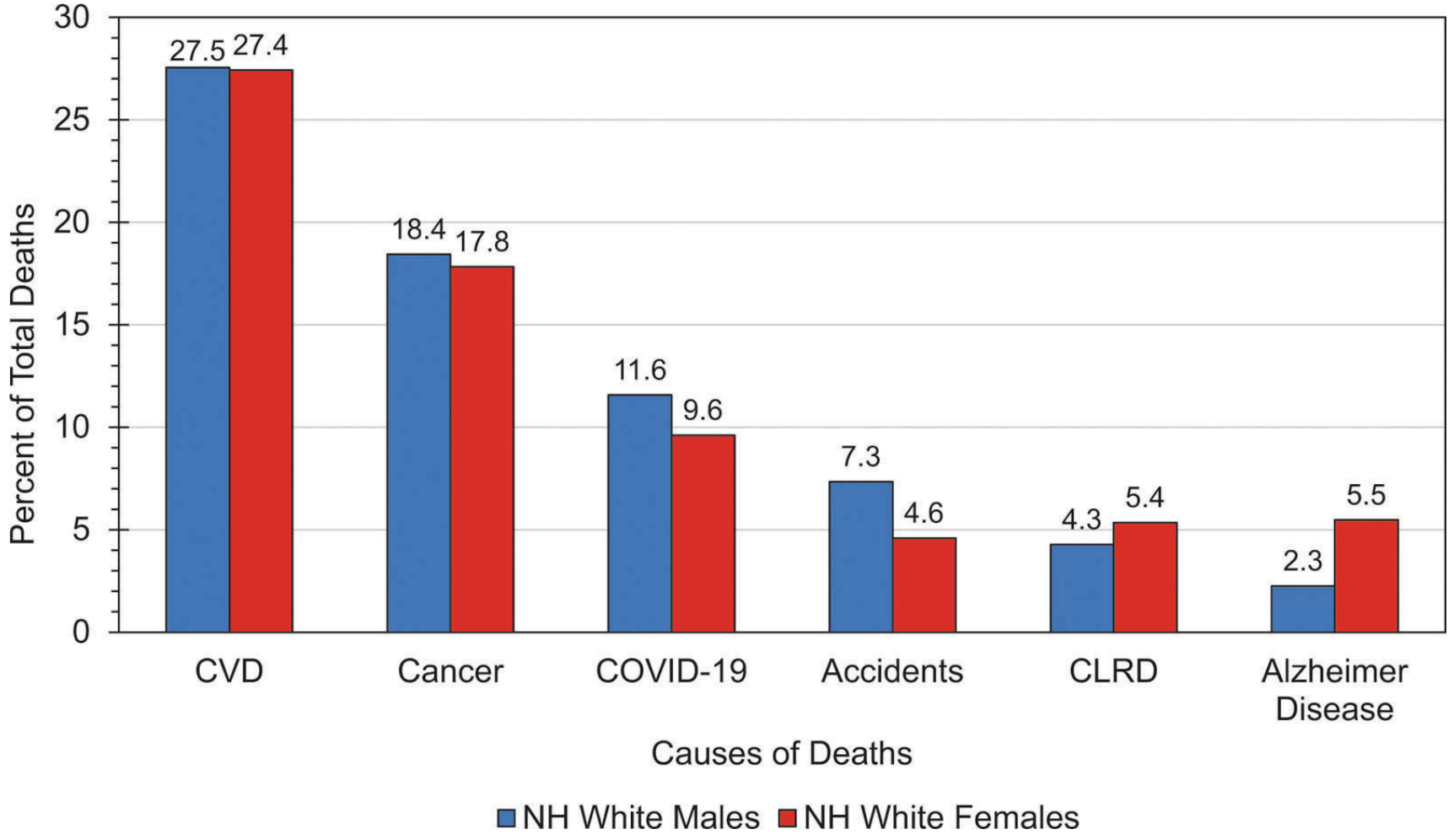
Current

- NIH NIGMS T34GM149472
- NIH NHLBI R01HL165193
- NSF CBET 2211966
- NSF CBET 1916997

Past (related to presented research)

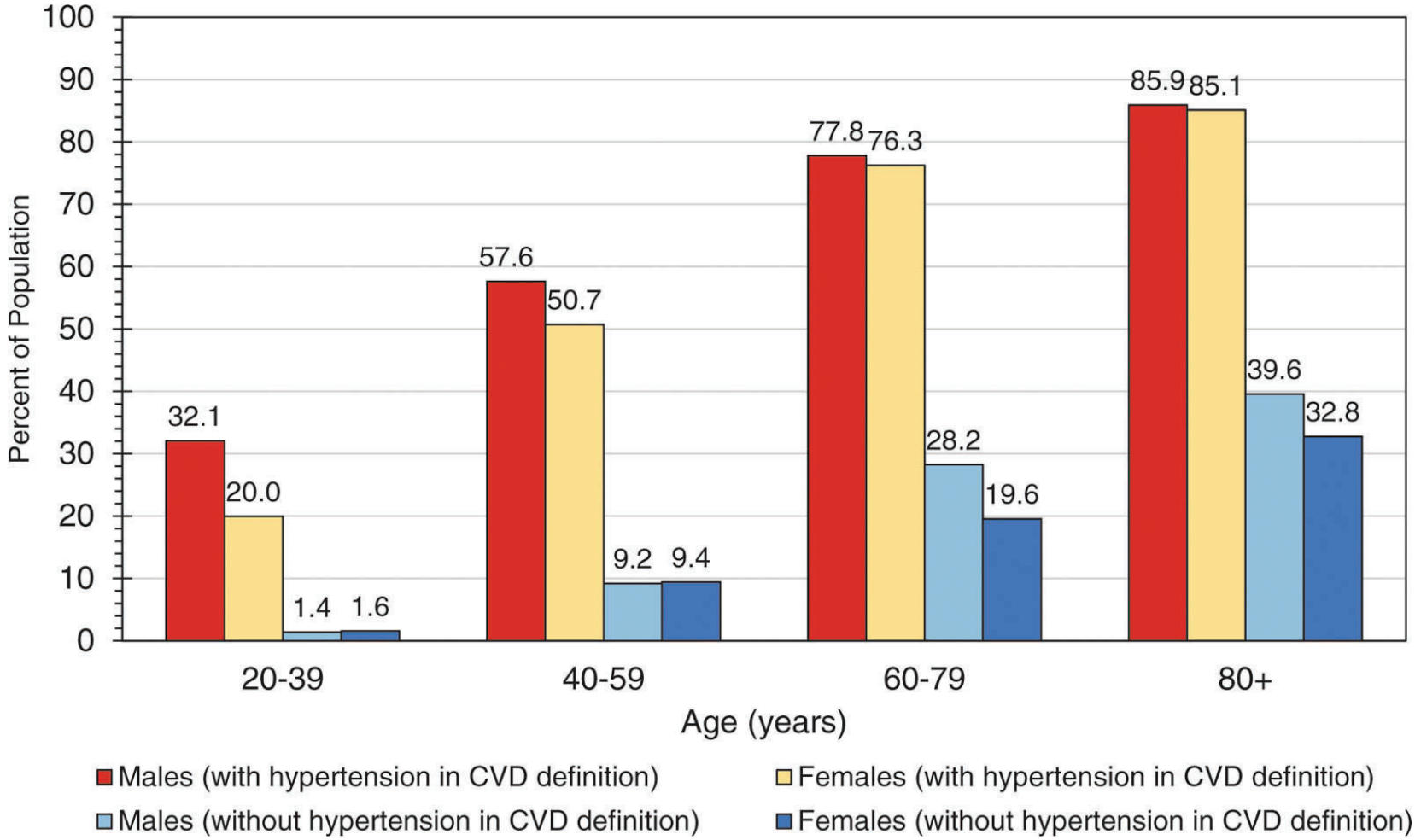
- UMD Brain and Behavior Initiative
- NIH R21EB028466
- NSF CMMI 1916814
- American Heart Association 17GRNT33460280

Heart disease remains the leading cause of death in the United States.



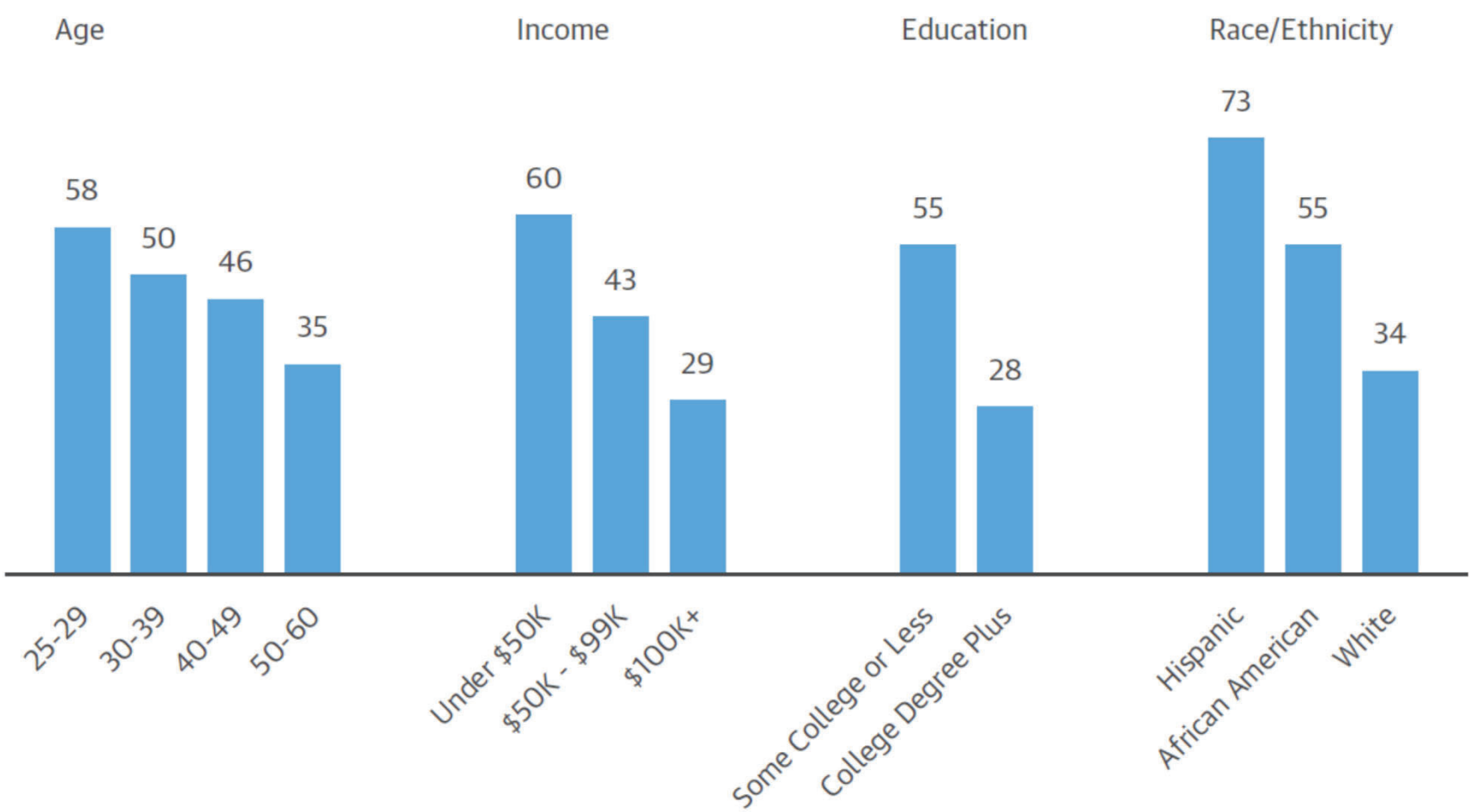
Martin *et al*. *Circulation.* 2024 Heart Disease and Stroke Statistics

CVD prevalence is similar for men and women across the lifespan.



Martin *et al*. *Circulation*. 2024 Heart Disease and Stroke Statistics

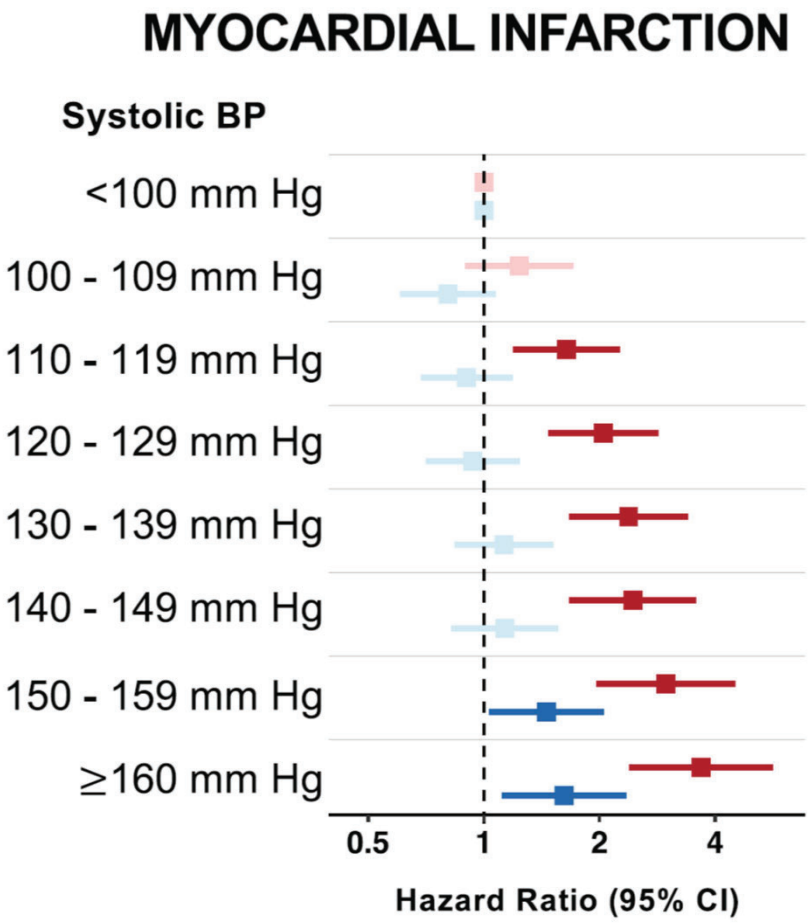
Many women are unaware that CVD is the #1 cause of death among women



Mertz *et al*. JACC. 2017

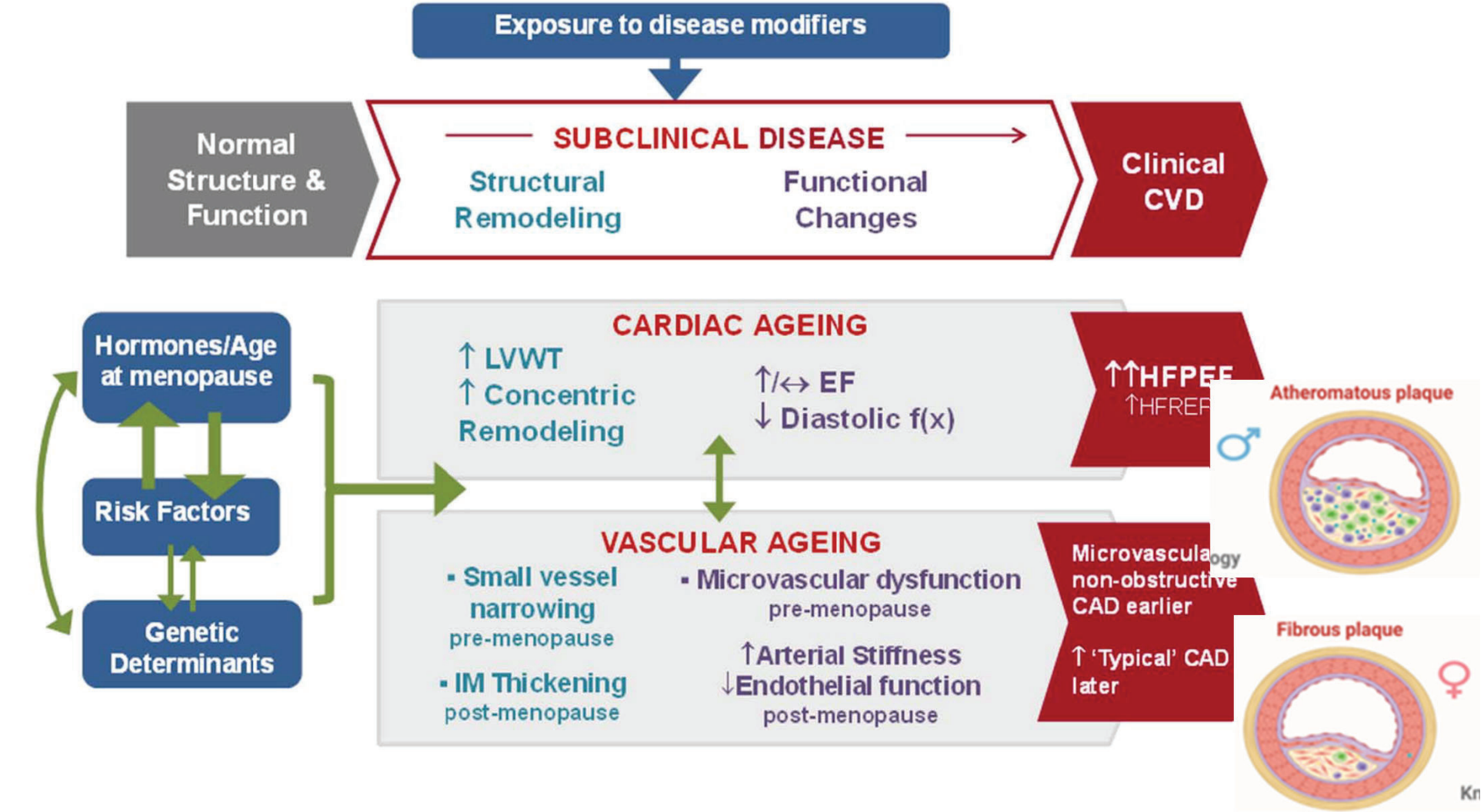
Women experience greater adverse effects from CVD risk factors.

- Diabetes increases cardiovascular risk by 3-7 fold in women as compared to 2-3 fold in men (Recarti *et al Curr. Hyperteans. Rep.* 2015)
- CVD mortality, MI, heart failure, and stroke are higher in women compared to men with diabetes (Angoulvant *et al Diabetes Metabol* 2021)
- Female smokers have a 25% greater CVD risk than male smokers (Huxley *et al Lancet* 2011)
- Women with obesity have a 64% increased risk of coronary heart disease compared to 46% in men with obesity (Wilson *et al Arch. Intern. Med.* 2002)



Ji *et al*. Circulation 2021

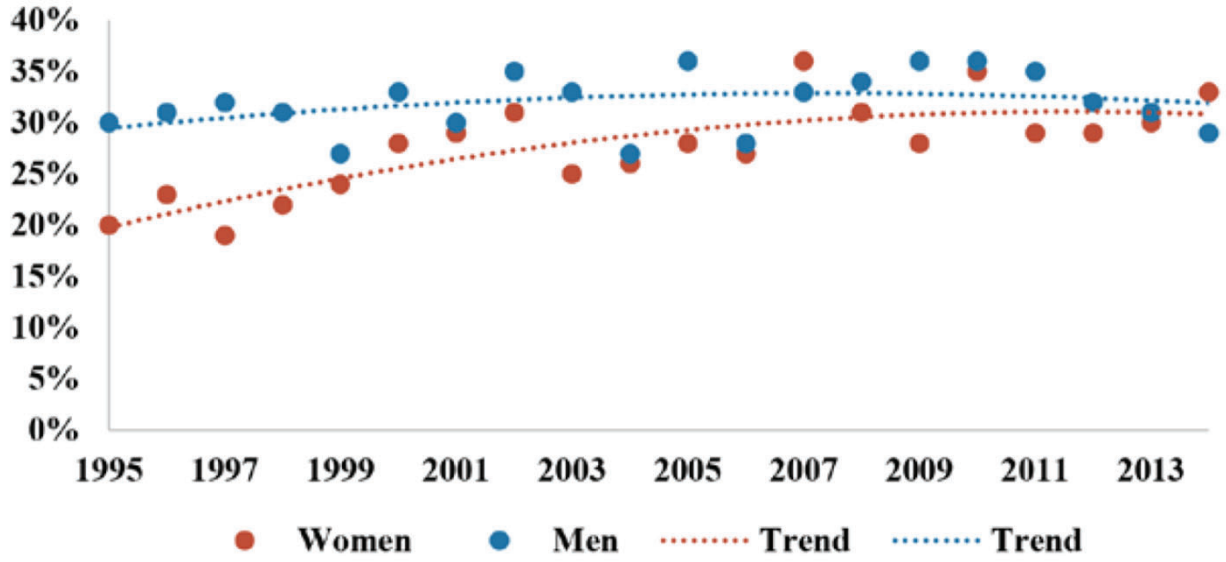
Women experience differences in cardiac and vascular ageing.



Sakkers *et al*. Atherosclerosis 2023

Merz and Cheng. *Heart* 2016

Women have lower probability of being appropriately treated for CVD.



%AMI hospitalizations attributable to young (35-54 years) patients

Therapy	Women vs Men (Ref.): Relative Probabilities*				Trend‡
	1995–1999	2000–2004	2005–2009	2010–2014	*P* Value
Aspirin	0.96 (0.86 - 1.02)	0.98 (0.90 - 1.04)	1.02 (0.93 - 1.06)	0.96 (0.86 - 1.03)	0.8
Non-aspirin antiplatelet†	—	0.95 (0.83 - 1.06)	0.75 (0.61 - 0.89)	0.79 (0.65 - 0.94)	0.3
Lipid lowering agent†	—	1.01 (0.89 - 1.11)	0.86 (0.72 - 0.98)	0.80 (0.67 - 0.91)	0.005
Beta blocker	0.94 (0.83 - 1.04)	1.00 (0.92 - 1.06)	0.98 (0.89 - 1.04)	0.92 (0.81 - 0.99)	0.2
Invasive angiography	0.94 (0.82 - 1.06)	0.95 (0.84 - 1.06)	0.91 (0.76 - 1.05)	0.88 (0.73 - 1.03)	0.3
Revascularization	0.97 (0.81 - 1.14)	0.88 (0.74 - 1.02)	0.63 (0.49 - 0.79)	0.72 (0.56 - 0.88)	0.002

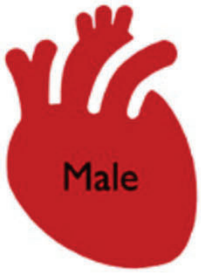
Arora *et al*. Circulation. 2019

Women have worse outcomes after CVD interventions.

- Women have worse outcomes after ST-segment–elevation myocardial infarction than men due to a worse risk factor profile, lower utilization of reperfusion therapies, lower use of medications, delayed symptom to presentation, and longer door-to-balloon time.
- Women have a worse outcome after percutaneous coronary intervention due to older age, higher rates of hypertension, diabetes, obesity, 3-vessel disease, and longer door-to-ballon time even in countries with universal health care.

Khraishah *et al*. ATVB. 2023

The female CV system is also biomechanically distinct from the male CV system.



	Male	Female	Sex Difference
↑ Lean body mass[1] (*kg*)	56.7 ± 7.9	36.5 ± 5.0	- 36 %
↑ Whole heart mass[2,3] (*g*)	331.0 ± 56.7	245.0 ± 52.0	- 26 %
↑ LV mass[4] (*g*)	173.9 ± 39.7	114.5 ± 23.5	- 34 %
↑ LV stroke volume[6] (*mL*)	89.75 ± 15.26	69.32 ± 19.69	- 23 %
LV ejection fraction[6] (%)	53.65 ± 6.47	57.17 ± 5.08	+ 7 %
↑ RV mass[7] (*g*)	52 ± 10	39 ± 5	- 25 %
↑ RV stroke volume[8] (*mL*)	88.3 ± 21.6	75.0 ± 17.9	- 15 %
↑ RV ejection fraction[8] (%)	62 ± 10	69 ± 10	+ 11 %
↑ Heart rate[11] (*bpm*)	74.3 ± 8.9	79.1 ± 8.2	+ 6 %
↑ Cardiac output[12] (*L/min*)	5.9 ± 1.4	4.6 ± 0.8	- 22 %

↑ indicates statistically significant difference, $P < 0.05$

St. Pierre *et al*. Frontiers in Physiology. 2022

However, women's health research largely focuses on reproductive/maternal health.

TRACK: Women's Health

SUB-TRACKS

- Reproductive and Maternal Health
  - Menstruation, Fertility Issues, Oncofertility, Contraception, Pregnancy, Childbirth, Maternal Health, and Postpartum Care
- Gynecological Health
  - PCOS, Endometriosis, Adenomyosis, Fibroids, Ovarian cysts, Pelvic Floor Disorders, Vaginitis, Gynecological Cancers
- Breast Health
  - Breast cancer, Benign Breast Conditions, Breast Surgery, Breast Reconstruction, Breast Imaging, Nipple Shields, Lactation
- Menopause and Postmenopausal Health
  - Management and Understanding of Menopause Symptoms, Osteoporosis, Osteoarthritis, Cardiovascular Health Post-menopause
- Athletic Health
  - Musculoskeletal Health, Injury Prevention, Exercise Across the Lifespan, Biomechanics, Athletic Performance and Lifetime Fitness
- Sex-related Differences in Health
  - Autoimmune Diseases, COVID-19, Cardiovascular Disease, Diabetes, Thyroid Disorders, Mental Health, Pain, Sleep
- Other / Non-specified

We use animal, human, and computational models to study CVD mechanics and metabolism.

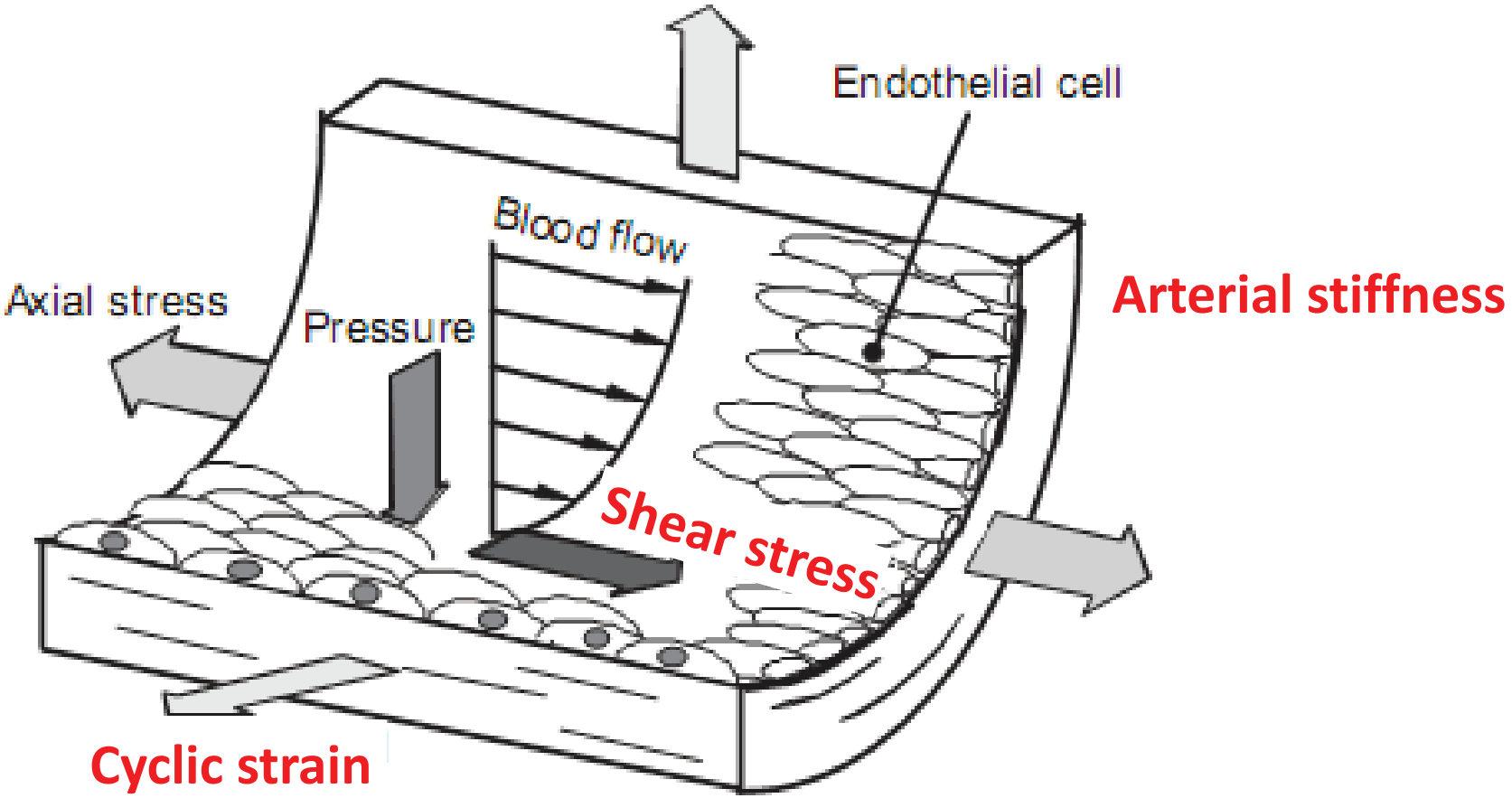
- Endothelial mechano-metabolism
- High-fat diet effect on perivascular adipose tissue in rats
- Stress effects on human endothelial cell function
- Computational models predict intracellular fluxes
- Human cardiovascular disease models for biomarker and therapeutic discovery

We use animal, human, and computational models to study CVD sex differences.

Endothelial mechano-metabolism

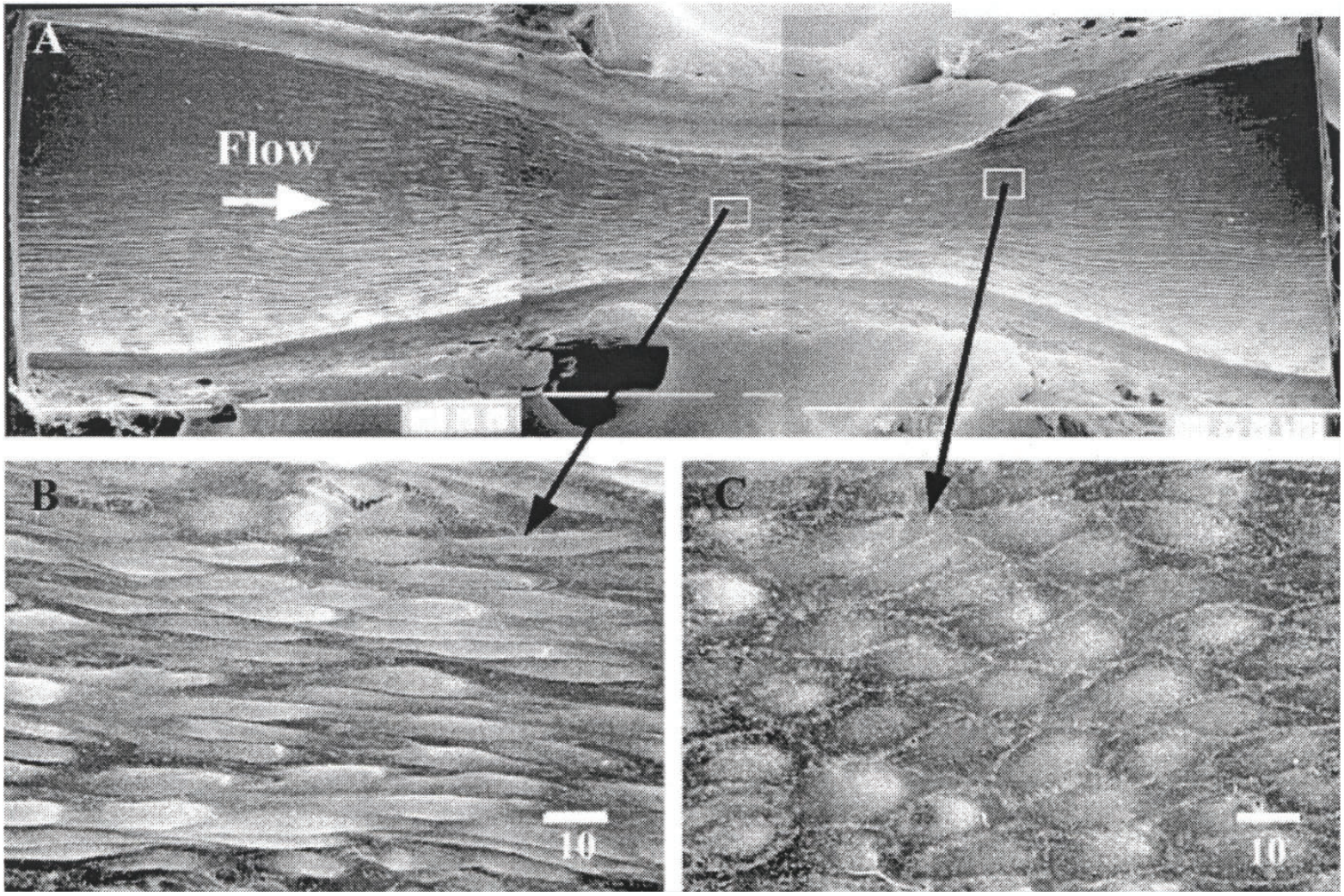
- High-fat diet effect on perivascular adipose tissue in rats
- Stress effects on human endothelial cell function
- Computational models predict intracellular fluxes
- Human cardiovascular disease models for biomarker and therapeutic discovery

Endothelial cells line blood vessels where they sense forces from the blood and the vessel wall.



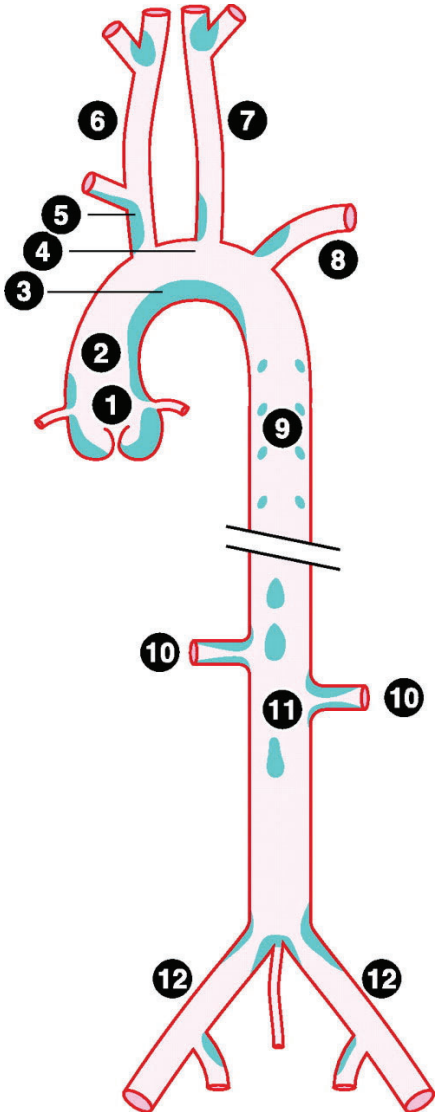
Ohashi, T. and M. Sato, (2005) *Fluid Dynamics Research*

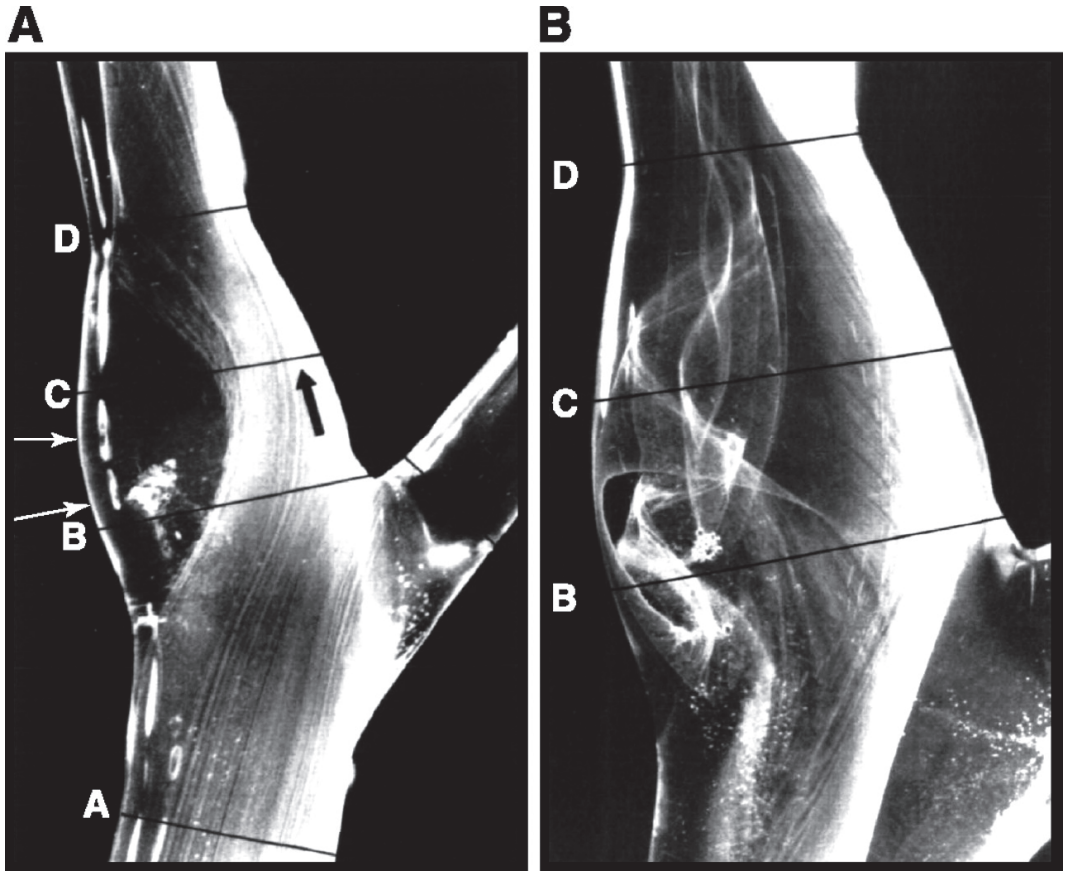
Endothelial cells elongate in high shear stress regions; disease occurs at low shear stress



Matsuda H *Microscopy Research and Technique* 2003

Atherosclerosis preferentially develops at arterial branches and curves where flow is disturbed.

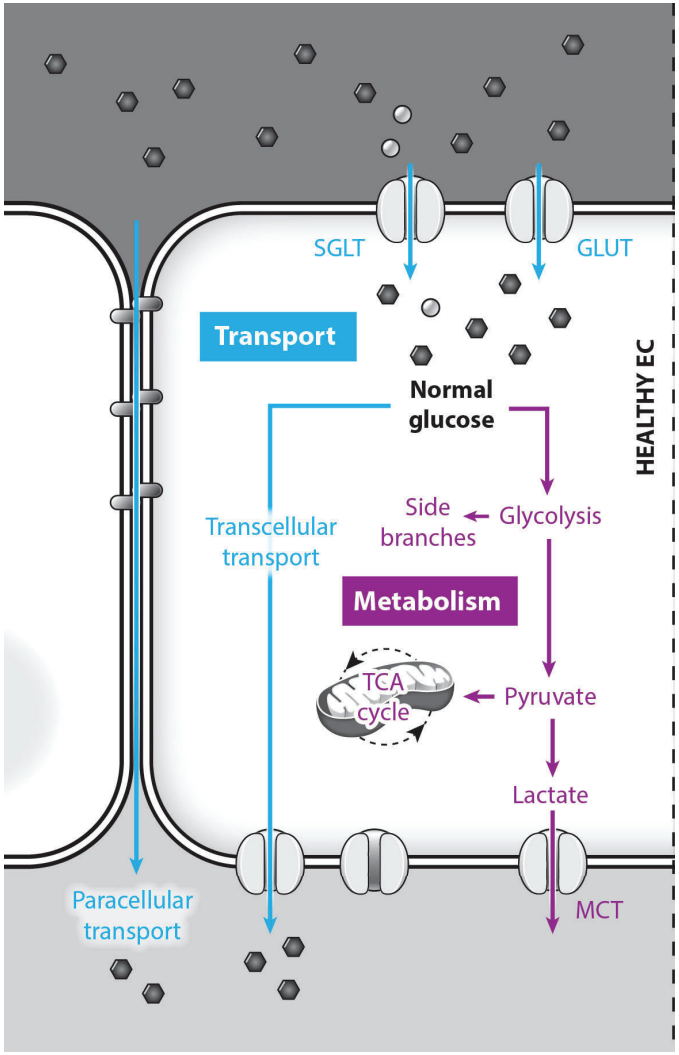




Jeng-Jiann Chiu, and Shu Chien Physiol Rev 2011;91:327-387

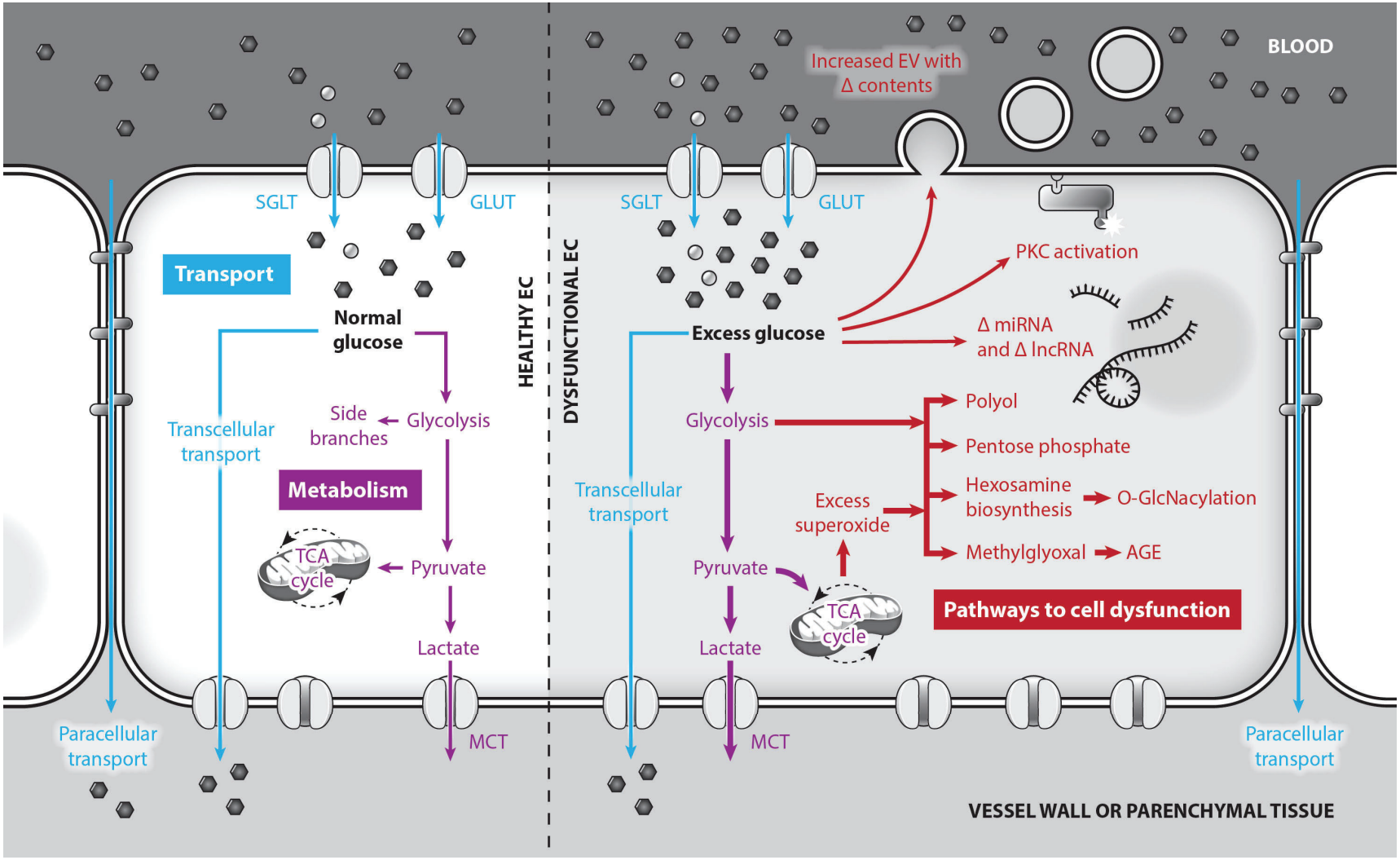
Zarins *et al*. Circ Res 1983; 53: 502–514

Endothelial cells also manage nutrient uptake and transport from blood to tissue.



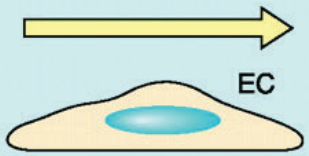
Clyne. *Biochem Soc Trans* 2021

Endothelial cells become dysfunctional in altered metabolic environments.



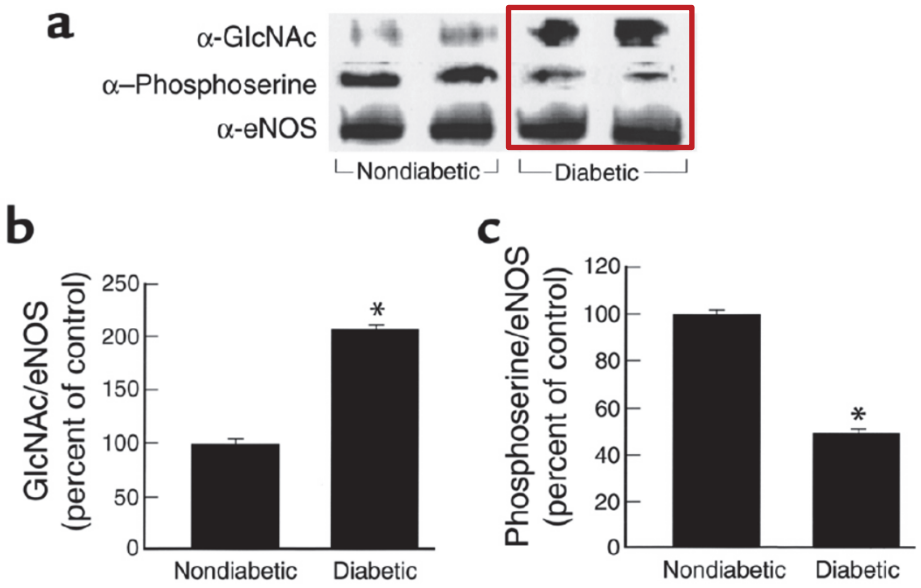
Clyne. *Biochem Soc Trans* 2021

The hallmark of endothelial cell dysfunction in disturbed flow is decreased nitric oxide production.

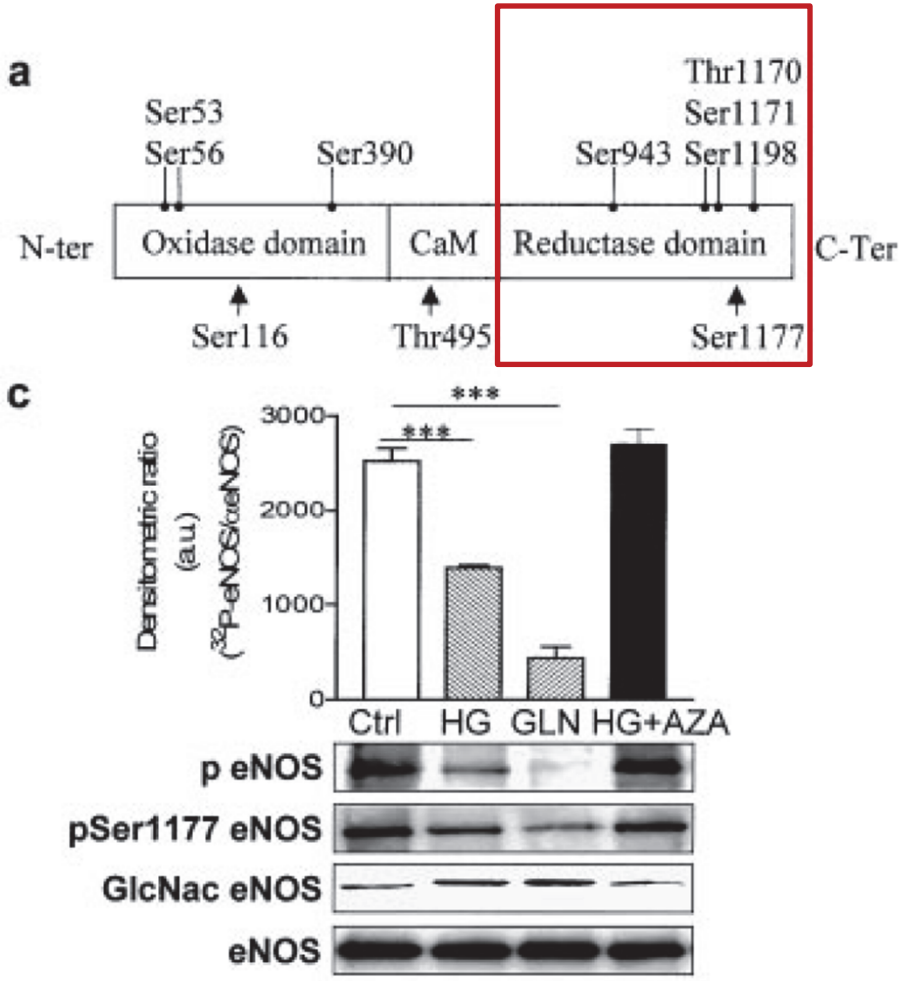
	Laminar flow / high shear stress 	Disturbed flow / low or reciprocating shear stress 
Vasoactivity	Vasodilation	Vasoconstriction
Turnover rate	Low	High
Macromolecular permeability & LDL uptake	Low	High
DNA synthesis	Low	High
Morphology	Elongated & aligned	Polygonal
Expression of adhesion molecules, inflammatory & chemokine genes	Low	High
Expression of antioxidant genes	High	Low
WBC adhesion and platelet aggregation	Inhibition	Promotion
Oxidative stress/ROS	Low	High (Sustained)
VSMC activation	Low	High
Wound repair: Endothelization	Promotion	Retardation
Heterogeneity	Low	High
Fibronectin/fibrinogen deposition	Low	High
Atherosclerosis & thrombosis	Prevention	Promotion

Jeng-Jiann Chiu, and Shu Chien Physiol Rev 2011;91:327-387

In diabetes, eNOS is thought to be GlcNAcylated at or near the Ser1177 phosphorylation site.

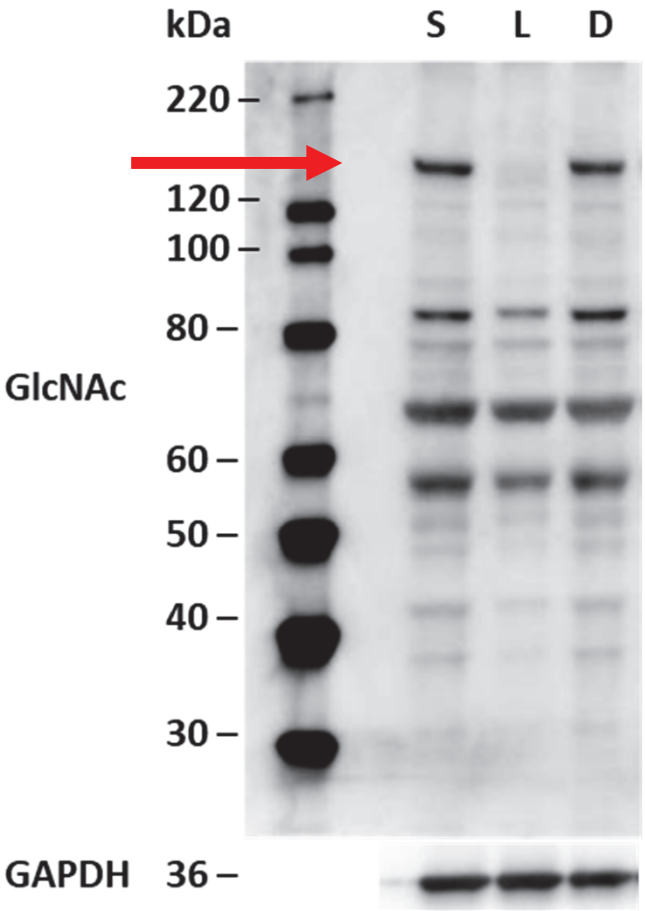


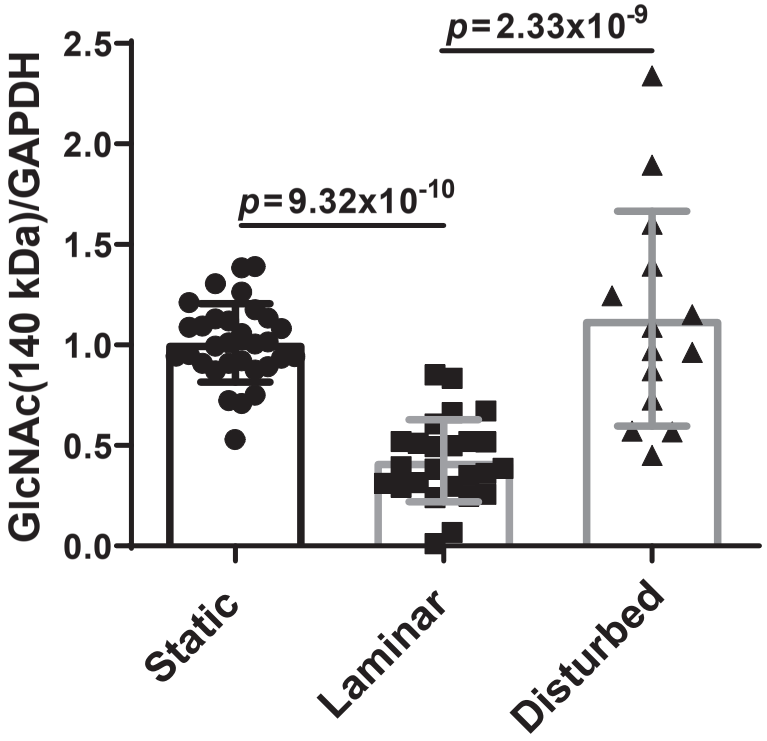
Du *et al*. *JCI* 2001



Federici *et al*. *Circulation* 2002

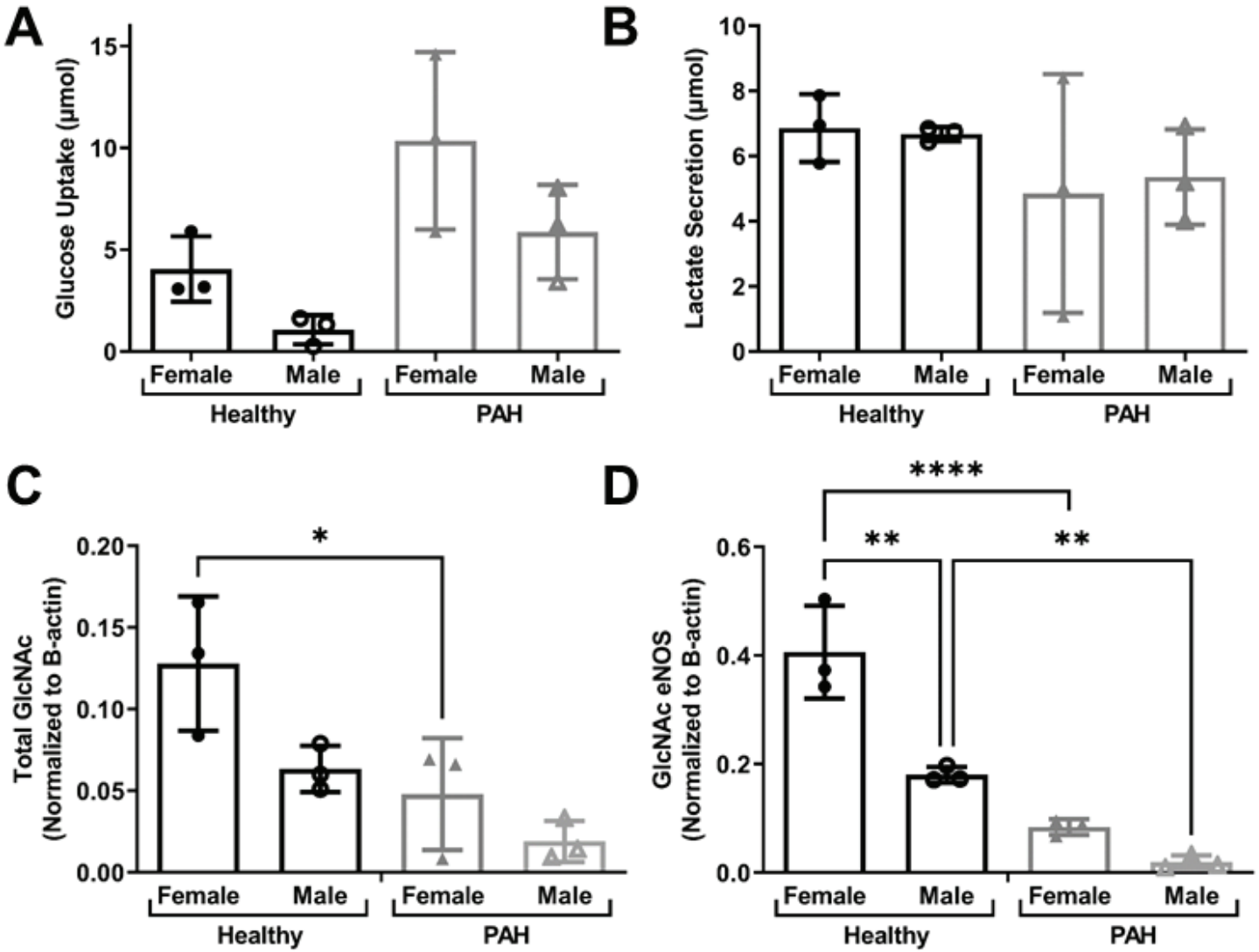
eNOS O-GlcNAcylation essentially disappeared in endothelial cells exposed to steady laminar flow.





Basehore and Clyne. *Circ Research* 2021

Female pulmonary artery endothelial cells had higher glucose uptake and eNOS O-GlcNAcylation.



Basehore and Clyne. *IJTM.* 2024

We use animal, human, and computational models to study CVD mechanics and metabolism.

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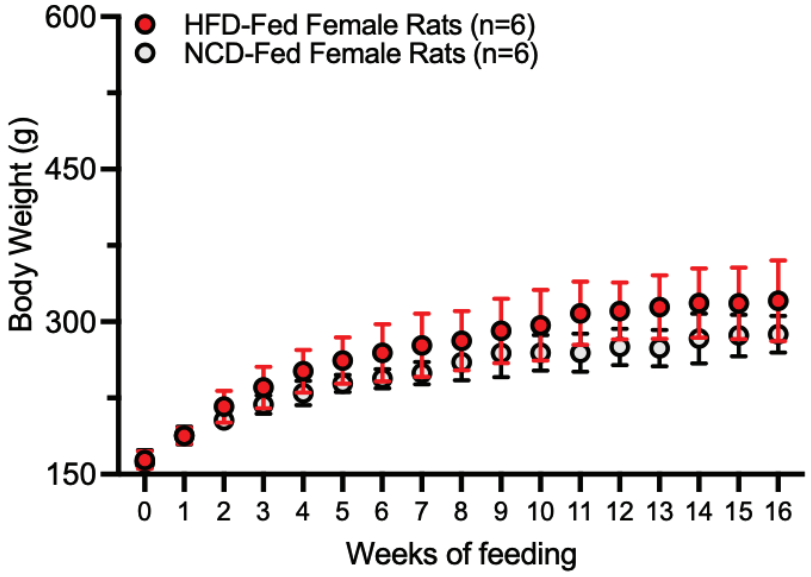
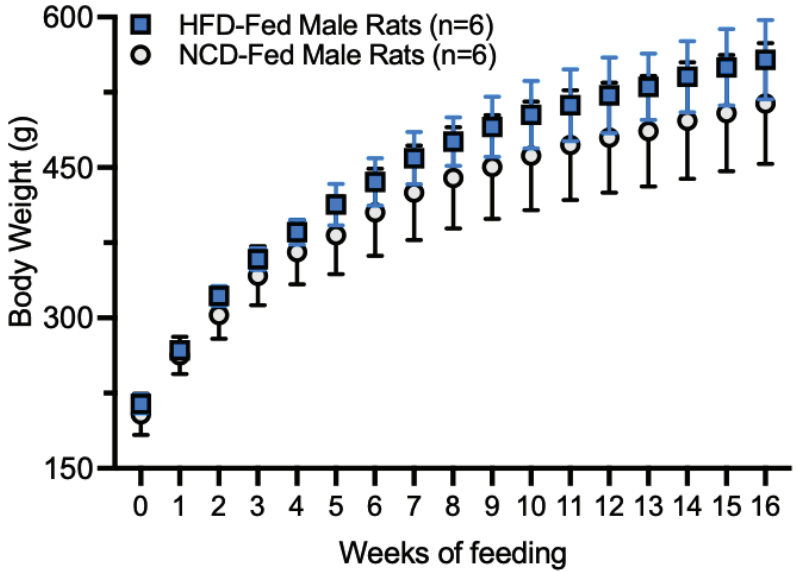
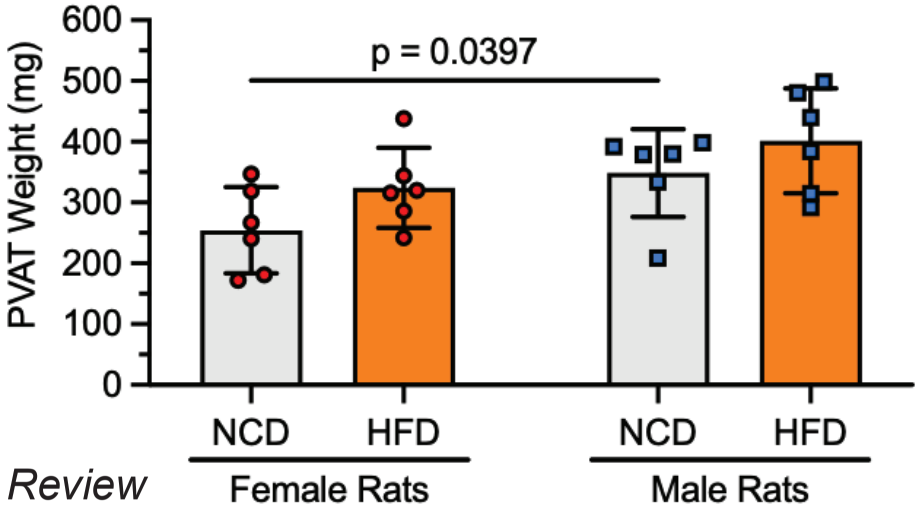
Both male and female rats gained weight and had more PVAT on a high fat diet.



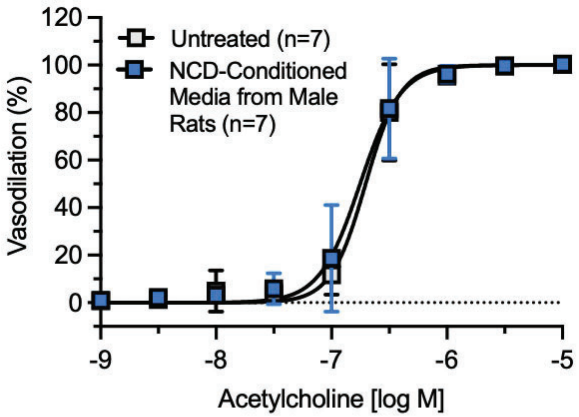




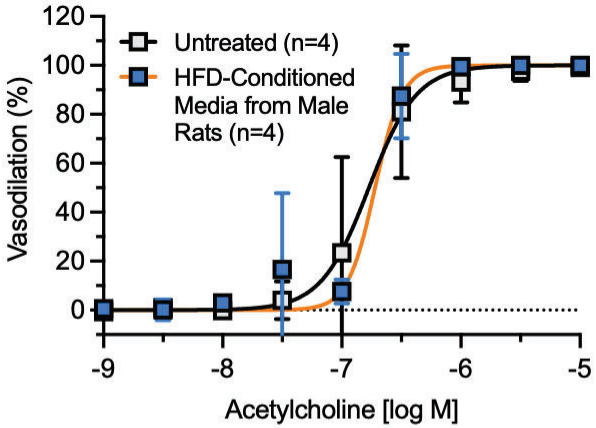
Sangha and Clyne. *In Review*

Vasodilation decreased in response to PVAT conditioned media of high fat diet-fed females only

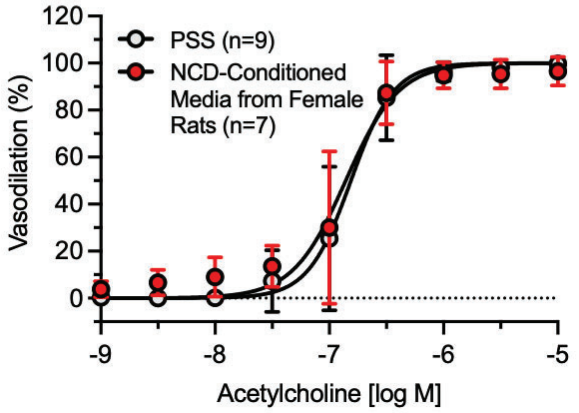
Male Normal Diet



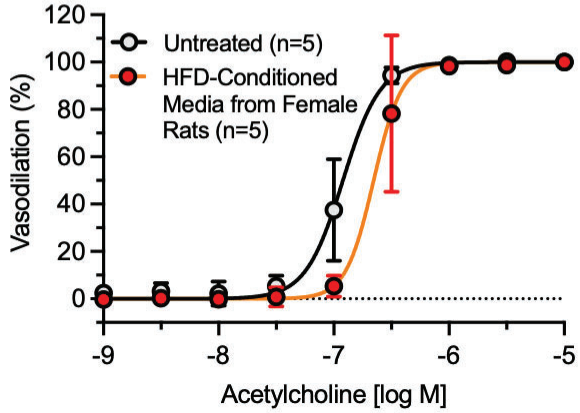
Male High Fat Diet

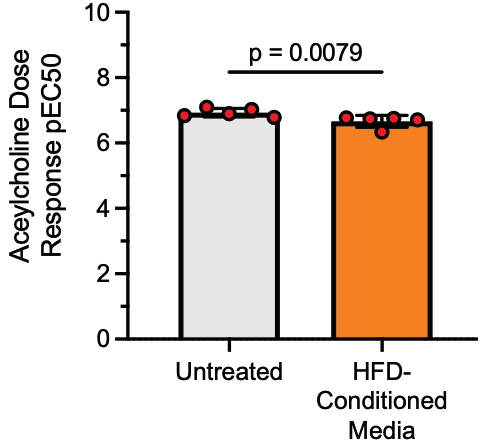


Female Normal Diet



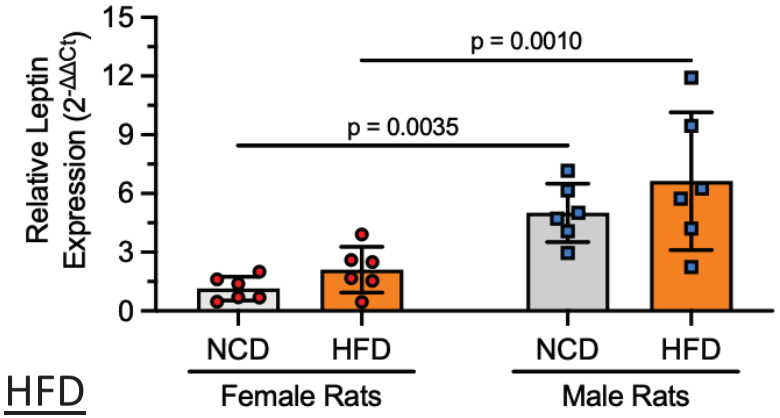
Female High Fat Diet

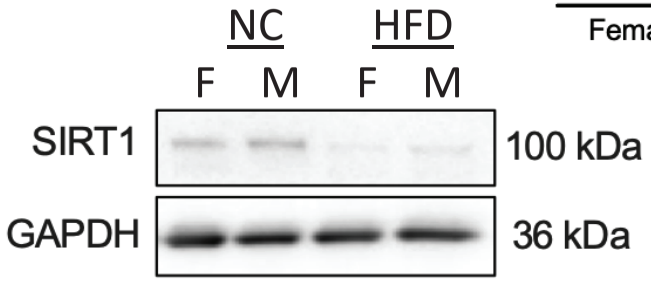


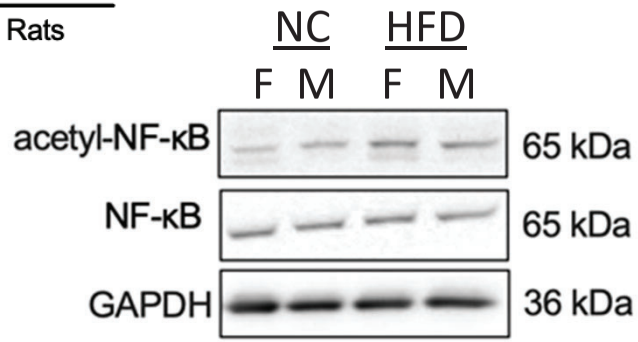


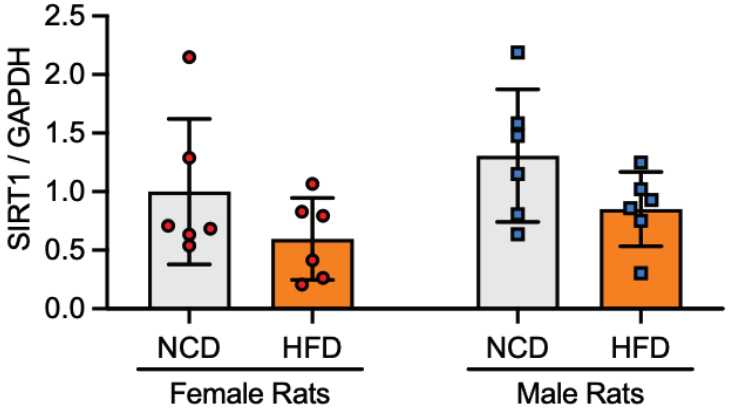
Sangha and Clyne. *In Review*

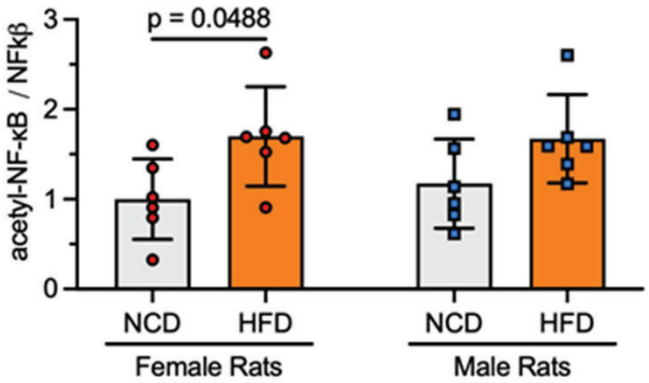
PVAT from rats on a high fat diet had higher leptin, lower SIRT1, and higher acetyl-NF-kB.



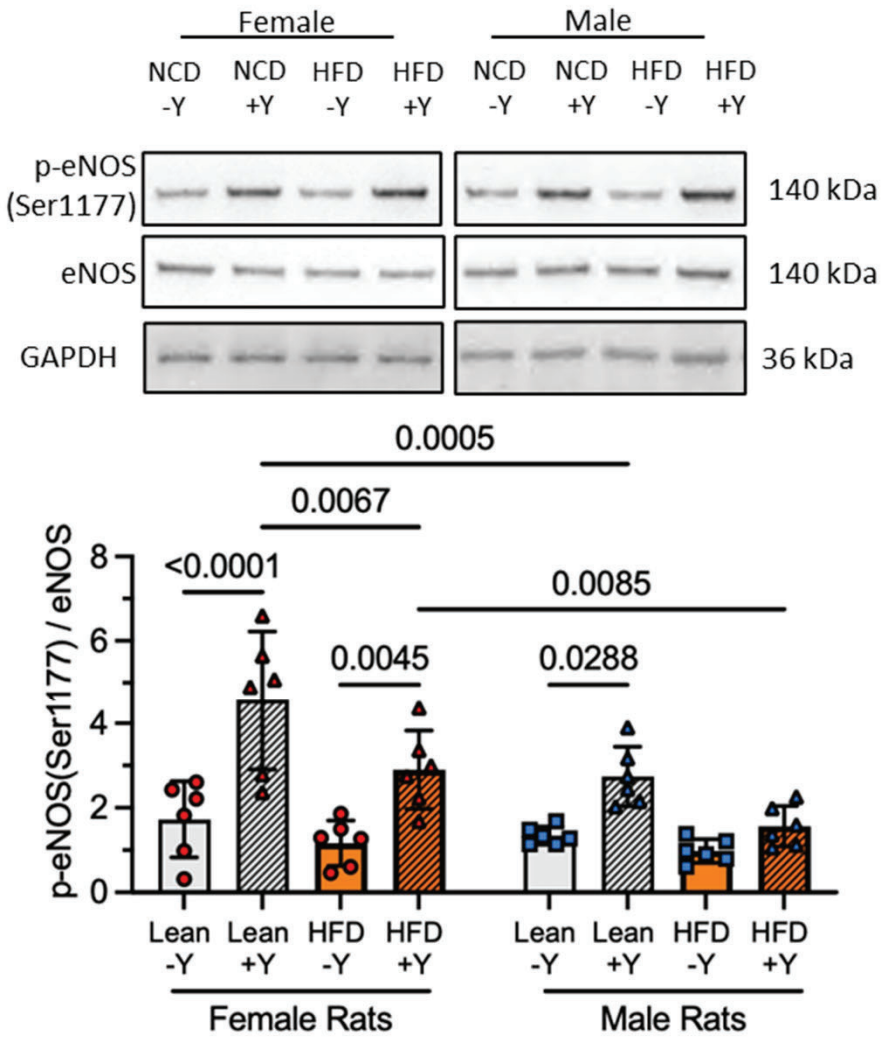


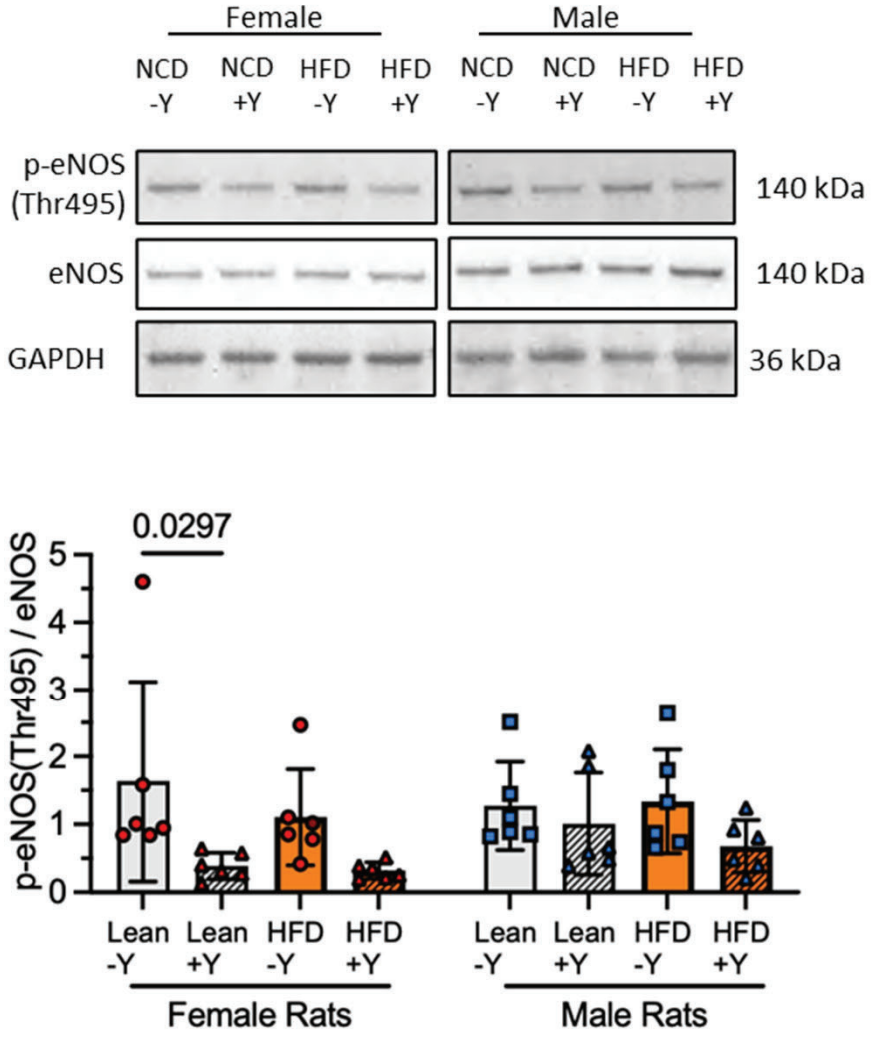






PVAT-conditioned media from high-fat diet-fed rats significantly impaired eNOS phosphorylation





Sangha and Clyne. *In Review*

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- **Stress effects on human endothelial cell function**
- Computational models predict intracellular fluxes
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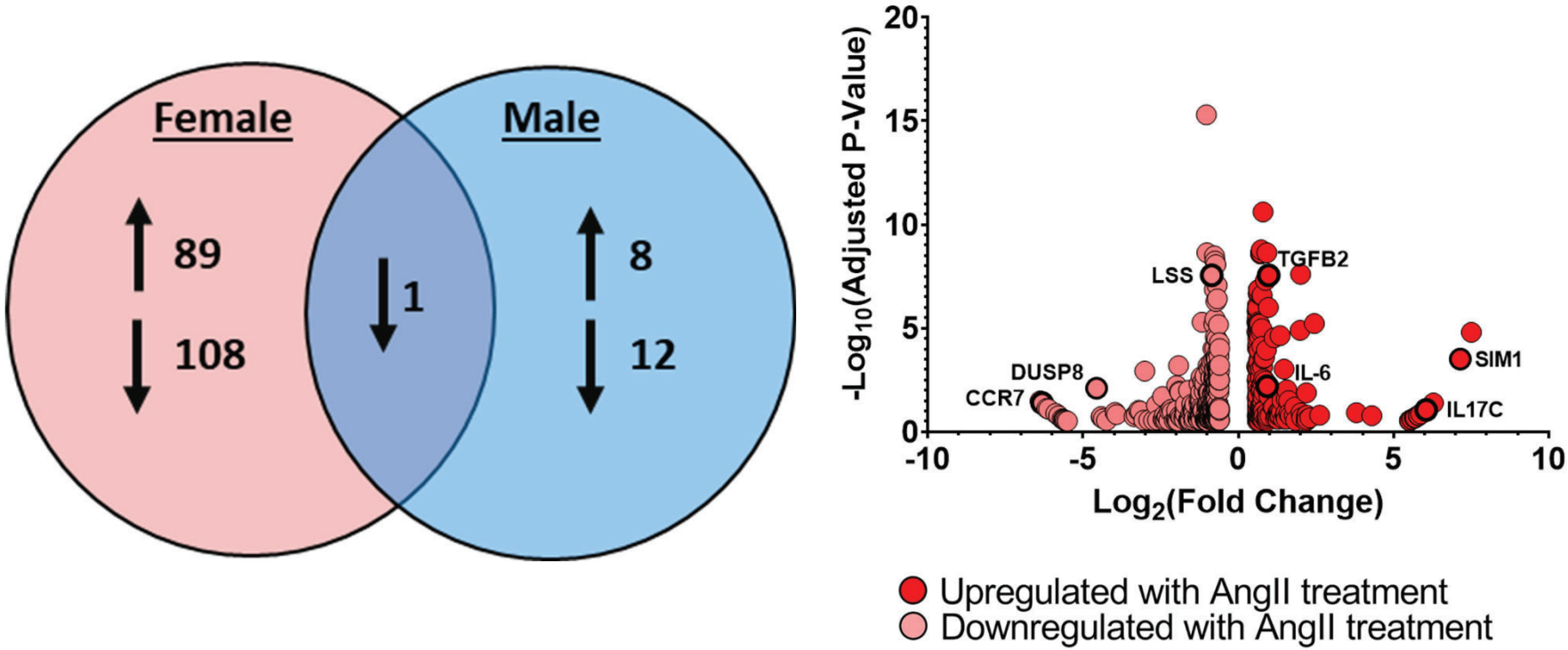
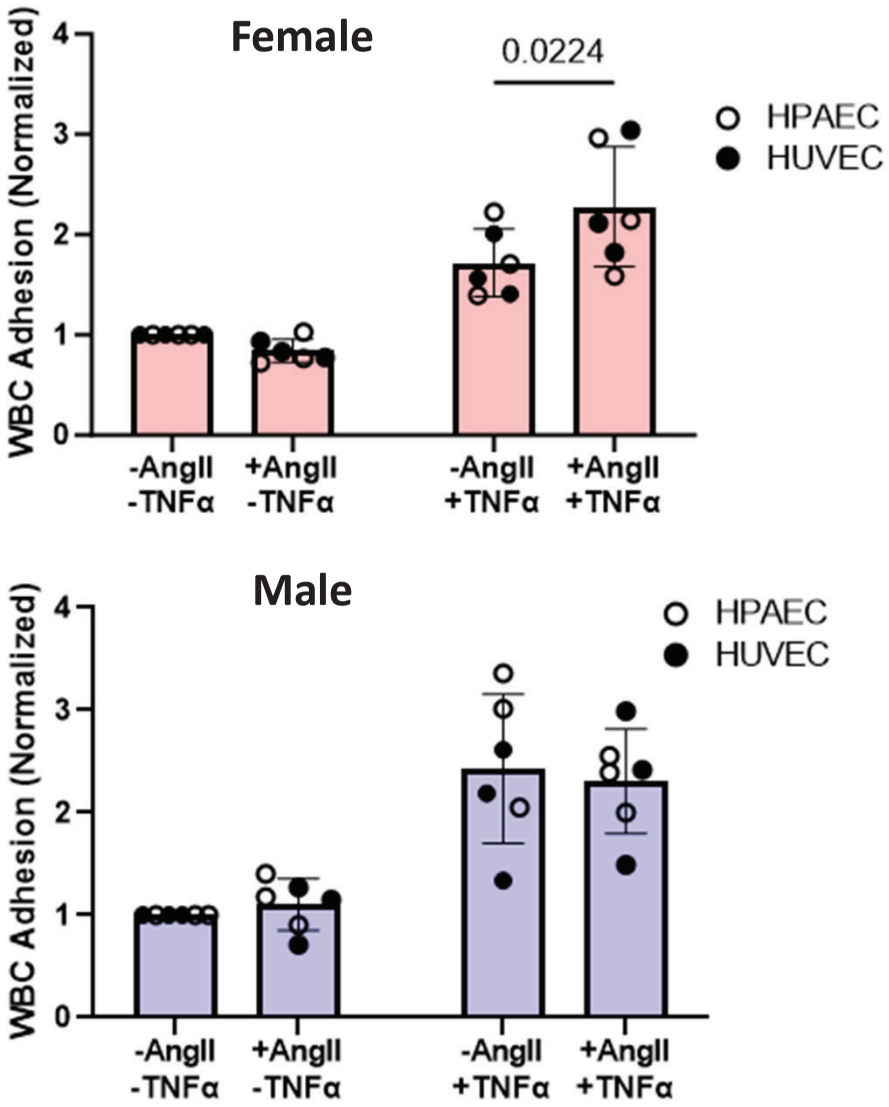
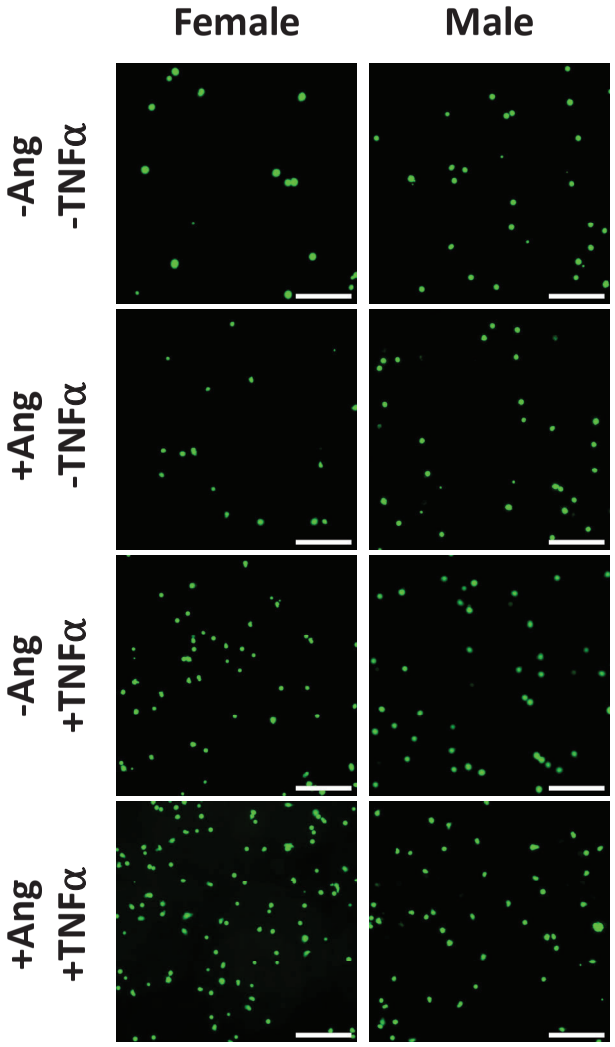
Female endothelial cells differentially expressed more genes in response to AngII treatment.



Weber and Clyne *CMBE* 2023

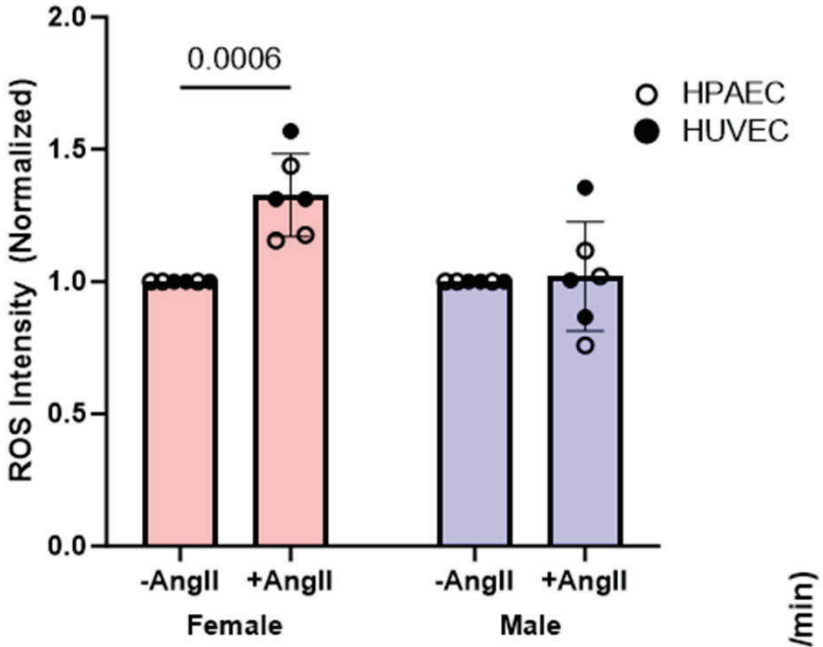
Female HUVEC treated with AngII and TNFα had higher inflammation

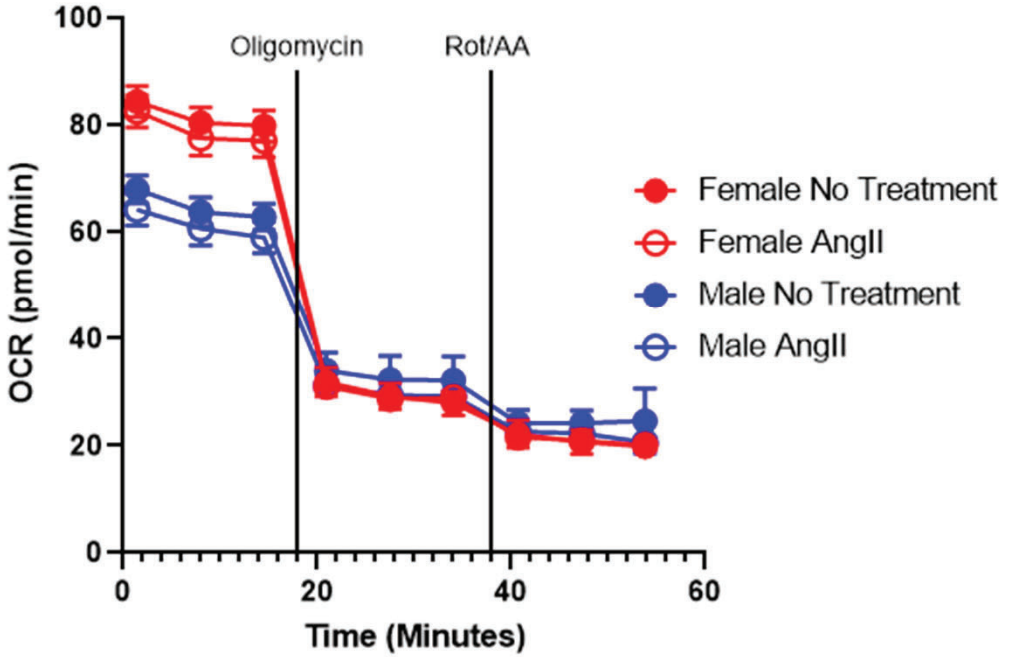




Weber and Clyne *CMBE* 2023

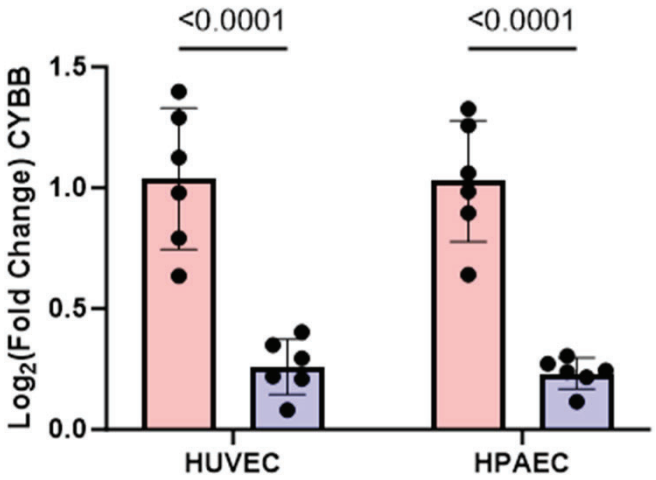
Female HUVEC had higher oxidative stress in response to AngII.

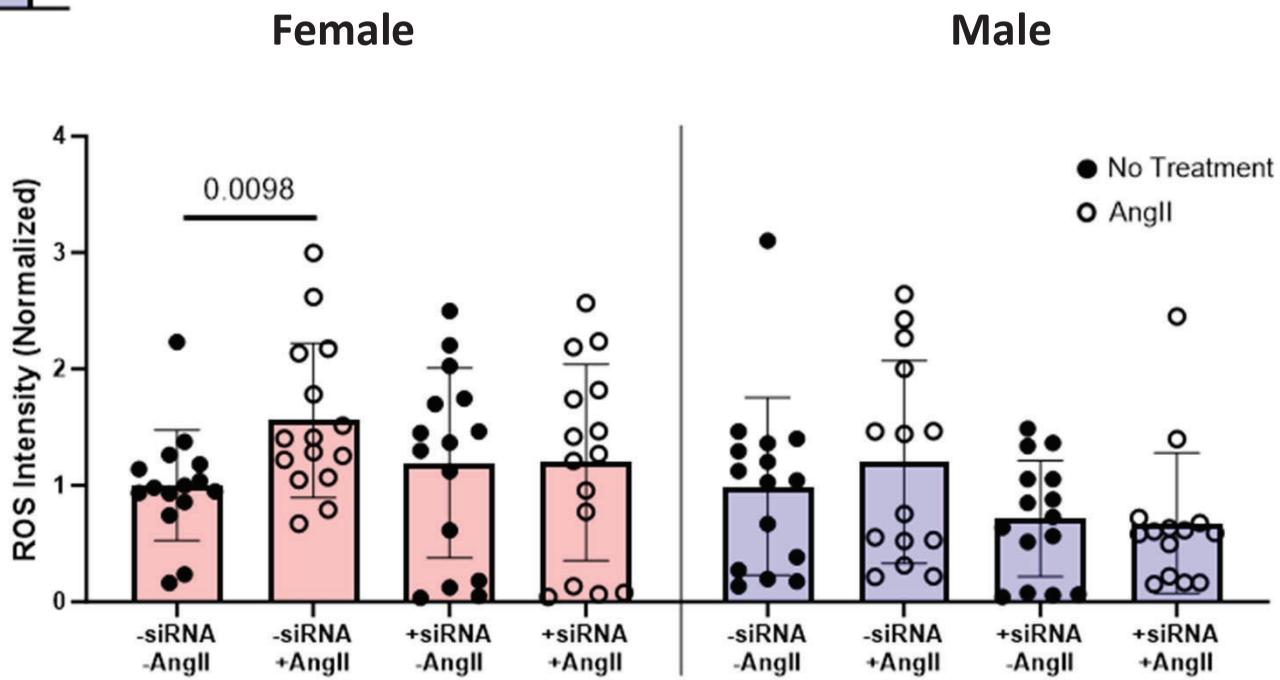




Weber and Clyne *CMBE* 2023

Higher oxidative stress in female endothelial cells was likely due to increase NADPH oxidases.





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We use animal, human, and computational models to study CVD mechanics and metabolism.

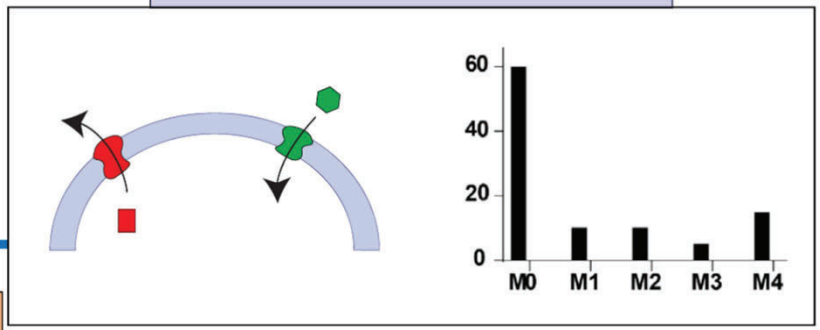
- Endothelial mechano-metabolism
- High-fat diet effect on perivascular adipose tissue in rats
- Stress effects on human endothelial cell function
- **Computational models predict intracellular fluxes**
- Human cardiovascular disease models for biomarker and therapeutic discovery

iMFA models fit LC/MS derived metabolites onto network models to predict intracellular fluxes

Network model

Reaction Name	Network Reactions	Atom Transition	Reaction Category
GLUT1	glc.e → glc.c	abcdef → abcdef	Source
HEX	glc.c → g6p.c	abcdef → abcdef	Internal
PFK	g6p.c ↔ f6p.c	abcdef ↔ abcdef	Internal
G6PDH	g6p.c → p5p.c + co2	abcdef → a + bcdef	Internal
ALDOLASE	f6p ↔ dhap.c + g3p.c	abc ↔ cba + def	Internal
Nucleotide synthesis	p5p.c → nuc.c	abcde → abcde	Sink
Lower glycolysis	g3p.c → pyr.c	abc → abc	Internal
ME/PK	mal.m → pyr.c	abc –> abc	Dilution/Internal
Lactate secretion	pyr.c → lac.e	abc → abc	Sink

Experimental data



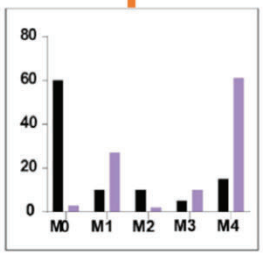
iMFA software

Generate flux guess

Calculate simulated MDVs

Compare simulated and experimental MDVs

Poor fit



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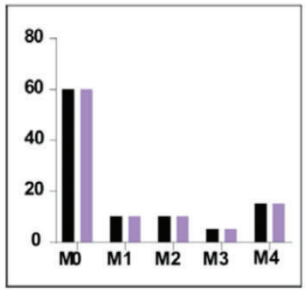
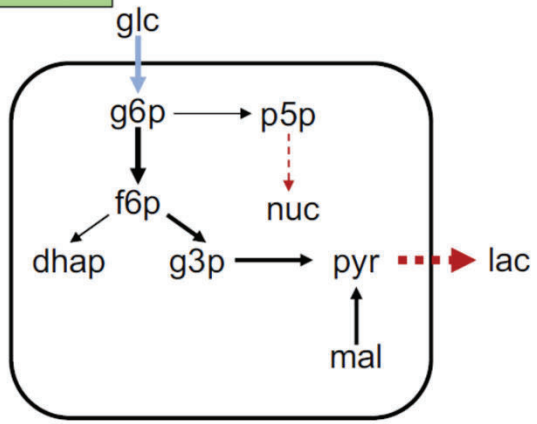
Compare simulated and experimental MDVs



Good Fit

Metabolic flux map

Reaction Name	Flux value (nmol/cell/hr)	Confidence Interval Range (nmol/cell/hr)
GLUT1	100	90-120
HEX	100	85-110
PFK	90	85-110
G6PDH	10	5-15
ALDOLASE	90	85-110
Nucleotide synthesis	10	5-15
Lower glycolysis	90	80-100
ME/PK	70	30-100
Lactate secretion	160	150-170



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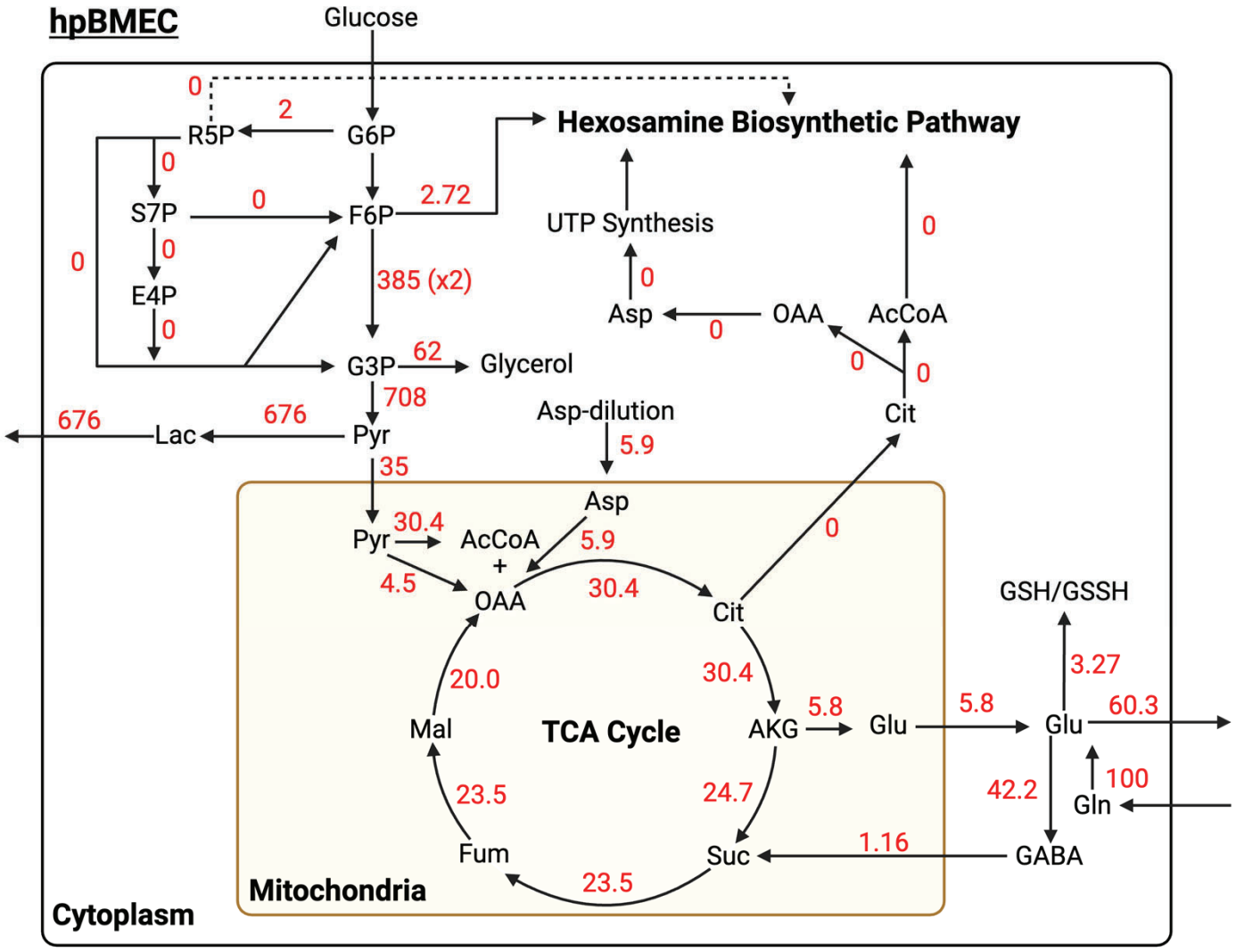
We used iMFA to compare glutamine metabolism between primary and iPSC-derived BMEC.



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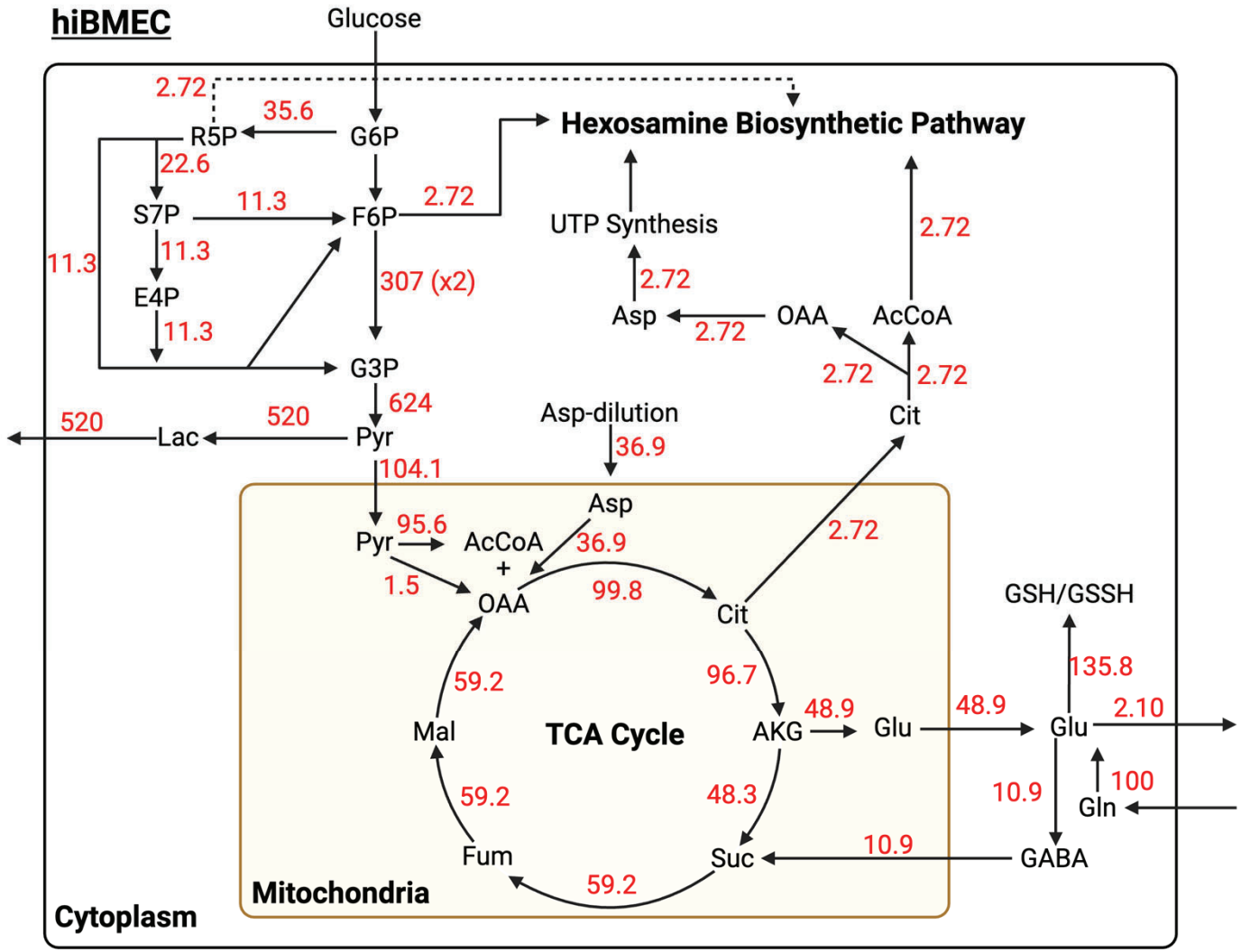

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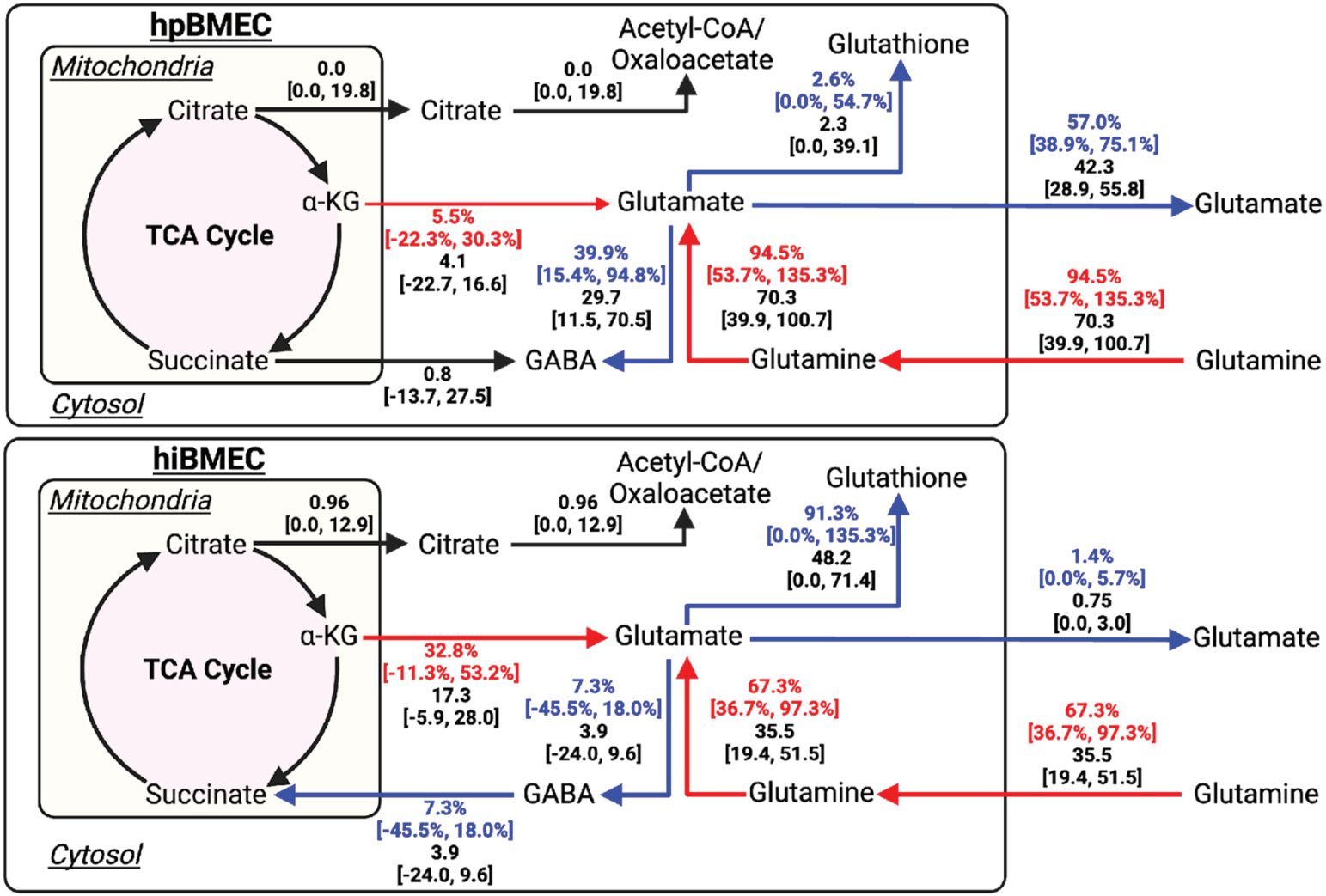
We can also use RNAseq data to predict intracellular fluxes via genome scale metabolic models.



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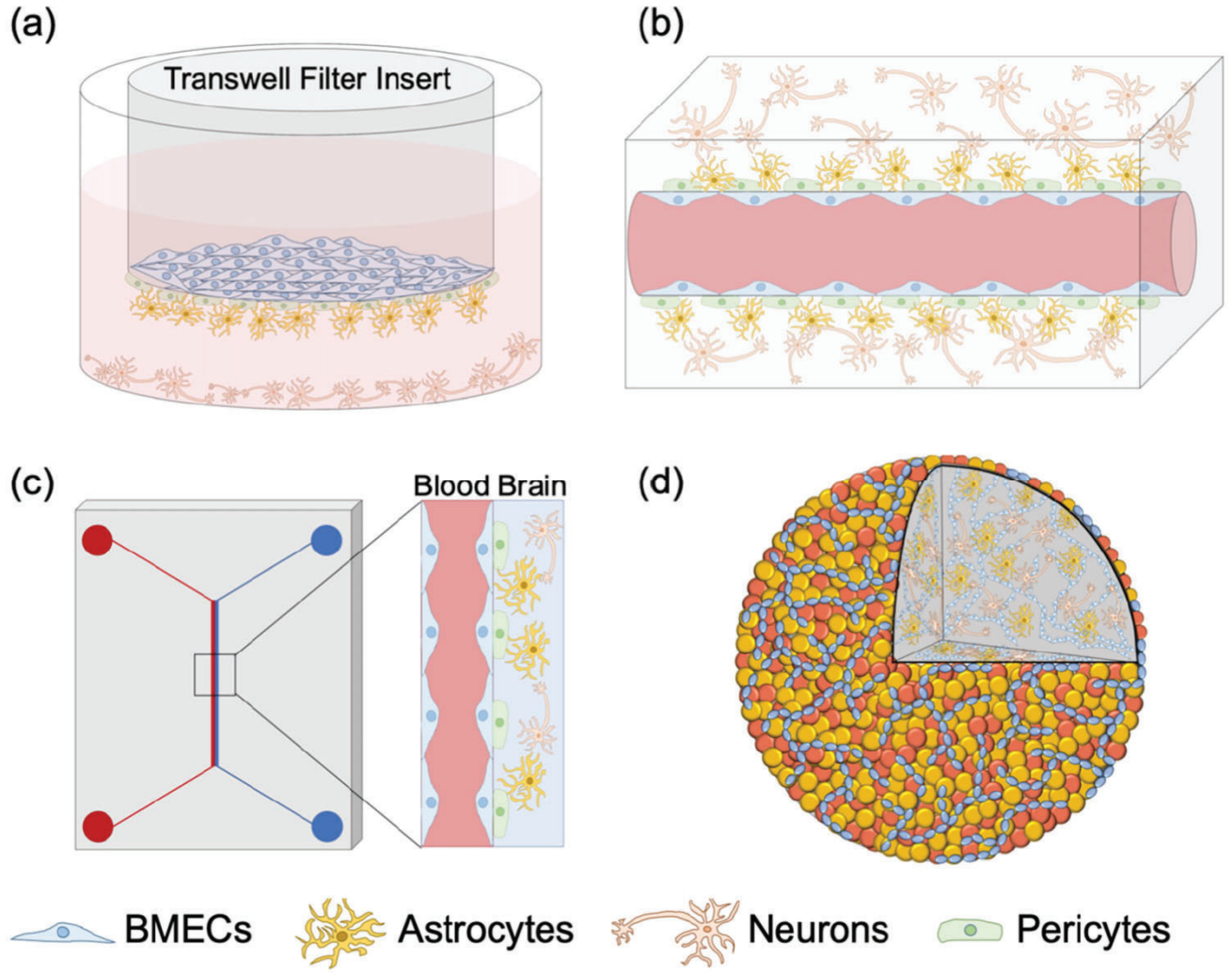
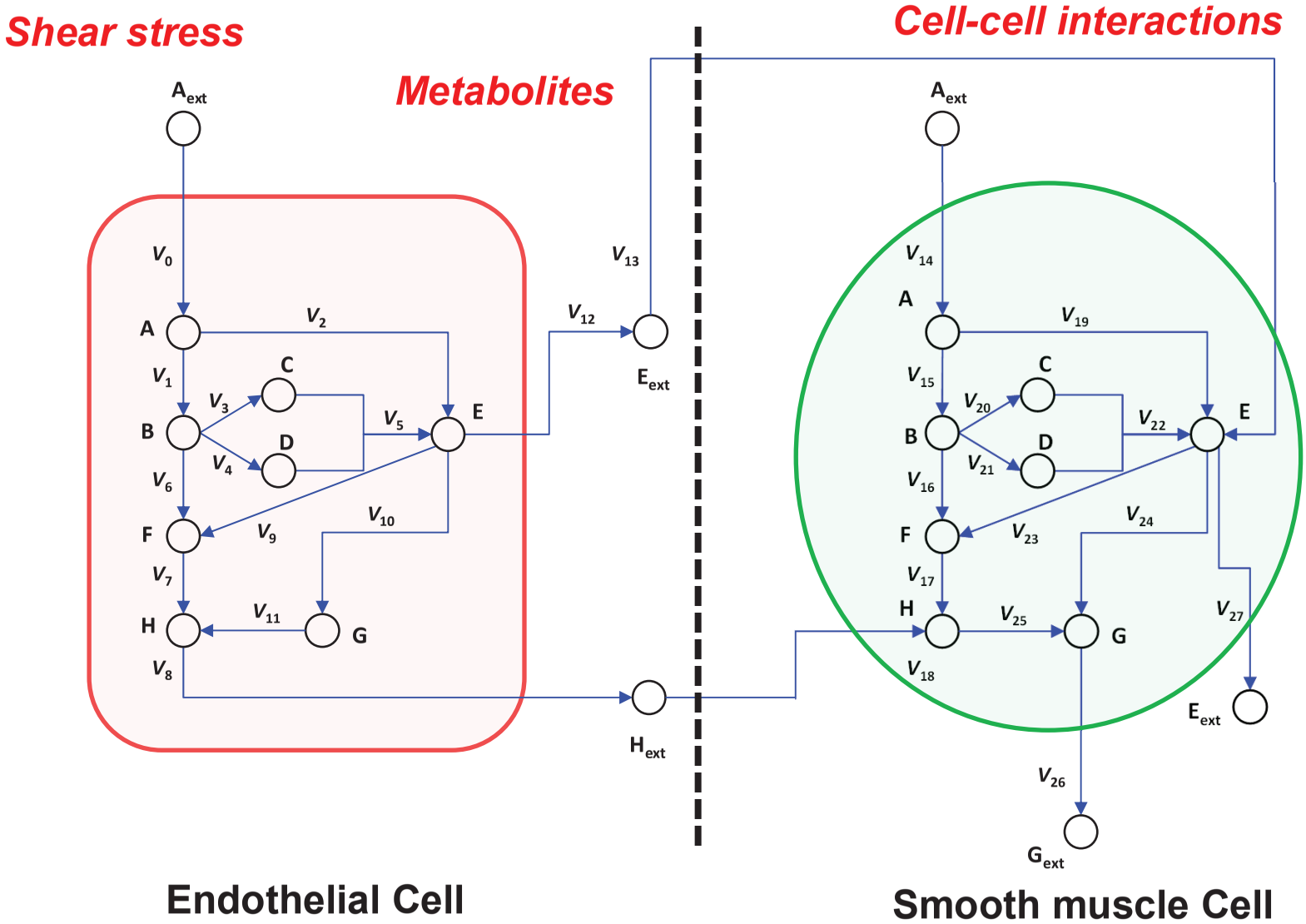
We need to create sex-specific cardiovascular *in vitro* systems across the human lifespan.

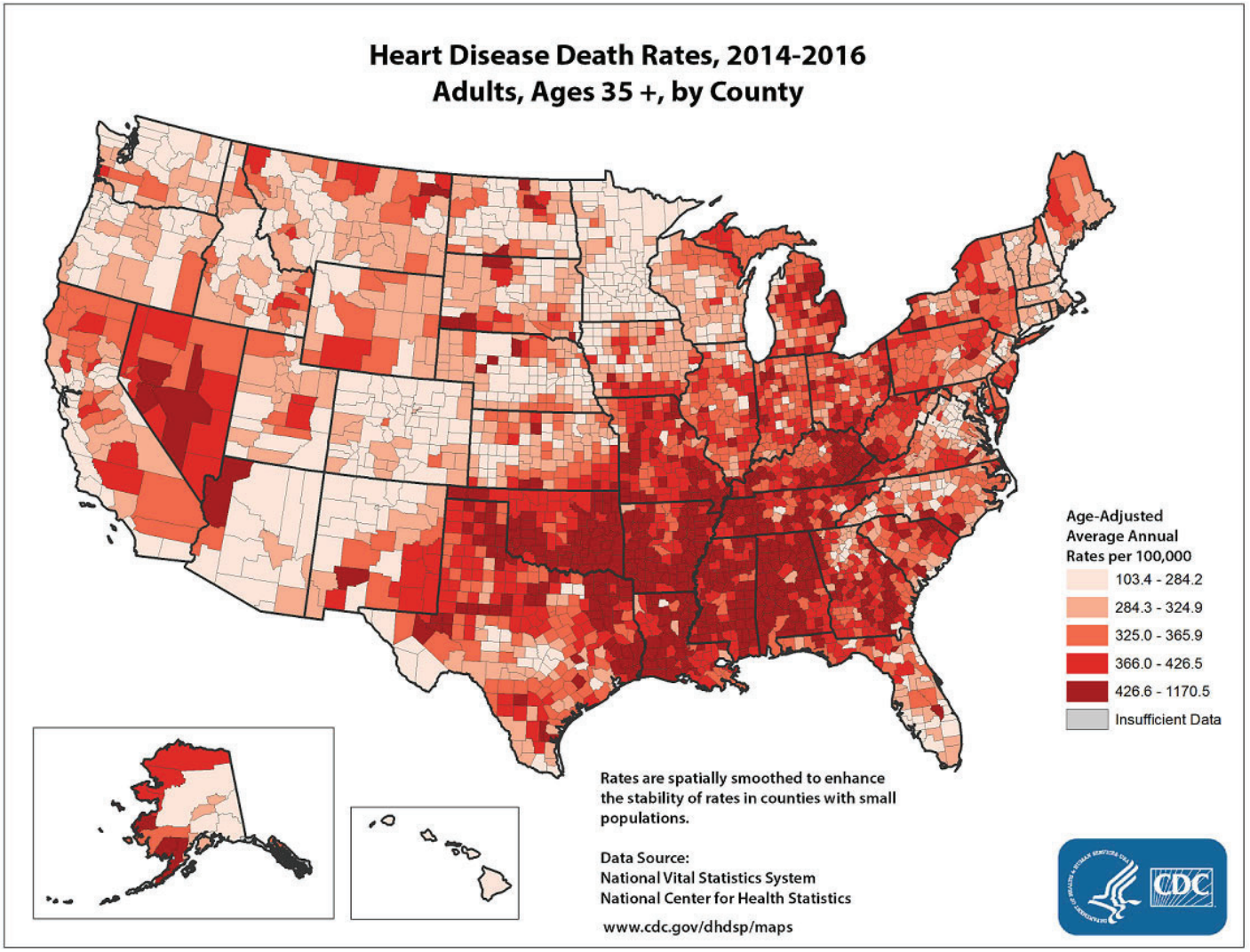


Weber and Clyne. APL Bioengineering 2021

We need computational models to predict and interpret experimental and human complexity.



Bioengineers can reduce disparities via experimental and computational models of human complexities.



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NIH National Institutes of Health

NSF

American Heart Association®