

University of Windsor

Scholarship at UWindor

Electronic Theses and Dissertations

Theses, Dissertations, and Major Papers

6-15-2023

Local Forearm Heating Does Not Alter Concentrations of Circulating Notch1 ECD and HSPG/CD44

Khushali Shrenik Parikh
University of Windsor

Follow this and additional works at: <https://scholar.uwindsor.ca/etd>



Part of the [Physiology Commons](#)

Recommended Citation

Parikh, Khushali Shrenik, "Local Forearm Heating Does Not Alter Concentrations of Circulating Notch1 ECD and HSPG/CD44" (2023). *Electronic Theses and Dissertations*. 9334.
<https://scholar.uwindsor.ca/etd/9334>

This online database contains the full-text of PhD dissertations and Masters' theses of University of Windsor students from 1954 forward. These documents are made available for personal study and research purposes only, in accordance with the Canadian Copyright Act and the Creative Commons license—CC BY-NC-ND (Attribution, Non-Commercial, No Derivative Works). Under this license, works must always be attributed to the copyright holder (original author), cannot be used for any commercial purposes, and may not be altered. Any other use would require the permission of the copyright holder. Students may inquire about withdrawing their dissertation and/or thesis from this database. For additional inquiries, please contact the repository administrator via email (scholarship@uwindsor.ca) or by telephone at 519-253-3000ext. 3208.

LOCAL FOREARM HEATING DOES NOT ALTER CONCENTRATIONS OF
CIRCULATING NOTCH1 ECD AND HSPG/CD44

By

Khushali Parikh

A Thesis
Submitted to the Faculty of Graduate Studies
through the Department of Integrative Biology and the Department of Biomedical
Sciences
in Partial Fulfillment of the Requirements for
the Degree of Master of Science
at the University of Windsor

Windsor, Ontario, Canada

2023

© 2023 Khushali Parikh

LOCAL FOREARM HEATING DOES NOT ALTER CONCENTRATIONS OF
CIRCULATING NOTCH1 ECD AND HSPG/CD44

by

Khushali Parikh

APPROVED BY:

K. Milne
Department of Kinesiology

A. Hubberstey
Department of Biomedical Sciences

A. Bain, Advisor
Department of Kinesiology

May 18, 2023

DECLARATION OF ORIGINALITY

I hereby certify that I am the sole author of this thesis and that no part of this thesis has been published or submitted for publication.

I certify that, to the best of my knowledge, my thesis does not infringe upon anyone's copyright nor violate any proprietary rights and that any ideas, techniques, quotations, or any other material from the work of other people included in my thesis, published or otherwise, are fully acknowledged in accordance with the standard referencing practices. Furthermore, to the extent that I have included copyrighted material that surpasses the bounds of fair dealing within the meaning of the Canada Copyright Act, I certify that I have obtained a written permission from the copyright owner(s) to include such material(s) in my thesis and have included copies of such copyright clearances to my appendix.

I declare that this is a true copy of my thesis, including any final revisions, as approved by my thesis committee and the Graduate Studies office, and that this thesis has not been submitted for a higher degree to any other University or Institution.

ABSTRACT

Endothelial dysfunction underlies the pathophysiology of cardiovascular disease. While advances have been made for treatment and management, many gaps in knowledge remain. Recently, heat therapy has gained attention for improving vascular endothelial function, tenably through increases in antegrade shear stress (SS). However, no study has examined the molecular mechanisms associated with SS and heating. Accordingly, the aim of this study was to determine how local forearm heating may impact Notch1 and Heparan Sulfate Proteoglycan (HSPG), which are transmembrane endothelial mechanosensors essential for preserving endothelial integrity and signalling. We hypothesized that 40-minutes of increased antegrade SS from forearm heating will increase the concentration of circulating Notch1 and HSPG/CD44. 9 healthy young adults underwent forearm heating by immersion in 42°C water for 40 minutes. Venous blood samples of the heated forearm were taken prior to and during heating. Concentrations of plasma Notch1 and HSPG/CD44 were subsequently determined by ELISA. Duplex ultrasound was used to determine SS in the brachial artery. Results: Antegrade SS was increased by heating ($P < 0.01$), however, the concentrations of Notch1 and HSPG/CD44 did not change throughout the heating protocol compared to baseline (P both > 0.05). These data indicate that the SS from forearm heating is not sufficient to increase Notch1 and HSPG/CD44, suggesting that the benefits of heating may not arise from the increases in antegrade SS, at least when quantified from circulating changes in Notch1 and HSPG/CD44, or local forearm heating is an insufficient stimulus to be used as heat therapy.

DEDICATION

To my grandpa – for being my biggest cheerleader, source of inspiration, and the person who sparked my love for the world of science. Thank you for everything,

Dada.

ACKNOWLEDGEMENTS

First and foremost, I would like to thank Dr. Anthony Bain for his unwavering support as my PI over the past two years. Thank you for your patience, mentorship, and for instilling in me a curiosity for the world of vascular physiology. Working under your supervision has provided me with invaluable opportunities for growth as a researcher and I am truly grateful to have had the opportunity to work with you.

I would also like to thank my committee members, Dr. Andrew Hubberstey and Dr. Kevin Milne. Thank you for offering your time to provide feedback on my thesis. Your support and guidance are greatly appreciated.

To Nick, Brooke, Renee, Olivia, and Melody, thank you for always helping with my project and making this master's a fun experience. I will miss our ultrasound practice sessions and endless conversations during our ELISA runs. There are also many other Bain group members who supported me throughout this journey. Thank you all for making it a memorable one, I will miss working alongside you.

Lastly, a special thank you to my friends and parents. Words cannot explain how grateful I am for your love and support. Thank you for believing in me, lending an ear when I needed it, and understanding why those endless hours in the lab were necessary. Thank you from the bottom of my heart, I couldn't have done it without you. Love you.

TABLE OF CONTENTS

DECLARATION OF ORIGINALITY.....	iii
ABSTRACT	iv
DEDICATION	v
ACKNOWLEDGEMENTS	vi
LIST OF TABLES	ix
LIST OF FIGURES	x
LIST OF ABBREVIATIONS	xii
1. REVIEW OF THE LITERATURE.....	1
Introduction	1
Endothelial Dysfunction.....	2
Atherosclerosis	3
Shear Stress and Endothelial Function	6
Shear Stress and Endothelial Mechanotransduction.....	7
Notch1	10
Heparan Sulfate Proteoglycan (CD44 Proteoglycan).....	11
Heat Therapy for Increases in Antegrade Shear Stress	13
2. AIMS AND HYPOTHESIS.....	18
Aim:	18
Hypothesis:	18
3. METHODOLOGY	19
Ethical Approval.....	19
Participants	19
Experimental Design	20
Measurement Techniques	21
Statistical Analysis	23
4. RESULTS	24

Heart Rate	24
Shear Rate.....	25
Notch1 ECD	27
HSPG/CD44	28
5. DISCUSSION	30
Notch1 as an endothelial mechanosensor.....	30
Heparan Sulfate Proteoglycan (CD44) as an endothelial mechanosensor	32
Study Considerations.....	34
6. CONCLUSION	38
REFERENCES	40
VITA AUCTORIS.....	50

LIST OF TABLES

Table 1: *A table of all included participant characteristics. ID=participant identification number; BMI=body mass index; SBP/DBP= resting systolic/diastolic blood pressure.*

LIST OF FIGURES

Figure 1: *The process of atherosclerosis from initial endothelial damage to potential plaque rupture. Figure adapted from NeurovascularMedicine, 2018.*

Figure 2: *A depiction of the endothelial mechanosensors. These exist on the apical surface, within junctions and at the basal level. There are many more known mechanosensors, and likely many more to be discovered. Highlighted in yellow are the mechanosensors of interest for this study. Figure adapted from Givens & Tzima, 2016.*

Figure 3: *A depiction of the experimental timeline. The syringe symbol represents sampling periods where both, blood draws and ultrasounds were conducted.*

Figure 4: *A screenshot of the ultrasound edge detection software video. The picture on the top is the brachial artery. The yellow lines represent the area where the software is detecting the arterial diameter. At the bottom is a graph of the blood velocity, with the yellow line tracking the envelope velocity.*

Figure 5: *Heart rate (beats per minute) at baseline, 20 and 40 minutes of heating, and 20 minutes following heating (post). Asterix (*) denotes significantly different ($p < 0.05$) compared to baseline. Data shown are individual values with mean \pm 95% CI.*

Figure 6: *Brachial artery shear rates at baseline, 20 and 40 minutes of heating, and 20 minutes following heating (post). Asterix (*) denotes significantly different ($p < 0.05$) readings compared to baseline. Data shown are individual values with mean \pm 95% CI.*

Figure 7: *A screenshot of the blood velocity at baseline. The x-axis represents time, and the y-axis represents blood velocity in cm/s.*

Figure 8: *A screenshot of the blood velocity at 20 mins into heating. The x-axis represents time, and the y-axis represents blood velocity in cm/s.*

Figure 9: *A screenshot of the blood velocity at 40 mins into heating. The x-axis represents time, and the y-axis represents blood velocity in cm/s.*

Figure 10: *A screenshot of the blood velocity at post heating. The x-axis represents time, and the y-axis represents blood velocity in cm/s.*

Figure 11: *Brachial artery Nocthl ECD concentrations at baseline, 20 and 40 minutes of heating, and 20 minutes following heating (post). Data shown are individual values with mean \pm 95% CI.*

Figure 12: *Brachial artery HSPG/CD44 concentrations at baseline, 20 and 40 minutes of heating, and 20 minutes following heating (post). Data shown are individual values with mean \pm 95% CI.*

LIST OF ABBREVIATIONS

ADAM	Disintegrin and Metalloproteases
COX	Cyclooxygenase
CVD	Cardiovascular Disease
CXCL	CXC Motif Chemokine Ligand 1
CXCR4	CXC Chemokine Receptor Type 4
ECD	Extracellular Domain
eNOS	Endothelial Nitric Oxide Synthase
GMP	Guanosine Monophosphate
GPCR	G-protein Coupled Receptor
HSP	Heat Shock Protein
HSPG	Heparan Sulfate Proteoglycan
ICAM 1	Intercellular Adhesion Molecule 1
KLF2	Kruppel-like Factor 2
LDL	Low-density Lipoprotein
MAPK	Mitogen Activated Protein Kinase
MMP	Matrix Metalloproteases
NEXT	Notch Extracellular Truncation
NF-kB	Nuclear Factor kappa B
NICD	Notch Intracellular Domain
NO	Nitric Oxide

PECAM-1	Platelet Endothelial Cell Adhesion Molecule-1
PO ₂	Partial Pressure of Oxygen
RBPJ	Recombination Signal Binding Protein for Immunoglobulin Kappa J Region
TGF- β	Transforming Growth Factor Beta
VEGFA	Vascular Endothelial Growth Factor A
VEGFR2	Vascular Endothelial Growth Factor Receptor-2
vWF	Von Willebrand Factor
ZO-1	Zonula Occludens

1. REVIEW OF THE LITERATURE

Introduction

Cardiovascular disease (CVD), defined as a spectrum of disorders that affect the heart and blood vessels (Ross, 1986), is the number one cause of morbidity and mortality worldwide (Vaduganathan et al., 2022). Globally, 17.9 million people died of CVD in 2019, accounting for 32% of all deaths (WHO, 2021). With such high rates of mortality, CVD poses an immense clinical and economic burden on the healthcare system. In Canada, the direct economic cost of CVD, which includes physician billing, hospital equipment, and lost wages, add up to more than \$22 billion annually (Smith, 2009). This number is expected to rise given the increased prevalence of obesity, which is a driving risk factor for the progression of vascular impairment and CVD (Smith, 2009). Moreover, CVD and its associated risk factors - importantly endothelial dysfunction - are associated with a myriad of other health complications, including increased cancer risk (Toya et al., 2020), and poor recovery prognostics following surgery (Rose et al., 2022; Schick et al., 2021). As such, better identifying the mechanisms associated with vascular endothelial impairments, and identifying novel therapies for CVD are necessary to prevent and manage this disease.

The overarching goal of this thesis is to examine the impact of limb heating on key biomarkers that are known to impact vascular endothelial function and in turn CVD. Under the overarching theme of endothelial (dys)function, this literature review will briefly discuss the pathogenesis of atherosclerosis and focus on the impacts of shear stress and mechanistic mechanotransduction of shear stress through Notch1 and Heparan

Sulfate Proteoglycans. The tenable impacts of heat stress for improved vascular endothelial function are then discussed.

Endothelial Dysfunction

The unifying precursor to CVD is endothelial dysfunction (Widmer & Lerman, 2014). All blood vessels are made up of endothelial cells as their innermost layer – i.e., the layer which is in contact with the circulating blood. These cells form a selectively permeable membrane with their tight cell-to-cell junctions, which is crucial to determining vascular tone, fluid homeostasis, and host defense (Zhang et al., 2018; Sun et al., 2020). Endothelial cells respond to external stressors and signals such as changes in hormones, neurotransmitters, shear stress, and temperature by releasing vasodilating/constricting biomolecules, inhibiting, or stimulating smooth muscle cell contractility, proliferation, and migration, thrombogenesis, and fibrinolysis (Davignon & Ganz, 2004; Sandoo et al., 2010). A healthy endothelium (which is the collective of all endothelial cells) is thus central to vascular homeostasis (Davignon & Ganz, 2004).

With respect to tissue blood flow and blood pressure, in normal conditions, the endothelium causes arterial smooth muscle dilatation primarily (albeit not exclusively) by releasing nitric oxide (NO), which is synthesized from endothelial nitric oxide synthase (eNOS) and the amino acid, L-arginine. This results in the production of cyclic GMP, causing relaxation of the vascular smooth muscle (Pasmanter et al., 2023; Widmer & Lerman, 2014). Under conditions of endothelial damage, which is caused by environmental factors such as turbulent blood flow (reduced shear stress, which will be covered in depth later in the thesis), NO consumption is disproportionately favoured over production (Widmer & Lerman, 2014). This imbalance increases platelet plus leukocyte

activation, increases adhesion of molecules such as circulating monocytes and low-density lipoproteins (LDLs) to the arterial wall and cytokine activation which increases the permeability of the vessel wall (Hall & Guyton, 2011). These subsequently cause the oxidation of lipoproteins and inflammatory mediators, which damage the arterial wall, and result in atherosclerotic plaque buildup (discussed below) (Widmer & Lerman, 2014), hypertension, impaired coronary microvascular function, and diastolic dysfunction (heart failure with preserved ejection fraction) (Brunt & Minson, 2021; Gamrat et al., 2020; Paulus & Tschöpe, 2013). Endothelial stressors also impair the endothelium repair mechanisms, which include the recruitment of T-lymphocytes, monocytes, and platelets. This impairment results in the initiation of atheroma buildup, or atherosclerosis (Insull, 2009).

Atherosclerosis

Atherosclerosis, the build-up of fatty lesions called atheromatous plaques in arteries, is a major cause of CVD deaths. (Davis, 2005; Ross, 1986). It is estimated that approximately 50 percent of Americans ages 45 and 84 have atherosclerosis (NHLBI, 2022). Atherosclerosis develops progressively through the accumulation of lipids and subsequent inflammatory processes affecting the endothelial, which cause increasingly complex changes to the artery. Often plaque buildup begins in early childhood; however, these changes are microscopic and can be minimized by a healthy lifestyle (Hong, 2010). Factors such as diet and medical history (eg: dyslipidemia and hypertension) result in the eventual progression to visible plaque formations (Insull, 2009). Dyslipidemia is caused by diet, genetics, and other factors. When it persists, it leads to an increase in circulating lipoproteins, which disrupt healthy lipid metabolism, leading to an increase in

triglycerides and cholesterol. Specifically, an elevation in LDLs occurs and these accumulate within atherosclerotic plaque (Lu et al., 2022). Not only do they cross into the arterial intima, but with endothelial dysfunction, they are bound and remain in this space, thus progressing plaque buildup (Tabas et al., 2007). With respect to hypertension, research has demonstrated that it accelerates the progression of atherosclerosis. Although both diseases have different biochemical and pathological changes, studies indicate that hypertension increases the risk of developing atherosclerosis. This occurs through damage of the arterial wall by processes such as increases in oxidative stress and inflammation (Alexander, 1995). Conversely, limited blood flow in arteries due to atheroma causes the heart to work harder and leads to hypertension, which is the leading risk factor for heart failure (Slivnick & Lampert, 2019).

The process of plaque buildup starts with endothelial damage. Endothelial stress causes endothelial cells to secrete adhesion molecules, inducing chemokine and chemoattractant expression (Abraham & Distler, 2007). This process attracts inflammatory molecules such as monocytes, lymphocytes, mast cells, and neutrophils to the site of stress (Insull, 2009). These inflammatory and fibroproliferative molecules leak into the arterial intima, which attracts LDLs. Macrophages then engulf these particles and become foam cells. The accumulation of foam cells moves the smooth muscle cells toward the artery interior; however, these changes can often be microscopic. Atherosclerosis is specifically when the fat accumulation appears contiguous (Insull, 2009). To now repair the endothelium, a smooth muscle and collagen cap is formed while white blood cells from the original response begin to die and create a necrotic core. Leukocytes and lipid fragments continue to enter at the shoulders, enlarging the lesion. A

larger plaque formation causes the arterial wall to stretch and small blood vessels (vasa vasorum) develop to maintain the plaque. Eventually, however, the arterial wall can no longer contain the plaque and it extends into the lumen, thus compromising blood flow and subsequently oxygen delivery, resulting in a variety of clinical repercussions (Davis, 2005; Ross, 1995). Thinning of the fibrous cap with fissuring of the endothelial surface may also occur. This formation of a thrombus (blood clot) occurs when the rate of fissuring surpasses the rate of repair, and lipid fragments as well as other cellular debris are released into the circulation. A thrombus released into the circulation can lead to a myocardial infarction or stroke (Davis, 2005).

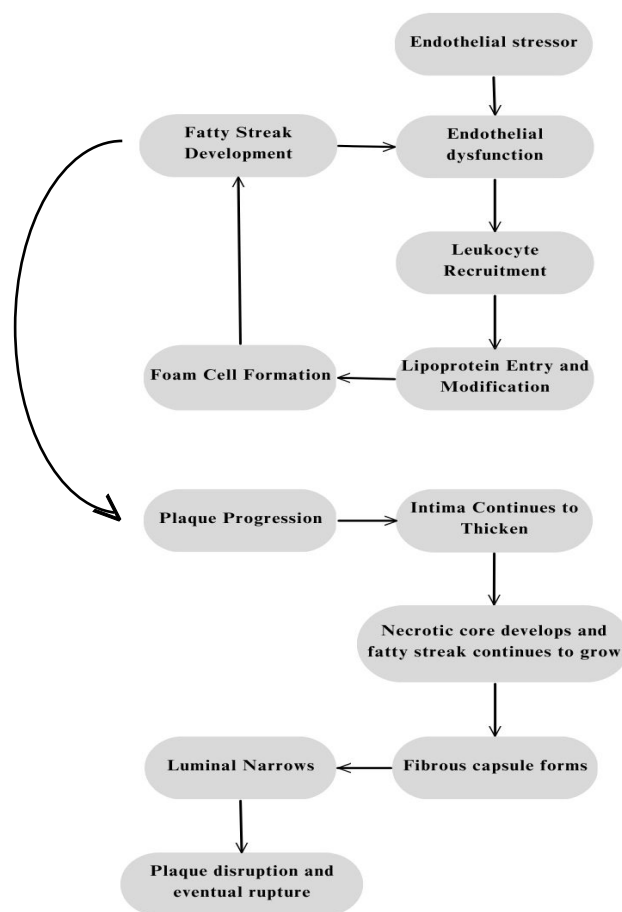


Figure 1: *The process of atherosclerosis from initial endothelial damage to potential plaque rupture. Figure adapted from NeurovascularMedicine, 2018.*

Accordingly, endothelial dysfunction is at the crux of both atherothrombotic disease (leading to thrombi) as well as vascular dilatory dysfunction (leading to elevated blood pressure and heart failure). Targeted prophylactic and treatment therapy for all forms of CVD should therefore, in some way, center on improving endothelial function.

Shear Stress and Endothelial Function

Shear stress is the force per unit area of blood acting on the endothelium of a vessel (Ballermann et al., 1998; Davies, 2009). The flow of blood can be categorized as retrograde/oscillatory (atheroprone) or antegrade/laminar (atheroprotective) shear stress. That is, antegrade shear stress is the forward movement of blood flow, which is beneficial to improving or preserving the health of the endothelium (Traub & Berk, 1998). Conversely, retrograde shear stress causes cyclical and disrupted movements of blood flow and has harmful effects.

The absolute shear stress on vessel walls is determined by the blood viscosity, blood velocity, and vessel diameter. In an artery, forward-moving blood does not move at the same velocity at all locations, rather blood flow is fastest in the center and slowest near the arterial walls. A slower velocity on the outside is due to the frictional force between the fluid and vessel wall, which is called wall shear stress. Shear stress is calculated based on how fast fluid velocity increases when moving from the vessel wall to the center. Mathematically, this is defined as (Shaaban & Duerinckx, 2000):

$$wall\ shear\ stress = viscosity \frac{velocity}{diameter}$$

The vessel anatomy plays a role in the type of shear (antegrade or retrograde) experienced. For example, in areas of vessel bifurcation, blood flow is slow near the outer wall and highest near the divider (Shaaban & Duerinckx, 2000). Higher retrograde shear stress is therefore observed in areas with strong curvatures and bifurcations. These areas also promote increased turbulent blood flow and are where atherosclerotic lesions are commonly found (Papaioannou & Stefanadia, 2005). Studies have shown that antegrade shear stress is lower (4 dyne/cm² compared to 12 dyne/cm²) in areas with bifurcations and plaque buildup (Malek et al., 1999). Additionally, human conduit arteries naturally experience retrograde shear stress and are therefore prone to plaque buildup (Thijssen et al., 2009).

Increases in retrograde shear cause a decrease in flow-mediated dilation of human arteries (Schreuder et al., 2014; Thijssen et al., 2009), create a pro-oxidative state (De Keulenaer et al., 1998) and cause atherogenesis. Overall, these studies suggest that increases in antegrade shear provide an atheroprotective environment (Malek et al., 1999).

Shear Stress and Endothelial Mechanotransduction

Endothelial cells respond to changes in blood flow/shear stress through mechanosensors on their surface (Mack et al., 2017). Mechanosensors are cellular proteins or molecules that translate mechanical stimuli to biochemical signals and transmit those to the interior of a cell, initiating various signaling pathways (Chen et al., 2016).

Shear stress can be detected at the apical surface of endothelial cells and transmitted through the cytoskeleton to cell-cell attachments and cell-matrix adhesions (Tzima et al., 2005). The mechanotransduction from shear stress on endothelial cells occurs in many steps: 1) the flow of blood causes a physical deformation of the cell surface 2) this results in intracellular signaling of the stress, 3) the conversion of mechanical force to chemical activity occurs, and 4) downstream signals are released (Davies, 2009). For example, antegrade shear stress aligns endothelial cells in the direction of the blood flow, resulting in less stress fibers and lower cell turnover. This causes the expression of anti-inflammatory genes and transcription factor KLF2, which regulates vascular endothelial cells (Turpaev, 2020). Conversely, retrograde shear stress results in unaligned endothelial cells. This causes activation of NF-kB-dependent genes, which encode the proteins intercellular adhesion molecule, vascular adhesion molecule, endothelial E-selectin, and platelet-driven growth factor. Stimulation of these genes has been shown to initiate atherosclerosis (Givens & Tzima, 2016; Tzima et al., 2005).

Endothelial mechanosensors exist on the apical surface, within junctions, and at the basal level. Within these categories, mechanosensors include, primary cilia, glycocalyx, heterotrimeric G-proteins and G-protein-coupled receptors (GPCRs), ion channels, caveolae, tie receptors, platelet endothelial cell adhesion molecule-1, VE-Cadherin, VEGF receptors, and integrins (Givens & Tzima, 2016). There are many more known mechanosensors, and likely many that have not been discovered yet.

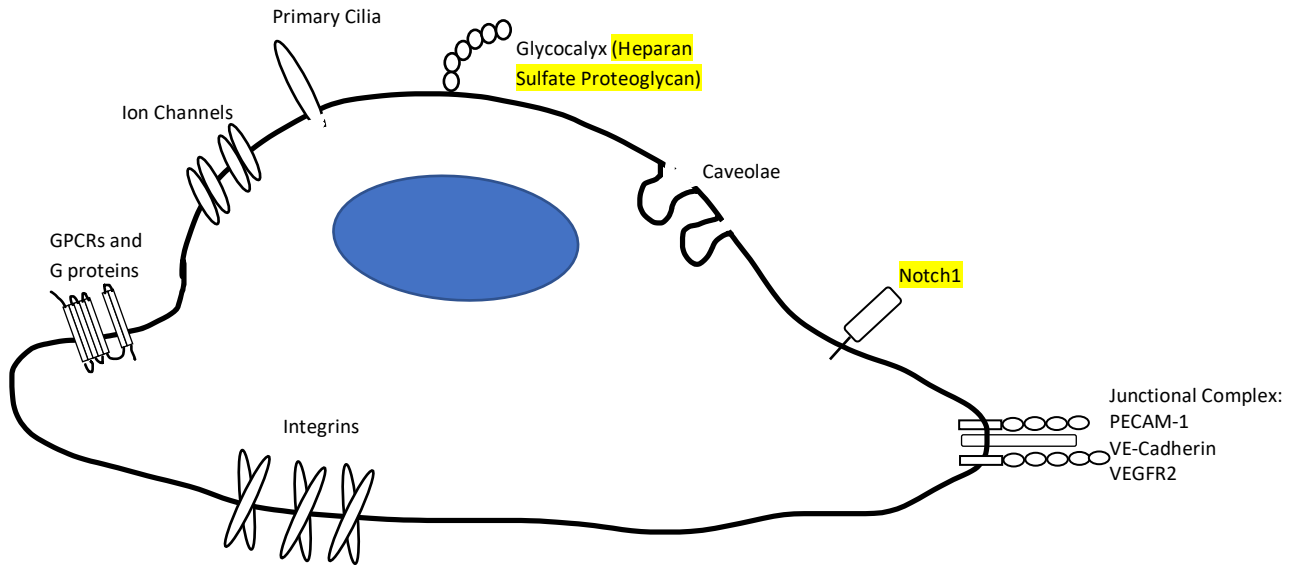


Figure 2: A depiction of the endothelial mechanosensors. These exist on the apical surface, within junctions and at the basal level. There are many more known mechanosensors, and likely many more to be discovered. Highlighted in yellow are the mechanosensors of interest for this study. Figure adapted from Givens & Tzima, 2016.

The most immediate response to shear stress comes from Ion channels (Chatterjee & Fisher, 2014). The most prominent channels are Ca^{2+} and K^{+} channels. Once activated they induce membrane hyperpolarization, leading to the release of vasodilators such as NO (Chen et al., 2016). GPCRs mediate Ras activation and NO production within one second of detecting antegrade shear stress and also cause the relaxation of vascular smooth muscle (flow-mediated dilation) (Hu et al., 2022). Examples of GPCRs that detect shear stress are endothelial glycocalyx, caveolae, and rapidly activated tyrosine kinases. Cell-to-cell junctions also have mechanosensory complexes composed of vascular-endothelial cadherin, platelet endothelial adhesion molecule, and vascular endothelial growth factor receptor 2 (VEGFR-2). Integrins are transmembrane receptors

responsible for cell-cell adhesion and communication with the extracellular matrix. They are responsible for downstream signaling with shear stress, such as the activation of the Mitogen-activated protein kinases (MAPK) pathway. Due to the complex nature of mechanosensing and limits in technical approaches, there is limited knowledge on which mechanosensors have the most impact (Chen et al., 2016). More recently, a study by Mack et al., 2017 discovered the importance of Notch1, a transmembrane receptor, in detecting antegrade shear stress.

Notch1

Four receptors in the Notch family are found in mammals. Notch1 and Notch4 are found in endothelial cells, while Notch2 and Notch3 are found on smooth muscle cells (Hofmann & Iruela-Arispe, 2007). These transmembrane receptors interact with five ligands (Jagged1,2; Delta-like 1, 3, and 4) (D'Souza et al., 2010). The activation of Notch occurs with ligand binding, causing the proteolytic cleavage of its extracellular domain by ADAM (disintegrin and metalloprotease). This creates a membrane-tethered intermediate called Notch extracellular truncation (NEXT), which is further cleaved by γ -secretase, and this results in the release of its intracellular domain (NICD) which is translocated to the nucleus. Here the NICD co-activates with recombination signal-binding protein for immunoglobulin kappa J (RBPJ) to act as a transcriptional activator for various genes that impact cell physiology (Kopan & Ilagan, 2009; Mack & Luisa Iruela-Arispe, 2018; Sander et al., 2006). Specifically, Notch1 signaling increases with increases in laminar flow, making it a potential marker for changes in shear stress (Mack et al., 2017).

While changes in shear stress can still be detected in the absence of Notch1, the ability of endothelial cells to remain mitotically quiescent and maintain junctional integrity is impaired (Mack & Luisa Iruela-Arispe, 2018). Activation of Notch1 results in cell-cycle arrest preserves vascular homeostasis, maintains EPHRIN B2 and arterial identity, maintains intracellular calcium homeostasis, and the regulation of pro-inflammatory genes related to atherosclerosis. Increases in Notch1, caused by increases in antegrade shear stress, have been shown to reduce the binding of the endothelium to inflammatory cells such as CXCL2, ICAM1, CXCR4, and many other CXCL ligands. Without Notch1, these inflammatory cells move past the endothelium and into the intima, beginning the atherosclerotic process (Mack et al., 2017). A study done on mice has also shown that reductions in endothelial Notch1 is an early indicator of vascular inflammation and atherosclerosis (Briot et al., 2015). Importantly, a recent study by Dr. Bain's group demonstrated an increase in Notch1 levels with acute increases in antegrade shear stress, for the first time in humans (Badour et al., 2022). Detectable changes in Notch1 from human plasma make it a potential, convenient marker of shear stress in research or clinical settings.

Heparan Sulfate Proteoglycan (CD44 Proteoglycan)

Another important endothelial mechanosensor is Heparan Sulfate Proteoglycan (HSPG), categorized as glycocalyx mechanosensors (Florian et al., 2003). These are heparan sulfates that have been modified with the addition of a glycosaminoglycan chain (Sarrazin et al., 2011). HSPGs can be divided into three categories- syndecans, glypicans, and perlecan. The syndecan is the most common and has an extracellular component that is cleaved during increases in antegrade shear stress (Florian et al., 2003). When exposed

to shear stress, HSPGs undergo a conformational change where its core proteins unfold, extending the heparan sulfate chain, allowing the delivery of sodium ions to transporter channels, therefore playing a role in the signaling pathway related to chemokines, interleukins, lipases, and more (Tarbell & Pahakis, 2006). HSPGs are also hypothesized to be important contributors to the production of NO that occurs in response to shear stress and is important to maintain vascular tone (Florian et al., 2003). Further, a study conducted on mouse embryonic stem cells demonstrates that HSPG is required for the shear-stress induced expression of vWF (a protein involved with blood clotting), VE-cadherin, ZO-1 (an important component of the blood-brain barrier), eNOS (required to generate NO), and COX-2 (an important component of the inflammatory process) (Nikmanesh et al., 2012). With respect to HSPG-mediated mechanotransduction and neovascularization, downstream signals are hypothesized to include VEGFA, TGF- β , MMP-1, -15 and -28, integrin, cadherin 11, E-selectin, and delta-like canonical Notch ligand 1, which halt neovascularization and stabilizing the vasculature during antegrade shear stress (Zhao et al., 2021). In addition to this, the lack of heparan sulfate in mice has been shown to increase neutrophil infiltration, a component of atherogenesis, during acute endothelial inflammation (Axelsson et al., 2012). This is due to the weaker binding of leukocyte (L)-selectin on neutrophils in the absence of HSPGs (Bishop et al., 2007; Wang et al., 2005). HSPGs also affect cell migration, proliferation, organization, and permeability (Zhao et al., 2021). Overall, HSPGs act as endothelial mechanosensors for shear stress and affect downstream signaling for various factors related to cardiovascular health.

Heat Therapy for Increases in Antegrade Shear Stress

Passive heat stress as a tenable therapy has been studied both in the lab and through observing cultural usage to prevent and manage various CVDs (Brunt & Minson, 2021). Passive refers to non-exercise induced heat stress and heat therapy is the chronic application of heat stress using methods such as hot baths or saunas (Brunt & Minson, 2021). The interest in using heat as a therapeutic has stemmed from basic animal studies and historic usages. Numerous studies conducted in lower-order organisms support the benefits of heat stress. For example, in *Caenorhabditis elegans* (worms) with mutations for thermotolerance and *Drosophila melanogaster* (fruit flies), elevated temperatures resulted in an increase in lifespan compared to controls experiencing normothermia (Khazaeli et al., 1997; Lithgow, 1995).

In humans, the interest in heat as a therapeutic began with observational studies associating habitual heating regimens with decreased incidences of CVD and all-cause mortality (Coombs & Tremblay, 2019). Common cultural uses of heat therapy include the Banyas of Russia, Indigenous sweat lodges, and the saunas of Finland (Patrick & Johnson, 2021). Other commonly studied methods of passive heating include Finnish dry saunas, Waon/far infrared sauna therapy, and hot water immersion.

Finnish dry saunas expose the body to temperatures ranging from 70-100°C at a relatively low humidity level (10-15%) (Keast & Adamo, 2000). This method typically increases esophageal temperatures by 1.5°C and rectal temperatures by 0.2-1°C. This form of heat therapy has been shown to have long-term cardiovascular improvements such as decreases in blood pressure in hypertensive patients and increases in left

ventricular ejection fraction in chronic heart failure patients (Brunt & Minson, 2021; Hannuksela & Ellahham, 2001).

Waon therapy uses a far-infrared-ray dry sauna, typically maintained at 60°C, where the entire body is heated for a short duration (Miyata & Tei, 2010). As large increases in air temperature do not occur with Waon therapy, the skin is not heated, and cutaneous blood flow is not increased to the same magnitude as external forms of heating (e.g., via warm water heating). This results in a lower burden on the cardiovascular system by mitigating the need for increased systemic vascular resistance and blood pressure, compared to dry saunas, and is, therefore, a preferred method of heat therapy for the cardiovascular compromised, such as people with heart failure (Brunt & Minson, 2021).

The second most popular form of heat therapy, hot water immersion, has technical benefits as it is easier to increase core temperatures faster due to 1) higher rates of heat transfer in water and 2) the ineffectiveness of the body's cooling (sweat evaporating) mechanism underwater. Another major benefit is the increase in hydrostatic pressure on the body which assists with the venous return of the blood back to the heart, improving cardiac filling pressure, cardiac output, mean arterial blood pressure, endothelial-mediated vasodilation, and increased arterial compliance (Brunt & Minson, 2021; Naylor et al., 2011).

Laboratory experiments highlighting the benefits of heat therapy can be seen in a study by Brunt et al., 2016. In young healthy, sedentary adults, 36 sessions of hot water immersion over 8 weeks improved endothelial-dependent dilation, arterial stiffness,

intima-media thickness, and blood pressure compared to individuals undergoing thermoneutral water therapy (control group) (Brunt et al., 2016). With respect to endothelial health, this study also demonstrated an improvement in flow-mediated dilation. In middle-aged to elderly Japanese individuals, hot water bathing (more than 5 times per week) lowered brachial-ankle pulse wave velocity (a measurement of arterial stiffness), central pulse pressure (higher values are related to adverse cardiovascular outcomes) and plasma B-type natriuretic peptide (higher levels indicate heart failure) (Roman et al., 2009). Longitudinally, hot water baths resulted in lower plasma B-type natriuretic peptide concentrations, reduced progression of brachial-ankle pulse wave velocity, and reduced carotid intima-media thickness (Kohara et al., 2018).

Often heat therapy has been studied in conjunction with exercise to improve cardiovascular outcomes. A study by Akerman et al., 2019 demonstrated that heat therapy (spa bathing) with calisthenics in those with peripheral artery disease led to reductions in diastolic blood pressure, mean arterial pressure, and systolic blood pressure, interestingly at comparable rates to the control group that only exercised.

Previous studies have shown improvements in cardiovascular function without increasing core temperature to large extents ($\sim 1^{\circ}\text{C}$). One such example is a study designed to heat only the lower limbs for 24 sessions over 8 weeks. A significant increase in antegrade shear stress and subsequent improvement in brachial artery endothelial function was seen (Carter et al., 2014).

Many other studies have shown improvements in cardiovascular function while heating only the lower body, such as using foot baths (Cheng et al., 2021; Neff et al.,

2016; Pellingier et al., 2019; Thomas et al., 2017). This is a major benefit of hot water immersion as it increases the accessibility of heat therapy such as for those with limited physical abilities. Lower body heating is also beneficial as large elevations in core temperatures can be undesirable for those with CVD. This is supported by the observation of a rise in deaths during heat waves, which are predominantly due to adverse cardiovascular outcomes rather than heat stroke (Kenney et al., 2014). In conditions of high temperature, the left ventricle works harder to increase blood flow to the skin and dissipate heat. Older individuals and those with CVD are limited in their ability to maintain stroke volume, increase cardiac output, and increase cutaneous blood flow, resulting in a fatal burden on their cardiovascular system (Kenny et al., 2014). In addition, many commonly prescribed medications such as diuretics, ACE inhibitors, antipsychotics, anticonvulsants, and antidepressants limit the body's ability to thermoregulate, further emphasizing the need to use heat therapy that does not cause large increases in core temperatures (Westaway et al., 2015).

While heat therapy has improved risk factors associated with CVD; the mechanisms underlying these improvements are not fully understood. Many have attributed the heat-induced cardiovascular improvements to reduced sympathetic activity (Cui et al., 2022; Ely et al., 2019) and reductions in oxidative stress and inflammation (Brunt & Minson, 2021). With respect to the changes that occur at a molecular level with heat therapy, some studies suggest the benefits relate to the upregulation of heat shock proteins (HSPs, e.g. HSP 70 and 90 (Brunt & Minson, 2021)). These chaperone proteins are necessary for folding newly synthesized proteins, the translocation of polypeptides, disassembly of protein complexes, and regulating protein activity. They also have anti-

oxidative and thermos-protective properties (Rosenzweig et al., 2019). In humans, heat stress may provide cardioprotective effects by upregulating HSPs (Faulkner et al., 2017); however, it is the intracellular HSP that provides a valuable measure of these positive properties. Currently, a reliable method for measuring iHSPs does not exist. Existing methods to quantify iHSP require the extraction of vascular cells. Procedures for this extraction change the cellular environments, potentially modifying the intracellular components. Extracellular HSPs can be easily measured through venous concentrations; however, eHSP levels are typically correlated with aspects different from the iHSP chaperon proteins, such as inflammation and bacterial or viral infections (Zilae et al., 2014). As such, looking for other biomarkers to gather information on the effects of heat stress is necessary.

An alternative explanation for the benefits of heat stress that is gaining attention is the ‘shearing’ properties of heat stress. Indeed, thermoregulatory induced increases in skin blood flow increase antegrade shear (Alali et al., 2020; Brunt & Minson, 2021; Thijssen et al., 2014). However, no study to date has examined whether a single bout of single arm heating elicits an antegrade shear response large enough to generate meaningful changes in mechanotransduction and in turn endothelial cell adaptations. Accordingly, this study will examine the mechanotransduction properties of local forearm heating, under the lens of whether the antegrade shear stimulus is large enough to increase circulating concentrations of Notch1 extracellular domain (ECD) and HSPGs.

2. AIMS AND HYPOTHESIS

Aim:

Determine the effects of local forearm heating on concentrations of Notch1 ECD and HSPGs in the forearm of young healthy adults.

Hypothesis:

Both Notch1 and HSPGs will be increased during forearm heating, dependent on the magnitude increase in antegrade shear stress.

3. METHODOLOGY

Ethical Approval

The Research Ethics Board at the University of Windsor (REB# 40815) as well as the Research Health and Safety Board approved all experimental procedures and protocols in adherence with the principles of the Tri-Council Policy Statement and the University of Windsor Guidelines for Research Involving Human Participants.

Participants

Of the fifteen participants recruited, three were excluded. One was excluded because of several failed attempts to establish a venous catheter, another was excluded due to high plasma triglyceride levels (visibly cloudy plasma, and later confirmed by a primary care provider), and one was excluded due to a vasovagal response during the catheter insertion. Additionally, the data displayed below is from the first nine participants, which was sufficient to confidently accept the null hypothesis. As such, data are presented as n=9 (3 M, 6 F) healthy young adults. Participant descriptive data are presented in Table 1. All participants were free from any overt cardiovascular or metabolic disease, or risk factors, including hypertension (>140/90)/hypotension (<90/60), presence of obesity, and history of smoking/nicotine use in the past twelve months. A medical pre-screening was conducted over Microsoft Teams to identify any potential exclusion factors and included a COVID-19 self-screen. Both, written and verbal consent was obtained from each participant following a briefing of the experimental protocol.

Experimental Design

A within participant repeated measures experimental design was employed. Participants arrived at the lab (HK Rm 221) at the University of Windsor in the morning, after observing an overnight, water-only fast. The participants had refrained from caffeine, alcohol consumption, and vigorous physical activity for 24 hours prior to the experiment. On testing day, resting blood pressure was determined in a seated position. Following this, venous catheters were placed in the superficial antecubital vein of the participant's left arm and secured for the duration of the study. Following an initial blood sample and ultrasound recording at normothermic baseline, the forearm was placed in a water bath, heated to 42°C by an Anova Sous Vide. Participants were seated at a 45° angle for the duration of the study. Another blood sample and ultrasound reading were taken at 20 minutes and 40 minutes of heating. Following this, the arm was removed from the water bath and allowed to sit for 20 minutes. After this period, the final blood sample and ultrasound recording was taken (see Figure 3).

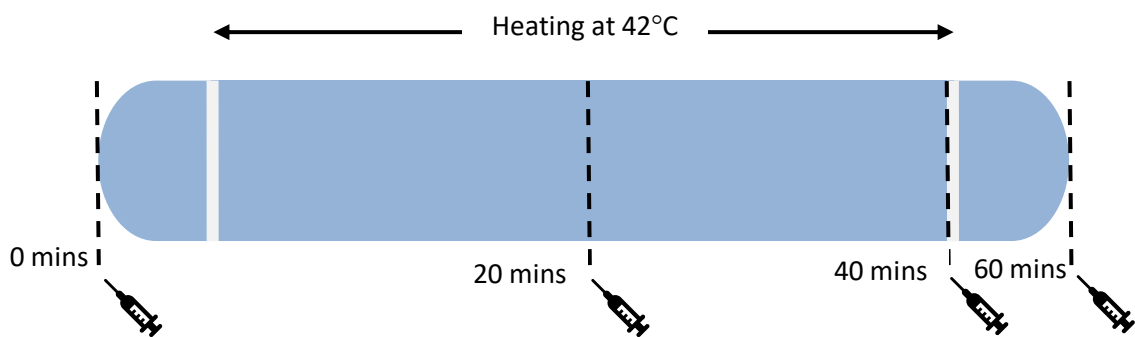


Figure 3: *A depiction of the experimental timeline. The syringe symbol represents sampling periods where both, blood draws and ultrasounds were conducted.*

Measurement Techniques

Shear stress was quantified in the brachial artery by linear array duplex ultrasound (Terason T3200) for ~30 seconds around each blood draw. Ultrasound videos were screen captured and analyzed using custom edge-detection software (Figure 4). Shear rate was calculated as the ratio between mean blood velocity (in cm/s) and artery diameter (in cm) [Shear Rate = mean velocity/diameter]. This value represents the peak shear rate.

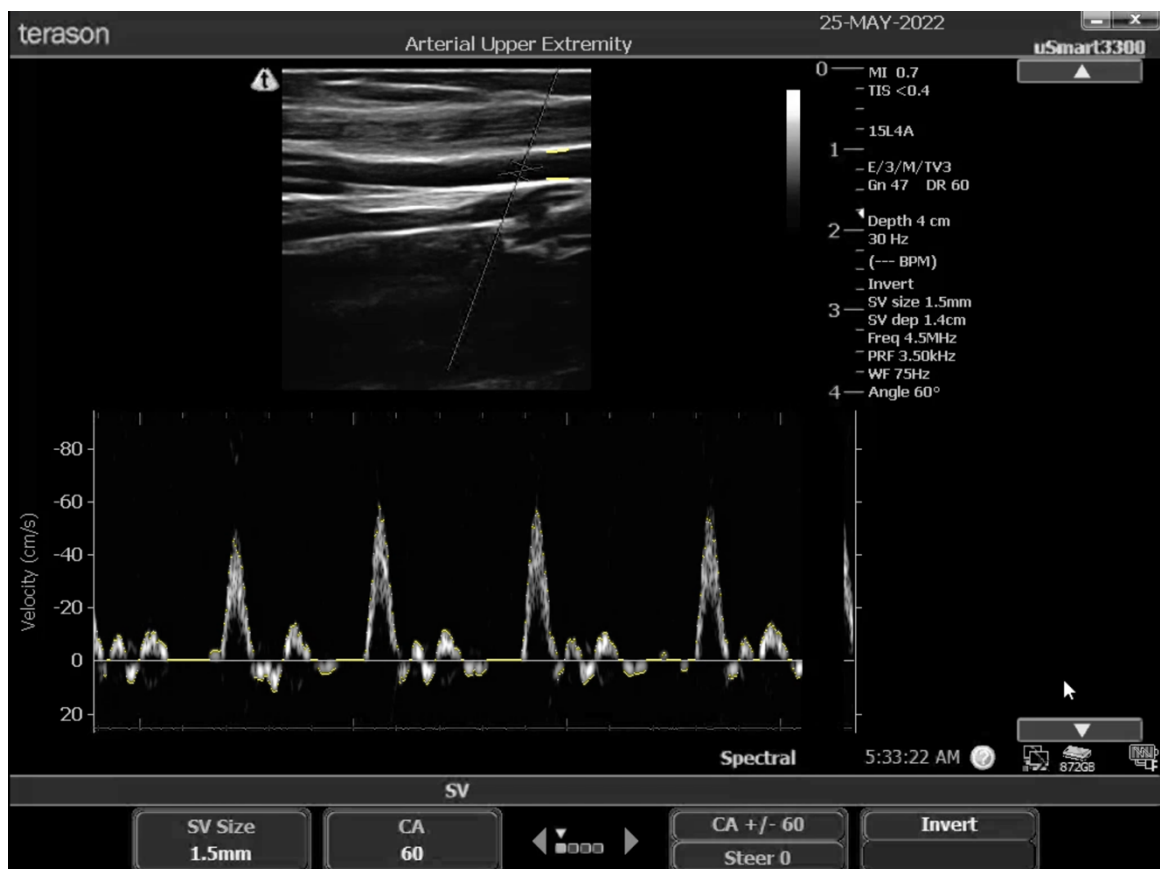


Figure 4: A screenshot of the ultrasound edge detection software video. The picture on the top is the brachial artery. The yellow lines represent the area where the software is detecting the arterial diameter. At the bottom is a graph of the blood velocity, with the yellow line tracking the envelope velocity.

Heart rate was calculated by counting cardiac cycles for 30 seconds from the ultrasound readings and multiplying this number by 2 to get a measurement in BPM.

Blood samples from the experimental arm were collected in sodium citrate tubes (BD Vacutainer, Cat # 363080) and kept on ice for the duration of the experiment. Immediately after the completion of the experiment, the tubes were centrifuged at 1200 g for 10 minutes to collect platelet-poor plasma. These samples were stored at -80°C. Notch1 ECD and HSPG/CD44 activity was quantified using a commercially available immunosorbent assay (RayBio, Cat #ELH-NOTCH1; RayBio, Cat #ELH-CD44) with an intra- and inter-assay CV of <10% and <12%, respectively. This ELISA uses a capture and detection antibody and is classified as a sandwich ELISA. The name “sandwich” refers to the antigen, which is sandwiched between both antibodies. The capture antibody, which is immobilized to the ELISA plate, binds to/captures the target antigen, while the detection antibody, reacts with the antigen and produces a fluorescent signal upon binding. The detection limit of the Notch1 assay is 20 pg/ml to 7000 pg/ml. The detection limit of the CD44 assay is 4.5 pg/ml to 1000 pg/ml. ELISA analysis was conducted according to the manufacturer’s instructions, except for the CD44 where plasma samples were diluted 15-fold, instead of the recommended 20-fold. All samples and reagents were brought to room temperature before mixing. Samples were read at 450nm on a microplate reader (BioTek Synergy HT) using the Gen5 software (Version 1.11).

Table 1: A table of all included participant characteristics. ID=participant identification number; BMI=body mass index; SBP/DBP= resting systolic/diastolic blood pressure.

ID	BMI (kg/m ²)	SBP (mmHg)	DBP (mmHg)	Sex (M/F)	Age (y)
1	25.9	126	83	M	24
2	22.6	111	75	F	21
3	18.8	90	71	F	19
4	21.7	113	81	F	22
5	24.3	122	73	M	21
6	24.2	109	71	F	20
7	26.6	126	66	F	22
8	28.0	96	72	F	21
9	23.2	103	70	M	22
Mean +/- SD	23.9 ± 2.8	110.7 ± 12.8	74 ± 5.4		21.3 ± 1.4

Statistical Analysis

All measures (Notch1, HSPG/CD44, shear rate, heart rate) were analyzed by one-way ANOVA using the four time points (baseline, 20 mins, 40 mins, post). When a significant interaction was found, the post-hoc Bonferroni's correction was performed. All analyses were performed on SPSS 26 (IBM). Significance was determined at an alpha of <0.05%.

4. RESULTS

Heart Rate

Figure 5 depicts the participants heart rate (BPM). There was a main time effect ($p < 0.005$). Compared to baseline (72 ± 6), the heart rate was increased at 20 mins by 18% ($p < 0.001$) and 40 mins by 18% ($p < 0.001$). At post, heart rate was statistically similar to baseline values ($p = 0.220$). The delta change in heart rate from baseline to 20 minutes was 13.1 BPM, to 40 minutes was 13.6 BPM, and to 20 mins post heating was 6.9 BPM.

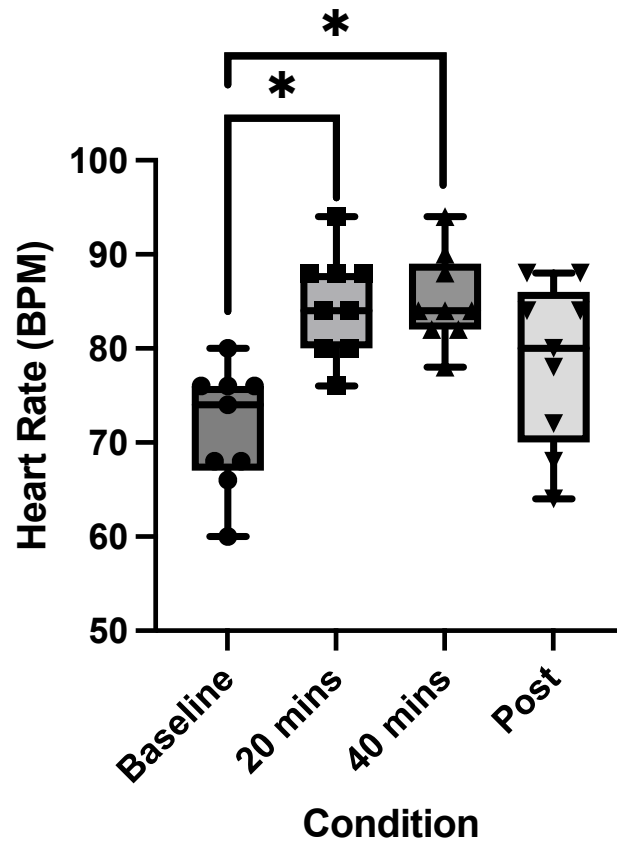


Figure 5: Heart rate (beats per minute) at baseline, 20 and 40 minutes of heating, and 20 minutes following heating (post). Asterix (*) denotes significantly different ($p < 0.05$) compared to baseline. Data shown are individual values with mean \pm 95% CI.

Shear Rate

Absolute shear rates (1/s) of the experimental arm at baseline, 20 mins into heating (20 mins), 40 mins into heating (40 mins), and 20 mins post heating (post) are depicted in Figure 6. There was a main time effect ($p < 0.005$). Compared to the baseline (53 ± 31), the shear rate was increased at 20 mins by 294% ($p < 0.001$) and 40 mins by 270% ($p < 0.001$). At post, shear rates were statistically similar to baseline ($p = 0.100$). The changes in shear, as seen on the ultrasound are depicted in Figure 7-10.

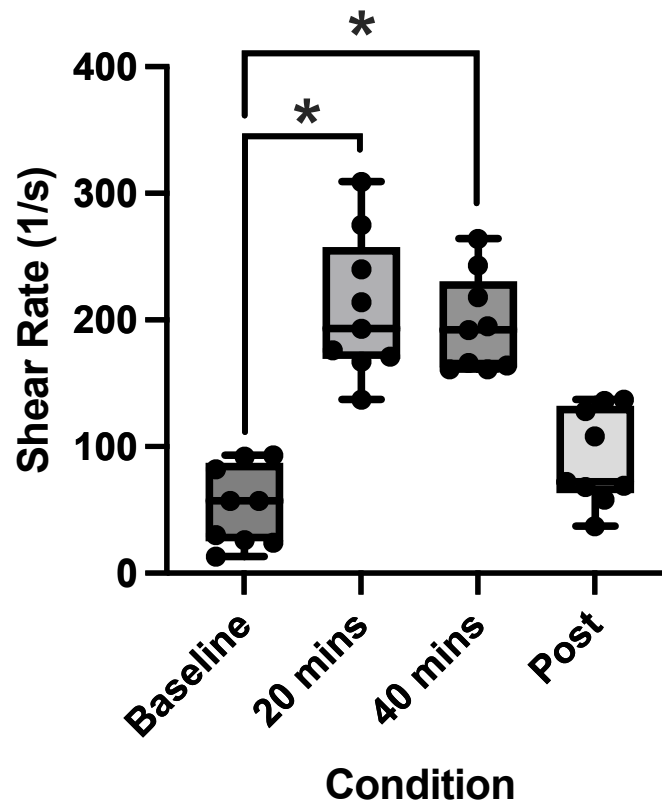


Figure 6: Brachial artery shear rates at baseline, 20 and 40 minutes of heating, and 20 minutes following heating (post). Asterix (*) denotes significantly different ($p < 0.05$) readings compared to baseline. Data shown are individual values with mean \pm 95% CI.

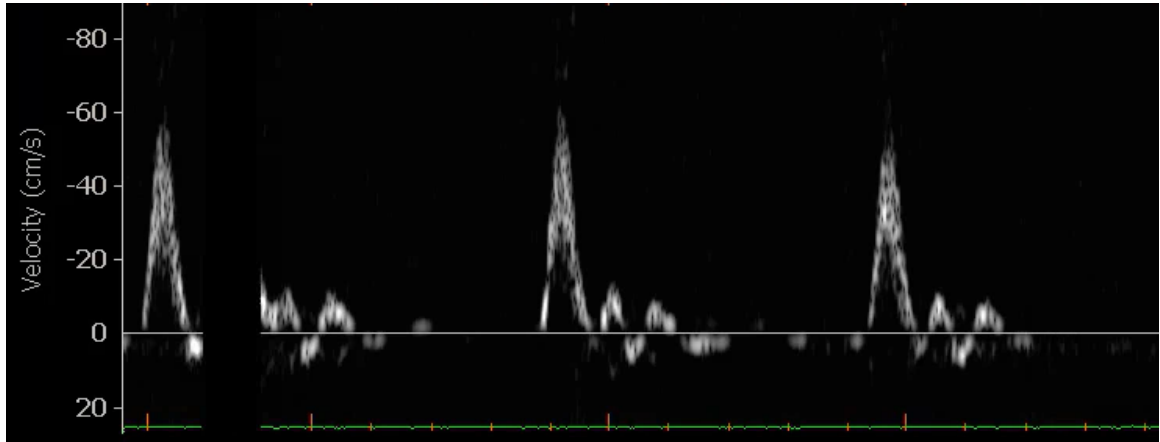


Figure 7: *A screenshot of the blood velocity at baseline. The x-axis represents time, and the y-axis represents blood velocity in cm/s.*

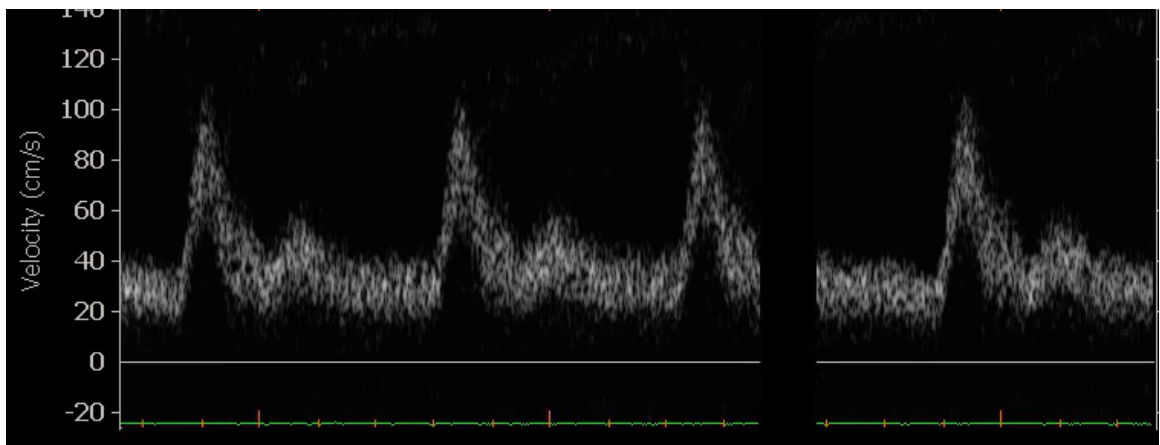


Figure 8: *A screenshot of the blood velocity at 20 mins into heating. The x-axis represents time, and the y-axis represents blood velocity in cm/s.*

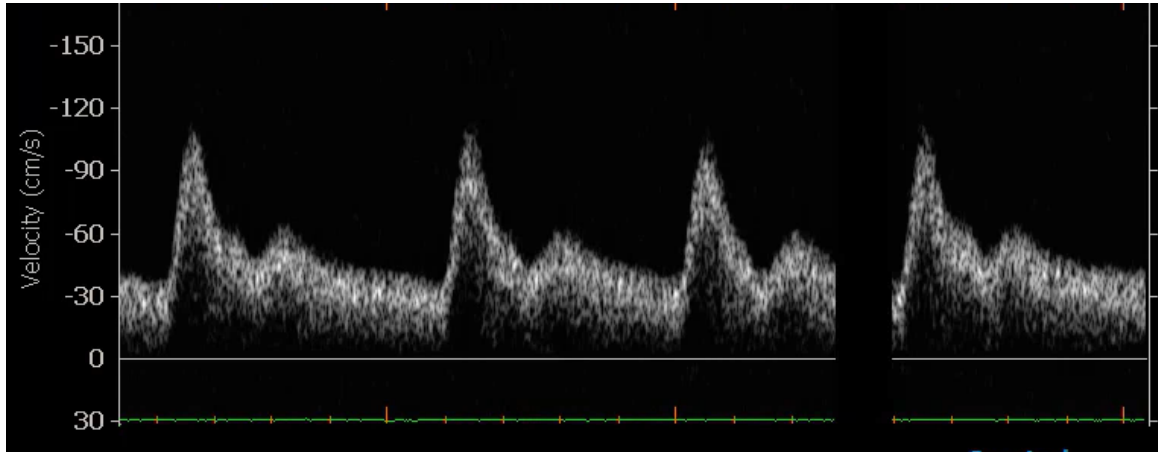


Figure 9: A screenshot of the blood velocity at 40 mins into heating. The x-axis represents time, and the y-axis represents blood velocity in cm/s.

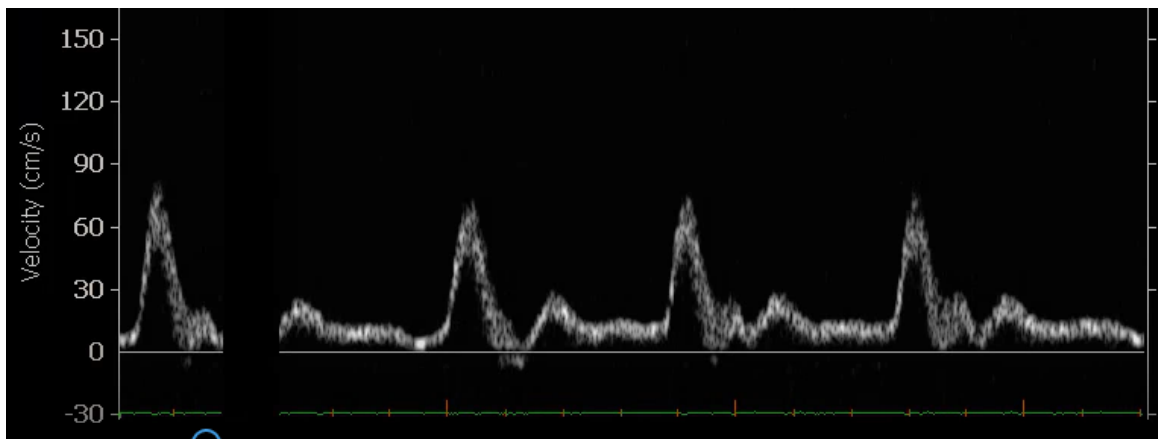


Figure 10: A screenshot of the blood velocity at post heating. The x-axis represents time, and the y-axis represents blood velocity in cm/s.

Notch1 ECD

Concentrations of Notch1 ECD in the experimental arm are shown in Figure 11.

There was no significant difference observed between any of the time points (p all >0.05). Average Notch1 ECD values at baseline were 509 ± 256 , 20 mins into heating were 400 ± 188 , 40 mins into heating were 465 ± 195 , post heating were 481 ± 219 .

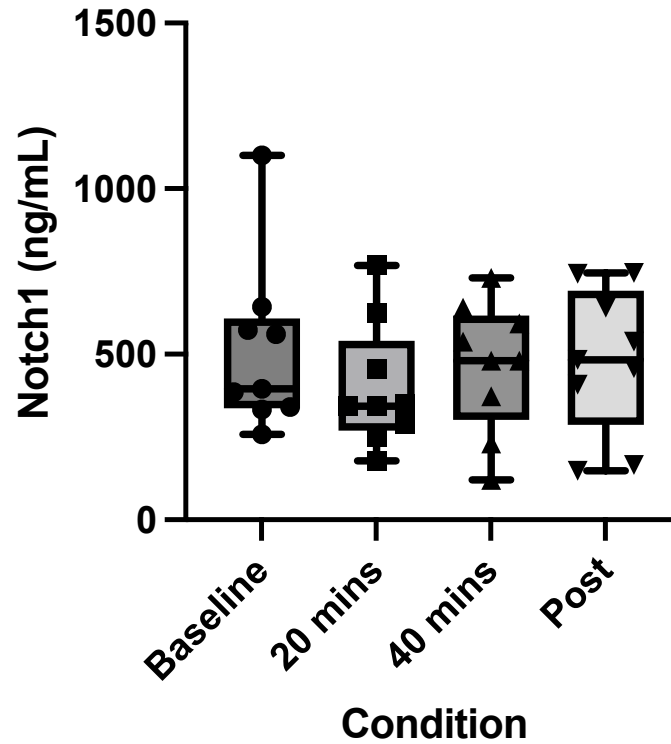


Figure 11: *Brachial artery Nocthl ECD concentrations at baseline, 20 and 40 minutes of heating, and 20 minutes following heating (post). Data shown are individual values with mean \pm 95% CI.*

HSPG/CD44

Concentrations of CD44 in the experimental arm are shown in Figure 12. CD44 was not found in detectable amounts at any time points for all but two participants. In the

two participants with detectable HSPG/CD44, there was no observable change from baseline to heating.

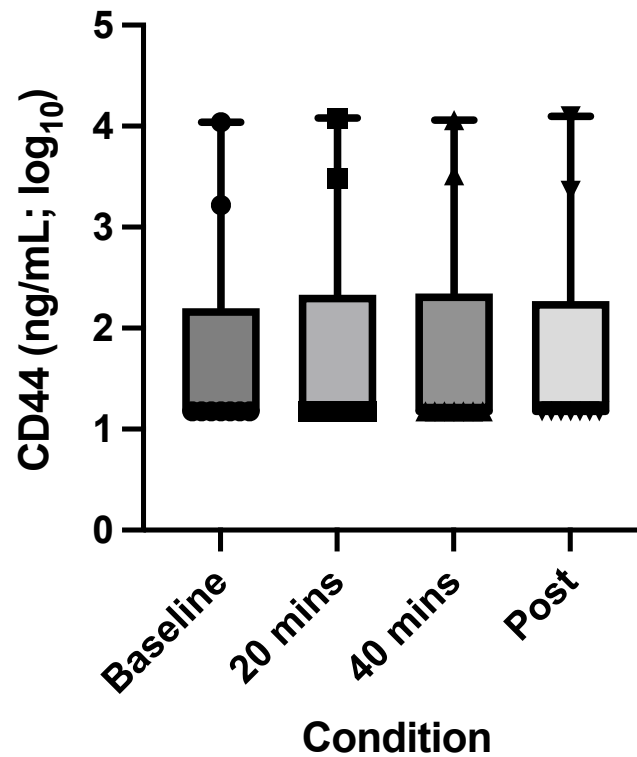


Figure 12: *Brachial artery HSPG/CD44 concentrations at baseline, 20 and 40 minutes of heating, and 20 minutes following heating (post). Data shown are individual values with mean \pm 95% CI.*

5. DISCUSSION

The findings of this thesis indicate that local forearm heating with 42°C water immersion for 40 mins increases antegrade shear stress in the brachial artery but does not alter levels of circulating Notch1 ECD and HSPG/CD44. Specifically, an average 270% increase in antegrade shear rate over 40 minutes resulted in no significant change in Notch1 ECD levels and no detectable amounts of HSPG/CD44 in plasma. To our knowledge, these are the first data to study in vivo alterations of Notch1 ECD and HSPG/CD44 during localized forearm hyperthermia. These data provide new insight into the field of thermal therapy for cardiovascular improvements, indicating that heating the forearm alone is likely insufficient to elicit a robust (or at least easily quantifiable) endothelial mechanosensory stimulus for adaptation.

Notch1 as an endothelial mechanosensor

Isolated cell prep studies indicate that Notch1 signaling increases under conditions of antegrade shear stress (Mack et al., 2017). Studies have indicated that antegrade shear stress results in the elongation and alignment of endothelial cells, along with a physical deformity on the apical surface of cells. This deformation results in the activation of various mechanosensors (Davies, 2009). Recently, our laboratory was the first to translate the in-vitro studies, by demonstrating that an acute increase in antegrade shear rate via forearm cuff inflation/deflation (i.e., reactive hyperemia), increases Notch1 ECD concentrations in the forearm (Badour et al., 2022). Notably, however (discussed below), the magnitude increases in antegrade shear rate in Badour et al., while shorter in duration, was much more pronounced than the current study.

Upon activation, ADAM (disintegrin and metalloprotease) cleave the Notch1 ECD, which produces a membrane-tethered intermediate called Notch extracellular truncation (NEXT), which is further cleaved by γ -secretase, translocating the intracellular Notch1 (NICD) domain to the nucleus. In the nucleus, the NICD co-activates with recombination signal-binding protein for immunoglobulin kappa J (RBPJ), activating downstream signaling pathways (Kopan & Ilagan, 2009). These pathways help to preserve vascular homeostasis, maintain EPHRIN B2 expression, arterial identity, and intracellular calcium homeostasis, and regulate the expression of pro-inflammatory genes associated with atherosclerosis. Examples of these pro-inflammatory genes are CXCL2, ICAM1, and CXCR4. During shear stress, Notch1 is also important for maintaining the stability of endothelial cell-to-cell junctions (Mack et al., 2017). Furthermore, many studies have linked the absence of Notch1 to increases in arterial inflammation, and plaque buildup, as reviewed in Rizzo & Ferrari, 2015. Collectively, it is now accepted that Notch1 serves as an endothelial mechanosensor of shear stress and increases in Notch1 levels are directly linked to improvements in vascular health (Mack et al., 2017; Rizzo & Ferrari, 2015; Briot et al., 2015). Our study, however, showed no significant changes in Notch1 levels with heat induced increases in antegrade shear stress.

One proposed explanation for our findings could be that the shear stress stimulus was not large enough to result in Notch1 ECD increases. Previous studies that have demonstrated the benefits of heat therapy have typically employed larger surface areas such as lower limb heating, or full body heating (Brunt et al., 2021). In the seminal study by Mack et. al, 2017, where Notch1 was first systematically demonstrated to exhibit mechanosensory properties in the endothelium, Notch1 levels were positively correlated

with the magnitude of shear stress, increasing logarithmically from 10 dynes/cm² and plateauing at 26 dynes/cm² (Mack et al., 2017). It is possible to compare these shear stress values (in dynes/cm²) with the shear rates measured in the current study (in 1/s) if we estimate a blood viscosity of 3.5×10^{-3} Pa·s. While blood viscosity will differ with varying hematocrit (reviewed in Nader et al., 2019), this blanket viscosity is valid to estimate the average ~1.9 dynes/cm² at normothermic rest, which increased to ~6.9 dynes/cm² at the 40 min mark of heating. Comparing this number to the values used in the Mack et al., 2017 study, shear stress around 10 dynes/cm² was categorized as a low shear producing minimal cell elongation, and lack of changes in Notch1 levels. In addition, in Mack et al., cells were exposed to differing levels of shear stress for 48 hrs. As such, it is likely that forearm heating alone for 40 min, did not produce a large enough increase in antegrade shear stress, or for a long enough time, to yield detectable changes in Notch1 ECD levels. In this respect, heating a larger surface area or combining other techniques of increasing antegrade shear stress alongside heating (e.g. exercise) is likely necessary to detect biomarkers of mechanotransduction from increased antegrade shear.

Heparan Sulfate Proteoglycan (CD44) as an endothelial mechanosensor

Previous studies have shown that HSPG functions as an endothelial glycocalyx mechanosensor for shear stress (Florian et al., 2003). HSPGs can be divided into three categories- syndecans, glypicans, and perlecan. Of these, the syndecan is the most common and has an extracellular component that is displaced during shear stress. The study by Florian et al., 2003 has shown that the glycosaminoglycan chain component of Heparan Sulfates is the shear stress sensor. To determine the function of HSPG as a shear sensor, this study used heparinase to remove heparan sulfate and increased shear stress.

With antegrade shear stress, research has established that the enzyme, eNOS is activated. This enzyme catalyzes reactions that produce NO, which is crucial to stimulating the relaxation of vascular smooth muscle and plays a role in eliminating free radicals and preventing plaque buildup (reviewed in Sriram et al., 2016). Florian et al., 2003 observed an inhibition of NO production with the removal of heparan sulfates. This finding supports the role of HSPGs as mechanosensors and participants in the NO-mediated response that occurs from shear stress. Subsequent studies have indicated that HSPGs are also critical for stabilizing vasculature and regulating arterial inflammation (Axelsson et al., 2012; Nikmanesh et al., 2012; Zhao et al., 2020). As such, upregulations in HSPG are typically associated with improved arterial health. We hypothesized that heat stress/increases in antegrade shear stress, would be detected by HSPG/CD44 and result in cleavage and release of its extracellular component into plasma. In this study, however, no detectable amounts of HSPG/CD44 were found in 7 of 9 participants. Additionally, no changes in HSPG/CD44 levels were found between normothermia and heating in the 2 participants where HSPG/CD44 was detected in their plasma.

It is unclear why HSPG/CD44 was not detectable in the majority of the participants. Samples were diluted only 15-fold, which was below the recommended 20-fold dilution. Moreover, in the two participants with detectable HSPG/CD44, their samples would have been oversaturated had the samples been less diluted (had a separate ELISA been run to potentially target those with low concentrations), therefore we suggest the lack of detectable HSPG/CD44 is due to participant variability, rather than sample dilution. Interestingly, there were no overt demographical differences between these 2

participants. Nonetheless, even in the participants with detectable HSPG/CD44 plasma concentrations, it is clear that forearm heating had no impact.

A study conducted by Zeng et al., 2013 showed that shear stress resulted in the clustering of heparan sulfate in the endothelial cell junctions. As such, it may be hypothesized that no significant increases in HSPG/CD44 from this study may be because this biomarker is also clustering in the cell junctions during shear stress, rather than being detectable in plasma. However, it is important to note that the Zeng et al., 2013 study was conducted on Rat fat pad endothelial cells, which possess a different phenotype than vascular endothelial cells and are therefore likely to have a separate response to HSPG mediated shear stress.

Study Considerations

While the data indicate that local forearm heating had no impact on endothelial mechanotransduction (at least through the biomarker lens of Notch1 and HSPG/CD44), and therefore may be an insufficient ‘stress’ to elicit positive vascular endothelial adaptations, heat stress may improve vascular function through alternate mechanisms, such as upregulating heat shock proteins (HSPs), specifically, HSP70, which has been studied for its thermal- and cardio-protective role (Latchman, 2001; Riezman, 2004). Therefore, heat therapy could improve cardiovascular function through an increase in levels of HSP70, rather than increasing antegrade shear stress. However, to measure the anti-inflammatory and beneficial effects of HSPs, it is necessary to measure intracellular HSP levels (Faulkner et al., 2017). While endothelial cells can be extracted to measure iHSPs, extraction substantially alters the cellular environment, making it challenging to accurately measure iHSPs in an in vivo human model. In addition to an increase in HSPs,

exposure to heat therapy also leads to heat acclimation, which increases plasma volume, reduces sympathetic activity, decreases blood pressure and arterial stiffness, and has many other benefits (reviewed in Brunt & Minson, 2021).

Another important consideration is the relatively small sample size ($n=9$). While samples were obtained from 12 participants, only 9 of those had their data analyzed. The data from 3 participants were not analyzed as no trends were observed for Notch1 ECD or CD44 levels. Specifically, running a post-hoc power test using G*power from the data of the 9 participants, revealed that a sample size of 458 participants would be required to achieve a power of >80 with an alpha of 0.05, assuming the variability and mean changes remained similar. As such, for economic reasons, the last three participants were omitted from analysis.

Furthermore, it is worth noting that biomarkers could not be trapped in the experimental arm through methods such as placing a pneumatic cuff placed on the upper arm, as used by Badour et al., 2022. Without the use of the cuff, it is possible that if there was an elevation in biomarkers from heat stress, they circulated throughout the body, leading to a decrease in plasma concentrations from the experimental arm. In this study, the pneumatic cuff could not be utilized as it would increase retrograde shear stress, therefore interfering with the ability to isolate the impacts of antegrade shear stress on endothelial function.

Another important consideration is that heat stress may have increased cell permeability, resulting in the quick re-uptake of biomarkers. That is, heat stress is a known stimulus that disrupts endothelial barrier function (Xu et al., 2015), which is

generally studied under the context of increased blood-brain barrier permeability (Bain et al., 2015). Heat stress may have also increased the ability of the endothelial cells to ‘engulf’ the Notch1 ECD and circulating HSPG/CD44, albeit the mechanism(s) associated with increased active uptake of the endothelial cells remains speculative. Nevertheless, considering this concept in the context of this study, it is plausible that Notch1 ECD and HSPG/CD44 levels were increased; however, they were quickly taken up by the cells due to the heat stress, resulting in no detectable plasma level changes during heating.

Another important consideration is the use of only venous blood in this study. Typically, studies use the difference in arterial and venous biomarker levels to determine cellular output vs. uptake. The endothelial cells in the arterial bed are those that are exposed to the greatest shear stress and indeed are those that are important for atherosclerosis progression and the target of this study. However, we were only able to collect blood from the venous circulation, under the assumption that Notch1 ECD and HSPG/CD44 released from the arterial endothelium would be detectable in the venous bed. As discussed above, it may be speculated that the absence of changes in venous biomarker levels may be due to the uptake of these biomarkers by cells on the arterial side. However, during the local forearm heating, the venous blood sample, was in fact, partly arterialized. That is, the arterio-venous anastomoses, which directly connect small arteries and veins under the skin, open in states of heat stress to increase the skin blood flow. This results in blood from the arterial side flowing into veins, causing a mixture of blood (Walløe, 2016). With respect to this study, blood collected from the superficial

antecubital vein was visibly 'pinker' in colour, therefore even though a PO₂ level was not measured, this visible inspection suggests the venous blood was indeed arterialized.

Relating to a 'control' for this experiment, it is important to note that a separate, thermoneutral experiment would have to be employed. This is because we observed an increase in heart rate (~13 BPM) due to heating. If this was converted to changes in core temperature (an increase in 7 BPM roughly equates to a 1°C increase in core temp) based on historical estimations of core temperature change from heart rate (Jose et al., 1970), our data shows that there was a systemic heating effect present. As such, the non-experimental arm would not be isolated enough for us to use as a true control and a separate thermoneutral control experiment would have to be employed. A more appropriate control would be by having a separate group of participants immerse their experimental arm in thermoneutral water and collecting samples at corresponding time intervals as the current study.

6. CONCLUSION

Cardiovascular disease is the number one cause of death globally. While commonly understood contributors to CVD are diet and lifestyle, underlying all of these is endothelial dysfunction. As the endothelium is in direct contact with circulating blood, the pattern of blood flow or shear stress can impact endothelial function. While retrograde or oscillatory shear stress is harmful to arterial health, antegrade or forward-moving blood flow has many positive vascular benefits. Recently, heat therapy has gained attention for its potential to improve cardiovascular health. However, to better understand the mechanisms underlying these improvements, much research remains to be done. Given that heat stress increases laminar blood flow, in this thesis it was hypothesized that biomarkers of endothelial mechanotransduction would be upregulated following 40 minutes of local forearm heating, providing evidence for the proposed shear-mediated therapeutic effects of heating. The transmembrane protein Notch1 and HSPG/CD44, a glycolytic sub-structure, were targeted, given the body of evidence indicating that their upregulation following antegrade shear stress is in part responsible for beneficial vascular endothelial adaptations. Notch1 and HSPG/CD44 were further targeted because of their unique substructure that is released into the circulation upon activation, compared to the other known mechanosensors (e.g. PECAM-1, integrins), where their increased activity is only detectable when measured para or intracellularly. However, while the forearm heating increased antegrade shear rate by (270%), it was not sufficient to elicit any detectable changes in Notch1 ECD and HSPG/CD44. These findings suggest that localized heating of the forearm is likely an insufficient stimulus to be considered a practical heat therapy, at least under the lens of mechanotransduction.

With CVD on the rise, finding therapies for prevention and management is becoming increasingly important. Therefore, two areas of future research would be to determine whether heating larger surface areas changes circulating endothelial mechanosensor levels and to identify if heat therapy works through alternative methods from shear stress.

REFERENCES

- Abraham, D., & Distler, O. (2007). How does endothelial cell injury start? The role of endothelin in systemic sclerosis. In *Arthritis Research and Therapy* (Vol. 9, Issue SUPPL. 2). <https://doi.org/10.1186/ar2186>
- Akerman, A. P., Thomas, K. N., Van Rij, A. M., Body, E. D., Alfadhel, M., & Cotter, J. D. (2019). Heat therapy vs. supervised exercise therapy for peripheral arterial disease: a 12-wk randomized, controlled trial. *Am J Physiol Heart Circ Physiol*, *316*, 1495–1506. <https://doi.org/10.1152/ajpheart.00151.2019.-Peripheral>
- Alali, M. H., Vianna, L. C., Lucas, R. A. I., Junejo, R. T., & Fisher, J. P. (2020). Impact of whole body passive heat stress and arterial shear rate modification on radial artery function in young men. *J Appl Physiol*, *129*, 1373–1382. <https://doi.org/10.1152/jappphysiol.00296.2020.-We>
- Alexander, R. W. (1995). Hypertension and the pathogenesis of atherosclerosis: Oxidative stress and the mediation of arterial inflammatory response: A new perspective. *Hypertension*, *25*(2), 155–161. <https://doi.org/10.1161/01.hyp.25.2.155>
- Axelsson, J., Xu, D., Kang, B. N., Nussbacher, J. K., Handel, T. M., Ley, K., Sriramara, P., & Esko, J. D. (2012). Inactivation of heparan sulfate 2-O-sulfotransferase accentuates neutrophil infiltration during acute inflammation in mice. *Blood*, *120*(8), 1742–1751. <https://doi.org/10.1182/blood-2012-03-417139>
- Badour, M. I., Stone, R. M., Parikh, K. S., Lester, N. J., Meloche, O. L., Wulterkens, R. N., & Bain, A. R. (2022). Circulating Notch1 in response to altered vascular wall shear stress in adults. *Experimental Physiology*, *107*(12), 1426–1431. <https://doi.org/10.1113/EP090749>
- Bain, A. R., Nybo, L., & Ainslie, P. N. (2015). Cerebral vascular control and metabolism in heat stress. *Comprehensive Physiology*, *5*(3), 1345–1380. <https://doi.org/10.1002/cphy.c140066>
- Ballermann, B. J., Dardik, A., Eng, E., & Liu, A. (1998). *Shear stress and the endothelium*.
- Bishop, J. R., Schuksz, M., & Esko, J. D. (2007). Heparan sulphate proteoglycans fine-tune mammalian physiology. In *Nature* (Vol. 446, Issue 7139, pp. 1030–1037). Nature Publishing Group. <https://doi.org/10.1038/nature05817>
- Briot, A., Civelek, M., Seki, A., Hoi, K., Mack, J. J., Lee, S. D., Kim, J., Hong, C., Yu, J., Fishbein, G. A., Vakili, L., Fogelman, A. M., Fishbein, M. C., Lusis, A. J., Tontonoz, P., Navab, M., Berliner, J. A., & Iruela-Arispe, M. L. (2015). Endothelial

- NOTCH1 is suppressed by circulating lipids and antagonizes inflammation during atherosclerosis. *Journal of Experimental Medicine*, 212(12), 2147–2163.
<https://doi.org/10.1084/jem.20150603>
- Brunt, V. E., Howard, M. J., Francisco, M. A., Ely, B. R., & Minson, C. T. (2016). Passive heat therapy improves endothelial function, arterial stiffness and blood pressure in sedentary humans. *Journal of Physiology*, 594(18), 5329–5342.
<https://doi.org/10.1113/JP272453>
- Brunt, V. E., & Minson, C. T. (2021). Heat therapy: mechanistic underpinnings and applications to cardiovascular health. In *Journal of Applied Physiology* (Vol. 130, Issue 6, pp. 1684–1704). American Physiological Society.
<https://doi.org/10.1152/japplphysiol.00141.2020>
- Carter, H. H., Spence, A. L., Atkinson, C. L., Pugh, C. J. A., Naylor, L. H., & Green, D. J. (2014). Repeated core temperature elevation induces conduit artery adaptation in humans. *European Journal of Applied Physiology*, 114(4), 859–865.
<https://doi.org/10.1007/s00421-013-2817-2>
- Chatterjee, S., & Fisher, A. B. (2014). Mechanotransduction in the endothelium: Role of membrane proteins and reactive oxygen species in sensing, transduction, and transmission of the signal with altered blood flow. In *Antioxidants and Redox Signaling* (Vol. 20, Issue 6, pp. 899–913). <https://doi.org/10.1089/ars.2013.5624>
- Chen, L.-J., Wang, W.-L., & Chiu, J.-J. (2016). *Molecular and Cellular Mechanobiology* (S. Chien, A. Engler, & P. Wang, Eds.; 1st ed.). Springer.
- Cheng, J. L., Williams, J. S., Hoekstra, S. P., & MacDonald, M. J. (2021). Improvements in vascular function in response to acute lower limb heating in young healthy males and females. *Journal of Applied Physiology*, 131(1), 277–289.
<https://doi.org/10.1152/japplphysiol.00630.2020>
- Coombs, G. B., & Tremblay, J. C. (2019). Passive heat therapy for cerebral protection: new ideas of age-old concepts. In *Journal of Physiology* (Vol. 597, Issue 2, pp. 371–372). Blackwell Publishing Ltd. <https://doi.org/10.1113/JP277314>
- Cui, J., Gao, Z., Leuenberger, U. A., Blaha, C., Luck, J. C., Herr, M. D., & Sinoway, L. I. (2022). Repeated warm water baths decrease sympathetic activity in humans. *Journal of Applied Physiology*, 133(1), 234–245.
<https://doi.org/10.1152/japplphysiol.00684.2021>
- Davies, P. F. (2009). Hemodynamic shear stress and the endothelium in cardiovascular pathophysiology. In *Nature Clinical Practice Cardiovascular Medicine* (Vol. 6, Issue 1, pp. 16–26). <https://doi.org/10.1038/ncpcardio1397>

- Davignon, J., & Ganz, P. (2004). Role of endothelial dysfunction in atherosclerosis. In *Circulation* (Vol. 109, Issue 23 SUPPL.).
<https://doi.org/10.1161/01.cir.0000131515.03336.f8>
- Davis, N. E. (2005). Atherosclerosis - An Inflammatory Process. In *Journal of Insurance Medicine J Insur Med* (Vol. 37).
- De Keulenaer, G. W., Chappell, D. C., Ishizaka, N., Nerem, R. M., Alexander, R. W., & Griendling, K. K. (1998). *Oscillatory and Steady Laminar Shear Stress Differentially Affect Human Endothelial Redox State Role of a Superoxide-Producing NADH Oxidase*. <http://ahajournals.org>
- D'Souza, B., Meloty-Kapella, L., & Weinmaster, G. (2010). Canonical and non-canonical notch ligands. In *Current Topics in Developmental Biology* (Vol. 92, Issue C). [https://doi.org/10.1016/S0070-2153\(10\)92003-6](https://doi.org/10.1016/S0070-2153(10)92003-6)
- Ely, B. R., Francisco, M. A., Halliwill, J. R., Bryan, S. D., Comrada, L. N., Larson, E. A., Brunt, V. E., Christopher, X., & Minson, T. (2019). Heat therapy reduces sympathetic activity and improves cardiovascular risk profile in women who are obese with polycystic ovary syndrome. *Am J Physiol Regul Integr Comp Physiol*, 317, 630–640. <https://doi.org/10.1152/ajpregu.00078.2019.-Polycystic>
- Faulkner, S. H., Jackson, S., Fatania, G., & Leicht, C. A. (2017). The effect of passive heating on heat shock protein 70 and interleukin-6: A possible treatment tool for metabolic diseases? *Temperature*, 4(3), 292–304.
<https://doi.org/10.1080/23328940.2017.1288688>
- Florian, J. A., Kosky, J. R., Ainslie, K., Pang, Z., Dull, R. O., & Tarbell, J. M. (2003). Heparan sulfate proteoglycan is a mechanosensor on endothelial cells. *Circulation Research*, 93(10). <https://doi.org/10.1161/01.res.0000101744.47866.d5>
- Gamrat, A., Surdacki, M. A., Chyrchel, B., & Surdacki, A. (2020). Endothelial dysfunction: A contributor to adverse cardiovascular remodeling and heart failure development in type 2 diabetes beyond accelerated atherogenesis. In *Journal of Clinical Medicine* (Vol. 9, Issue 7, pp. 1–15). MDPI.
<https://doi.org/10.3390/jcm9072090>
- Givens, C., & Tzima, E. (2016). Endothelial Mechanosignaling: Does One Sensor Fit All? In *Antioxidants and Redox Signaling* (Vol. 25, Issue 7, pp. 373–388). Mary Ann Liebert Inc. <https://doi.org/10.1089/ars.2015.6493>
- Hall, J., & Guyton, A. (2011). *Guyton and Hall Textbook of Medical Physiology* (11th ed.). Saunders/Elsevier.

- Hannuksela, M. L., & Ellahham, S. (2001). *Benefits and Risks of Sauna Bathing*.
- Hofmann, J. J., & Iruela-Arispe, M. L. (2007). Notch signaling in blood vessels: Who is talking to whom about what? In *Circulation Research* (Vol. 100, Issue 11, pp. 1556–1568). <https://doi.org/10.1161/01.RES.0000266408.42939.e4>
- Hong, Y. M. (2010). Atherosclerotic cardiovascular disease beginning in childhood. In *Korean Circulation Journal* (Vol. 40, Issue 1, pp. 1–9). Korean Society of Cardiology. <https://doi.org/10.4070/kcj.2010.40.1.1>
- Hu, Y., Chen, M., Wang, M., & Li, X. (2022). Flow-mediated vasodilation through mechanosensitive G protein-coupled receptors in endothelial cells. In *Trends in Cardiovascular Medicine* (Vol. 32, Issue 2, pp. 61–70). Elsevier Inc. <https://doi.org/10.1016/j.tcm.2020.12.010>
- Insull, W. (2009). The Pathology of Atherosclerosis: Plaque Development and Plaque Responses to Medical Treatment. *American Journal of Medicine*, 122(1 SUPPL.). <https://doi.org/10.1016/j.amjmed.2008.10.013>
- Jose, A. D., St, F., Collison, M. D., & Sydney, M. (1970). Experimental and laboratory reports The effects of exercise and changes in body temperature on the intrinsic heart rate in man. In *American Heart Journal April* (Vol. 79, Issue 4).
- Keast, M. L., & Adamo, K. (2000). The Finnish Sauna Bath and Its Use in Patients With Cardiovascular Disease. *Journal of Cardiopulmonary Rehabilitation*, 20(4), 225–230.
- Kenney, W. L., Craighead, D. H., & Alexander, L. M. (2014). Heat waves aging and human cardiovascular health. *Medicine and Science in Sports and Exercise*, 46(10), 1891–1899. <https://doi.org/10.1249/MSS.0000000000000325>
- Khazaeli, A. A., Tatar, M., Pletcher, S. D., & Curtsinger, J. W. (1997). Heat-Induced Longevity Extension in *Drosophila*. I. Heat Treatment, Mortality, and Thermotolerance. In *Journal of Gerontology: BIOLOGICAL SCIENCES* (Vol. 52). <https://academic.oup.com/biomedgerontology/article/52A/1/B48/550151>
- Kohara, K., Tabara, Y., Ochi, M., Okada, Y., Ohara, M., Nagai, T., Ohyagi, Y., & Igase, M. (2018). Habitual hot water bathing protects cardiovascular function in middle-aged to elderly Japanese subjects. *Scientific Reports*, 8(1). <https://doi.org/10.1038/s41598-018-26908-1>
- Kopan, R., & Ilagan, M. X. G. (2009). The Canonical Notch Signaling Pathway: Unfolding the Activation Mechanism. In *Cell* (Vol. 137, Issue 2, pp. 216–233). Elsevier B.V. <https://doi.org/10.1016/j.cell.2009.03.045>

- Latchman, D. S. (2001). Heat shock proteins and cardiac protection. In *Cardiovascular Research* (Vol. 51). www.elsevier.com/locate/cardiore
- Lithgow, G. (1995). *Thermotolerance and extended life-span conferred by single-gene mutations and induced by thermal stress*.
- Lu, Y., Cui, X., Zhang, L., Wang, X., Xu, Y., Qin, Z., Liu, G., Wang, Q., Tian, K., Lim, K. S., Charles, C. J., Zhang, J., & Tang, J. (2022). The Functional Role of Lipoproteins in Atherosclerosis: Novel Directions for Diagnosis and Targeting Therapy. In *Aging and Disease* (Vol. 13, Issue 2, pp. 491–520). International Society on Aging and Disease. <https://doi.org/10.14336/AD.2021.0929>
- MacK, J. J., & Luisa Iruela-Arispe, M. (2018). NOTCH regulation of the endothelial cell phenotype. In *Current Opinion in Hematology* (Vol. 25, Issue 3, pp. 212–218). Lippincott Williams and Wilkins. <https://doi.org/10.1097/MOH.0000000000000425>
- Mack, J. J., Mosqueiro, T. S., Archer, B. J., Jones, W. M., Sunshine, H., Faas, G. C., Briot, A., Aragón, R. L., Su, T., Romay, M. C., McDonald, A. I., Kuo, C. H., Lizama, C. O., Lane, T. F., Zovein, A. C., Fang, Y., Tarling, E. J., De Aguiar Vallim, T. Q., Navab, M., ... Iruela-Arispe, M. L. (2017). NOTCH1 is a mechanosensor in adult arteries. *Nature Communications*, 8(1). <https://doi.org/10.1038/s41467-017-01741-8>
- Malek, A. M., Alper, S. L., & Izumo, S. (1999). *Hemodynamic Shear Stress and Its Role in Atherosclerosis*. <http://jama.jamanetwork.com/>
- Miyata, M., & Tei, C. (2010). Waon therapy for cardiovascular disease: Innovative Therapy for the 21st Century. In *Circulation Journal* (Vol. 74, Issue 4, pp. 617–621). <https://doi.org/10.1253/circj.CJ-09-0939>
- Nader, E., Skinner, S., Romana, M., Fort, R., Lemonne, N., Guillot, N., Gauthier, A., Antoine-Jonville, S., Renoux, C., Hardy-Dessources, M. D., Stauffer, E., Joly, P., Bertrand, Y., & Connes, P. (2019). Blood rheology: Key parameters, impact on blood flow, role in sickle cell disease and effects of exercise. In *Frontiers in Physiology* (Vol. 10, Issue OCT). Frontiers Media S.A. <https://doi.org/10.3389/fphys.2019.01329>
- Naylor, L. H., Carter, H., Fitzsimons, M. G., Cable, N. T., Thijssen, D. H. J., & Green, D. J. (2011). Repeated increases in blood flow, independent of exercise, enhance conduit artery vasodilator function in humans. *Am J Physiol Heart Circ Physiol*, 300, 664–669. <https://doi.org/10.1152/ajpheart.00985.2010>.-This
- Neff, D., Kuhlenhoelter, A. M., Lin, C., Brett, X., Wong, J., Motaganahalli, R. L., & Roseguini, B. T. (2016). Thermotherapy reduces blood pressure and circulating

- endothelin-1 concentration and enhances leg blood flow in patients with symptomatic peripheral artery disease. *Am J Physiol Regul Integr Comp Physiol*, 311, 392–400. <https://doi.org/10.1152/ajpregu.00147.2016>.-Leg
- Nikmanesh, M., Shi, Z. D., & Tarbell, J. M. (2012). Heparan sulfate proteoglycan mediates shear stress-induced endothelial gene expression in mouse embryonic stem cell-derived endothelial cells. *Biotechnology and Bioengineering*, 109(2), 583–594. <https://doi.org/10.1002/bit.23302>
- PAPAIOANNOU, T., & STEFANADIS, C. (2005). Basic principles of shear stress. *Hellenic J Cardio*, 9–15.
- Pasmanter, N., Iheanacho, F., & Muhammad, H. (2023). *Biochemistry, Cyclic GMP*. StatPearls.
- Patrick, R. P., & Johnson, T. L. (2021). Sauna use as a lifestyle practice to extend healthspan. In *Experimental Gerontology* (Vol. 154). Elsevier Inc. <https://doi.org/10.1016/j.exger.2021.111509>
- Paulus, W. J., & Tschöpe, C. (2013). A novel paradigm for heart failure with preserved ejection fraction: Comorbidities drive myocardial dysfunction and remodeling through coronary microvascular endothelial inflammation. In *Journal of the American College of Cardiology* (Vol. 62, Issue 4, pp. 263–271). Elsevier USA. <https://doi.org/10.1016/j.jacc.2013.02.092>
- Pellinger, T. K., Neighbors, C. B., & Simmons, G. H. (2019). Acute Lower Leg Heating Increases Exercise Capacity in Patients With Peripheral Artery Disease. *Journal of Cardiovascular Nursing*, 34(2), 130–133. <https://doi.org/10.1097/JCN.0000000000000510>
- Riezman, H. (2004). Why do cells require heat shock proteins to survive heat stress? In *Cell Cycle* (Vol. 3, Issue 1, pp. 60–62). Taylor and Francis Inc. <https://doi.org/10.4161/cc.3.1.625>
- Rizzo, P., & Ferrari, R. (2015). The Notch pathway: A new therapeutic target in atherosclerosis? *European Heart Journal, Supplement*, 17, A74–A76. <https://doi.org/10.1093/eurheartj/suv011>
- Roman, M. J., Devereux, R. B., Kizer, J. R., Okin, P. M., Lee, E. T., Wang, W., Umans, J. G., Calhoun, D., & Howard, B. V. (2009). High Central Pulse Pressure Is Independently Associated With Adverse Cardiovascular Outcome. The Strong Heart Study. *Journal of the American College of Cardiology*, 54(18), 1730–1734. <https://doi.org/10.1016/j.jacc.2009.05.070>

- Rose, G. A., Davies, R. G., Appadurai, I. R., Williams, I. M., Bashir, M., Berg, R. M. G., Poole, D. C., & Bailey, D. M. (2022). 'Fit for surgery': the relationship between cardiorespiratory fitness and postoperative outcomes. In *Experimental Physiology* (Vol. 107, Issue 8, pp. 787–799). John Wiley and Sons Inc. <https://doi.org/10.1113/EP090156>
- Rosenzweig, R., Nillegoda, N. B., Mayer, M. P., & Bukau, B. (2019). The Hsp70 chaperone network. In *Nature Reviews Molecular Cell Biology* (Vol. 20, Issue 11, pp. 665–680). Nature Publishing Group. <https://doi.org/10.1038/s41580-019-0133-3>
- Ross, R. (1986). The Pathogenesis of Atherosclerosis. *The New England Journal of Medicine*, 488–500.
- Ross, R. (1995). CELL BIOLOGY OF ATHEROSCLEROSIS. In *Annu. Rev. Physiol* (Vol. 57). www.annualreviews.org
- Sander, G. R., Kryszinska, H., & Powell, B. C. (2006). *Developmental Signaling Networks: The Notch Pathway*.
- Sandoo, A., Veldhuijzen Van Zanten, J. J. C. S., Metsios, G. S., Carroll, D., & Kitas, G. D. (2010). The Endothelium and Its Role in Regulating Vascular Tone. In *The Open Cardiovascular Medicine Journal* (Vol. 4).
- Sarrazin, S., Lamanna, W. C., & Esko, J. D. (2011). Heparan sulfate proteoglycans. *Cold Spring Harbor Perspectives in Biology*, 3(7), 1–33. <https://doi.org/10.1101/cshperspect.a004952>
- Schick, V., Boensch, M., van Edig, M., Alfitian, J., Pola, T., Ecker, H., Lindacher, F., Shah-Hosseini, K., Wetsch, W. A., Riedel, B., & Schier, R. (2021). Impaired vascular endothelial function as a perioperative risk predictor – a prospective observational trial. *BMC Anesthesiology*, 21(1). <https://doi.org/10.1186/s12871-021-01400-y>
- Schreuder, T. H. A., Green, D. J., Hopman, M. T. E., & Thijssen, D. H. J. (2014). Acute impact of retrograde shear rate on brachial and superficial femoral artery flow-mediated dilation in humans. *Physiological Reports*, 2(1). <https://doi.org/10.1002/phy2.193>
- Shaaban, A. M., & Duerinckx, A. J. (2000). Wall shear stress and early atherosclerosis: A review. In *American Journal of Roentgenology* (Vol. 174, Issue 6, pp. 1657–1665). American Roentgen Ray Society. <https://doi.org/10.2214/ajr.174.6.1741657>

- Slivnick, J., & Lampert, B. C. (2019). Hypertension and Heart Failure. In *Heart Failure Clinics* (Vol. 15, Issue 4, pp. 531–541). Elsevier Inc.
<https://doi.org/10.1016/j.hfc.2019.06.007>
- Smith, E. (2009). *The Canadian Heart Health Strategy and Action Plan*. www.chhs-scsc.ca
- Sriram, K., Laughlin, J. G., Rangamani, P., & Tartakovsky, D. M. (2016). Shear-Induced Nitric Oxide Production by Endothelial Cells. *Biophysical Journal*, 111(1), 208–221. <https://doi.org/10.1016/j.bpj.2016.05.034>
- Sun, H. J., Wu, Z. Y., Nie, X. W., & Bian, J. S. (2020). Role of endothelial dysfunction in cardiovascular diseases: The link between inflammation and hydrogen sulfide. In *Frontiers in Pharmacology* (Vol. 10). Frontiers Media S.A.
<https://doi.org/10.3389/fphar.2019.01568>
- Tabas, I., Williams, K. J., & Borén, J. (2007). Subendothelial lipoprotein retention as the initiating process in atherosclerosis: Update and therapeutic implications. In *Circulation* (Vol. 116, Issue 16, pp. 1832–1844).
<https://doi.org/10.1161/CIRCULATIONAHA.106.676890>
- Tarbell, J. M., & Pahakis, M. Y. (2006). Mechanotransduction and the glycocalyx. *Journal of Internal Medicine*, 259(4), 339–350. <https://doi.org/10.1111/j.1365-2796.2006.01620.x>
- Thijssen, D. H. J., Atkinson, C. L., Ono, K., Sprung, V. S., Spence, A. L., Pugh, C. J. A., & Green, D. J. (2014). Sympathetic nervous system activation, arterial shear rate, and flow-mediated dilation. *J Appl Physiol*, 116, 1300–1307.
<https://doi.org/10.1152/japplphysiol.00110.2014.-The>
- Thijssen, D. H. J., Dawson, E. A., Tinken, T. M., Cable, N. T., & Green, D. J. (2009). Retrograde Flow and Shear Rate Acutely Impair Endothelial Function in Humans. *Hypertension*, 53(6), 986–992.
<https://doi.org/10.1161/HYPERTENSIONAHA.109.131508>
- Thomas, K. N., Van Rij, A. M., Lucas, S. J. E., & Cotter, J. D. (2017). Lower-limb hot-water immersion acutely induces beneficial hemodynamic and cardiovascular responses in peripheral arterial disease and healthy, elderly controls. *Am J Physiol Regul Integr Comp Physiol*, 312, 281–291.
<https://doi.org/10.1152/ajpregu.00404.2016.-Passive>
- Toya, T., Sara, J. D., Corban, M. T., Taher, R., Godo, S., Herrmann, J., Lerman, L. O., & Lerman, A. (2020). Assessment of peripheral endothelial function predicts future

- risk of solid-tumor cancer. *European Journal of Preventive Cardiology*, 27(6), 608–618. <https://doi.org/10.1177/2047487319884246>
- Traub, O., & Berk, B. C. (1998). *Laminar Shear Stress Mechanisms by Which Endothelial Cells Transduce an Atheroprotective Force Brief Review*. <http://ahajournals.org>
- Turpaev, K. T. (2020). Transcription Factor KLF2 and Its Role in the Regulation of Inflammatory Processes. In *Biochemistry (Moscow)* (Vol. 85, Issue 1, pp. 54–67). Pleiades journals. <https://doi.org/10.1134/S0006297920010058>
- Tzima, E., Irani-Tehrani, M., Kiosses, W. B., Dejana, E., Schultz, D. A., Engelhardt, B., Cao, G., DeLisser, H., & Schwartz, M. A. (2005). A mechanosensory complex that mediates the endothelial cell response to fluid shear stress. *Nature*, 437(7057), 426–431. <https://doi.org/10.1038/nature03952>
- Vaduganathan, M., Mensah, G. A., Turco, J. V., Fuster, V., & Roth, G. A. (2022). The Global Burden of Cardiovascular Diseases and Risk: A Compass for Future Health. In *Journal of the American College of Cardiology* (Vol. 80, Issue 25, pp. 2361–2371). Elsevier Inc. <https://doi.org/10.1016/j.jacc.2022.11.005>
- Walløe, L. (2016). Arterio-venous anastomoses in the human skin and their role in temperature control. *Temperature*, 3(1), 92–103. <https://doi.org/10.1080/23328940.2015.1088502>
- Wang, L., Fuster, M., Sriramaraio, P., & Esko, J. D. (2005). Endothelial heparan sulfate deficiency impairs L-selectin- and chemokine-mediated neutrophil trafficking during inflammatory responses. *Nature Immunology*, 6(9), 902–910. <https://doi.org/10.1038/ni1233>
- Westaway, K., Frank, O., Husband, A., McClure, A., Shute, R., Edwards, S., Curtis, J., & Rowett, D. (2015). Medicines can affect thermoregulation and accentuate the risk of dehydration and heat-related illness during hot weather. *Journal of Clinical Pharmacy and Therapeutics*, 40(4), 363–367. <https://doi.org/10.1111/jcpt.12294>
- Widmer, R. J., & Lerman, A. (2014). Endothelial dysfunction and cardiovascular disease. In *Global Cardiology Science and Practice* (Vol. 2014, Issue 3). HBKU Press. <https://doi.org/10.5339/gcsp.2014.43>
- Xu, Q., Liu, J., Wang, Z., Guo, X., Zhou, G., Liu, Y., Huang, Q., & Su, L. (2015). Heat stress-induced disruption of endothelial barrier function is via PAR1 signaling and suppressed by Xuebijing injection. *PLoS ONE*, 10(2). <https://doi.org/10.1371/journal.pone.0118057>

- Zeng, Y., Waters, M., Andrews, A., Honarmandi, P., Ebong, E. E., Rizzo, V., & Tarbell, J. M. (2013). Fluid shear stress induces the clustering of heparan sulfate via mobility of glypican-1 in lipid rafts. *Am J Physiol Heart Circ Physiol*, 305, 811–820. <https://doi.org/10.1152/ajpheart.00764.2012>. -The
- Zhang, X., Sessa, W. C., & Fernández-Hernando, C. (2018). Endothelial Transcytosis of Lipoproteins in Atherosclerosis. In *Frontiers in Cardiovascular Medicine* (Vol. 5). Frontiers Media S.A. <https://doi.org/10.3389/fcvm.2018.00130>
- Zhao, P., Liu, X., Zhang, X., Wang, L., Su, H., Wang, L., He, N., Zhang, D., Li, Z., Kang, H., Sun, A., Chen, Z., Zhou, L., Wang, M., Zhang, Y., Deng, X., & Fan, Y. (2021). Flow shear stress controls the initiation of neovascularization: via heparan sulfate proteoglycans within a biomimetic microfluidic model. *Lab on a Chip*, 21(2), 421–434. <https://doi.org/10.1039/d0lc00493f>
- Zilae, M., Ferns, G. A. A., & Ghayour-Mobarhan, M. (2014). Heat shock proteins and cardiovascular disease. In *Advances in Clinical Chemistry* (Vol. 64, pp. 73–115). Academic Press Inc. <https://doi.org/10.1016/B978-0-12-800263-6.00002-1>

VITA AUCTORIS

NAME: Khushali Parikh

PLACE OF BIRTH: Mumbai, IN

YEAR OF BIRTH: 1999

EDUCATION: Vincent Massey Secondary School, Windsor, ON,
2017

University of Windsor, B.Sc., Windsor, ON, 2021

University of Windsor, M.Sc., Windsor, ON, 2023